GENETIC DIFFERENTIATION OF SIX IMPROVED TROPICALLY ADAPTED CHICKEN BREEDS IN IMO STATE NIGERIA USING MITICHONDRIAL DNA

BY

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CERTIFICATION

I certify that the work- Genetic differentiation of six improved tropically adapted chicken breeds (TABs) in Imo State Nigeria, using mitochondrial DNA was carried out by OKANI ONYEJIAKA, MIRIAN CHIKODI, (20154940308) in partial fulfilment for the award of the degree of Master of Science (MSc) in Animal Breeding and Genetics in the Department of Animal Science and Technology of the Federal University of Technology, Owerri.

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DEDICATION

I dedicate this thesis to God Almighty, who alone anchored and inspired me in the course of this study, and to my late aunty; Mrs Juliana Uzoagba (Nee-Okani).

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX

ABSTRACT

This study investigated the genetic differentiation (degree of divergence) of six Improved Tropically Adapted Chicken Breeds (iTABs), raised under semi-intensive management system in Imo State. 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 the blood samples in the same ratio for the same populations, respectively. A 592-bp of mtDNA D-loop region was amplified followed by partial sequencing of the mtDNA D-loop region. A 315 bp long sequence fragment was subsequently realized and used for the various analysis using different softwares. Results showed that 14 haplotypes were identified from 62 polymorphic sites. The highest (H=10) and the lowest (H=5) number of haplotypes were found within FUNAAB-Alpha, and Shika Brown/Noiler, respectively. The haplotype diversity (Hd) and nucleotide diversity (Pi) within population ranged from 0.731 (Sasso) - 0.970 (FUNAAB Alpha), and 0.383 (Sasso) – 0.471 (FUNAAB Alpha), respectively while the Hd and Pi among population were 0.796±0.025 and 0.386±0.013, respectively. The phylogenetic analysis grouped the iTABs into three main clades out of the seven clades identified in Asian domestic chicken with 97.39 % of the total maternal genetic variations occurring within population. Sasso and Shika Brown had the least (0.385) genetic distance. The analysis of mutation type associated with SNP revealed a total of 157 SNPs in the six breeds. There was an abundance of non-synonymous transition mutations within and across the breeds' except in Shika brown that have higher abundance of synonymous transition mutations. There was a moderate to high genetic differentiation between the six populations of iTABs as revealed by Arlequin 3.5.1.3 software, but significant value was observed only between Sasso and FUNAAB-Alpha (**0.517**). Tajima's D and Fu's F statistics were positive both within and among populations but was only statistically significant among the populations. Significant Fu's F was observed within the population of Noiler, Shika Brown, Kuroiler, and Fulani. Diversity indices of this study revealed that mtDNA polymorphism was high within populations and low among populations. All the breeds shared a common ancestry with Sasso while Shika Brown was more closely related. The iTABs originated from three distinct maternal lineages from Southeast Asia, Indian subcontinent and Southwest China. Results of the mutation analysis gives an indication that there is a conservation substitution in which case the alteration of the observed SNPs are less likely to have any severe effect on the protein structure and function. Therefore, it is recommended that the findings of this study could be utilized for the long term genetic improvement and stabilization of the breeds.

Key words: Smallholder poultry, mtDNA, Genetic Differentiation, Polymorphism, Phylogenetics, Geneflow.

CHAPTER ONE

INTRODUCTION

1.6 Background information

The livestock sector forms a vital part of the agricultural output in both the developed and developing world. Of the animal species used, poultry considerably contributes to the total food and agricultural production, (Guaye, 2000). In 2002, the Food and Agriculture Organization of the United Nations estimated the overall meat production to be 245 million tons, and about 30% there of was poultry meat, mainly from chickens. More than half of this was produced in developing countries inclusive of Africa. For eggs, developing countries' portion of the total production was even higher, (FAO 2002). Unlike other farm animals such as cattle, pigs and the likes, poultry and in particular chickens play an important role in the smallholder farming systems in developing countries specifically in Africa. Quite often local poultry stocks serve as a major source of animal protein to the poor since they are accessible even to landless households. However, in Africa, specifically in Sub-Saharan regions, business opportunities in the area of local and tropically adapted breeds in semi-intensive and extensive systems have been overlooked (James, 2006).

The latest brief by the African Chicken Genetic Gains (ACGG) projects; led by the International Livestock Research Institute (ILRI), seeks to increase access of poor smallholder farmers in sub - Saharan Africa to high producing however agro – ecologically acceptable chickens. The project is a platform for testing, delivery and continuously improving tropically adapted chickens for productivity growth under low- input systems (http://www.africacgg.net - June 2nd 2017). Five states in Nigeria are involved in the project and these states include Imo, Rivers, Kwara, Nasarawa and Kebbi. Six (6) Improved Tropically Adapted Chicken Breeds (iTABs) which includes; Shika brown, FUNAAB-Alpha,

Fulani, Sasso, Kuroiler and Noiler are tested in Nigeria to demonstrate differential productivity, preference, income growth and household consumption of the various Chicken breeds. Four of these breeds (Shika brown, FUNAAB-Alpha, Fulani and Noiler) were developed in Nigeria, while the other two (Sasso and Kuroiler) were introduced into Nigeria from other countries (http://www.africacgg.net - June 2nd 2017). Among the four breeds developed in Nigeria, Fulani has widely been known as the only ancient and Nigerian indigenous ecotype chicken. It has been in existence long before the breeding of Shika Brown started in Nigeria in 1985, at the National Animal Production Research Institute, Zaria (Adebambo, 1992). It is known as a heavy type chicken and had stood the test of time as it (Fulani ecotype chicken) is widely distributed in the rural areas of the tropics. The chicken is characterized by self-reliant and hardiness with the capacity to withstand harsh weather conditions and adaptation to an adverse environment. Horst, (1989), reported that the Fulani chicken possesses qualities such as the ability to hatch on their own, brood and scavenge for major parts of their food. It also possesses appreciable immunity from endemic diseases. Its existence has persisted over time because their products are preferred by most Nigerians because of the pigmentation, taste, leanness, and sustainability for special dishes Horst, (1989). Their output (egg and meat) are readily available to villagers and people in semi-urban areas thus serves as a good source of dietary protein and income. According to (Horst, 1989), the Fulani ecotype chickens are also characterized by extensive genetic diversity that allows for the rearing of poultry under varied environmental conditions thus providing a range of products and functions. Thus, it has been adopted for the African Chicken Genetic Gains project alongside the five developed Improved Tropically Adapted Chicken Breeds specifically known as iTABs. The Improved Tropically Adapted chicken Breeds are dual-purpose chicken used for both meat and egg production and are developed to adapt to the adverse climatic conditions of the tropical environment and low management input under

family-based– scavenging management systems (http://www.africacgg.net - June 2nd 2017). Therefore, to support the development of smallholder poultry production in Nigeria, it becomes necessary to understand the genetic worth and degree of divergence of these improved tropically adapted chicken breeds. Such findings would be critical for making informed recommendations to the smallholder poultry farmers over their choice of breeds' selection for breeding and production purposes.

To improve the performance of a flock, breeders make use of two principles, selection and crossbreeding. The benefit of using crossbreeding in animal production including chicken is well-documented (Sorensen *et al*., 2008) and due to heterosis, crossbred animals are more robust and economically efficient compared with the parental breeds (Pederson and Christensen, 1989; Making – Tanila, 2007). Adebambo *et al*. (2009) also reported that crossbreeding of local stock with an exotic commercial stock could take advantage of artificial selection for productivity in the exotic birds and natural selection for hardiness in the indigenous birds. Certainly, crossbreeding allows genetic progress but could lead to the dilution of genetic structure between breeds. Also, intermixing of breeds may pose a danger of maintaining breed purity in the long run (Haifa *et al.*, 2012). It is therefore essential to determine the level of differentiation and gene flow among these improved tropically adapted chickens breeds, for the purpose of a planned breeding-program.

Genetic diversity studies, which assess the variation existing in a population as it relates to the allelic differences, have recently become an integral part of agricultural programs as a tool for selection in breeding and identification of endangered species for possible conservation measures (De Silver, 2015). The genetic diversity observed between breeds and population is a result of various evolutionary forces, such as mutation and recombination, along with genetic drift, natural and artificial selection, migration and population expansion

as well as population contraction (Lush, 1994). The genetic diversity found in domestic chicken breeds permits farmers to develop new characteristics in response to changes in environment, disease or market conditions (Lush, 1994). However, diversity within breeds is probably reduced due to intercrossing and can be described at different levels from phenotypic observations to molecular data (Steffen et al., 2004). Molecular characterization, therefore, 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 covered extensive insight into phylogenetic and genetic distance analysis.

Mitochondrial are structures within cells that generate energy from food for metabolic activities of the cell. Most DNA is found in the chromosome within the nucleus, but mitochondrial also have their DNA called mitochondrial DNA (mtDNA). The haploid mtDNA, carried by the mitochondrial in the cell cytoplasm, has a maternal mode of inheritance (individuals inherit the mtDNA from their dams not from their sires, (ftp://ftp.fao.org/docrep/fao/010/91250e/a1250e17.pdf - June 2nd 2017). These characteristics enable biologists to reconstruct evolutionary relationships between and within species by assessing the patterns of mutations in mtDNA (ftp://ftp.fao.org/docrep/fao/010/91250e/a1250e17.pdf - June 2nd 2017). MtDNA markers

may additionally offer a rapid way of detecting hybridization between farm animal species or subspecies (Nijman *et al*., 2003). D-loop is, therefore, the most variable region of the mtDNA genome (Meyer, 1993).

Moreover, 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). Muchadeyi *et al*. (2008) reported that at least two ancient mtDNA genome have been indicated in East African, while only one major genome is observed in West Africa, specifically Nigeria (Adebambo *et al*., 2010). Therefore, identification of genetic diversity of these improved tropically adapted chicken breeds using variations in D–loop region of the mitochondrial will be useful in making recommendations for a planned breeding program aimed at improving and continual distribution of these breeds in Nigeria specifically in the South East.

1.2 Problem statement

Many past efforts to make smallholder chicken production more productive in sub–Saharan Africa have failed to deliver impacts largely because of the use of high producing genotypes created for intensive temperate feeding system. These exotic birds are often not suited to local conditions and demanded high investments in feeds, veterinary support, and labor; while local breeds are characterized by small body size, small egg size, and slow growth rate, thus making it less productive, non–attractive and undesirable in a competitive economic situation (Ibe, 1990).

Sonaiya (2015) reported that the introduction of the improved tropically adapted breeds' germplasm is capable of increasing productivity in the smallholder farming culture (SFC) of the rural poor in Nigeria. Therefore, when poultry at the household level becomes more productive and profitable, the need might arise for increased diversity and/or mixing up of germplasm which might result in the genetic erosion or extinction of the improved tropically adapted chicken breeds (*http://www.africacgg.net* June 2nd 2017).

Hence the general question becomes;

- How can we determine and/or access the appropriate improvement and conservation techniques that could be conceived and carried out for these iTABs to ensure adequate sustainability?

In designing answers to such critical question, there arises the need to first address the following questions;

- Which genetic marker could be more suitably used to access the level of genetic variation among these iTABs?
- How do we determine the genetic structure and distinctiveness among these iTABs?
- Could there be any interaction of traits or striking similarities in the evolutionary history of these iTABs that would enable us to determine the realized dispersal distances among them?
- How can we better explain the evolutionary history of these iTABs?
- What genetic process can generate changes that might endanger these iTABs to extinction?
- What level of equivalent allele frequency can be obtained among these iTABs? Therefore, the objective of this study is as follows;

1.3 Objective of the study

1.3.1 General objective

The main objective of this study is to evaluate the genetic differentiation of six improved tropically adapted chicken breeds (iTABs) in Imo State Nigeria using mitochondrial DNA (D-loop) as a molecular marker.

1.3.2 Specific Objectives

- To determine the level of mitochondrial DNA polymorphism among the populations.
- To estimate the genetic distance (diversity) within and among the chicken population sampled.
- To establish the phylogenetic relationships among the chicken population of the study.
- To ascertain the maternal lineage and origin of the breeds.
- To determine the mutation type associated with SNPs in the chicken population.
- To determine the extent of gene flow between the breeds.

1.4 Justification of the study

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). However, adoption of the improved tropically adapted chicken breeds (iTABs) into the tropically farming system has the potential for achieving this (Soinaya, 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 in Imo State. 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). However, little or no work has been reported to explore the genetic differentiation of these iTABs in Imo State. Although, 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.

Therefore, this entails a thorough understanding of the extent and nature of genetic diversity among and within breeds and populations, based on a wide range of information necessary for effective management and utilization of genetic resources in chicken as well as in other farm animal species (Steffen *et al*. 2012). Such information may include; keeping proper records of the genetic resources and the various ecosystems where they are found, identifying, characterization of these resources to estimate the genetic variation and conservation potential of these breeds' resources, monitoring genetic resources for their best short–term use, whilst ensuring their long term–ready availability (Steffen *et al*. 2012).

1.5 Scope of the Study

The scope of this work is centered mainly on understanding the contributions of some evolutionary processes for shaping variations in six improved tropically adapted chicken breeds (iTABs) raised in Imo state. The six iTABs used include; FUNAAB-Alpha, Noiler,

Shika brown, Kuroiler, Sasso and Fulani (Nigerian Indigenous) chickens. The study covered a period of five months and was grouped into three stages:

Stage 1: Blood collection

Stage 2: DNA extraction

Stage 3: Partial sequencing of the mtDNA D-loop region.

The evolutionary processes analyzed include:

1: The level of mitochondrial DNA polymorphism, for understanding the extent of genetic diversity within and among the populations.

2: Genetic distance, for understanding the nature of the chicken populations (genetic structure) and level of distinctiveness among the iTABs.

3: Phylogenetic relationships, for ascertaining the level of gene interaction and/or similarities in the evolutionary history of the six iTABs.

4: Matrilineal origins, to obtain an insight on the evolutionary history of the iTABs relative to other poultry species?

5: Mutational SNPs, for evaluating the confounding effects of natural selection on the iTABs.

6: Extent of gene flow, to be able to predict the level of adaptability and sustainability of the iTABs to different climatic changes (regions) under varying population sizes.

7: Inference of F-statistics (F_{ST}), for ascertaining the overall extent of genetic differentiation.

8: Demographic indices, for understanding the risk status of the iTABs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Smallholder chicken production in the tropics

Smallholder chicken production in most tropical countries is based mainly on scavenging production systems. It has been estimated that 80% of the chicken population in Africa is found in traditional scavenging systems (Gueye, 1998; Gueye, 2000). Women and children are generally in charge of poultry husbandry under the traditional scavenging system (Anon, 2002). The birds scavenge in the vicinity of the homestead during the daytime where they may be given sorghum, millet, maize bran, broken grains, or other waste products as supplementary feed. The level of productivity is very low compared to high-input systems; scavenging hens lay 30 eggs per year, while industrialized battery hens lay up to 300 eggs annually. Furthermore, it may take up to 12 months to raise a scavenging/local chicken for consumption. These production systems are titled ‘low input-low output' systems (Pandey, 1992). A range of factors such as sub-optimal management, lack of supplementary feed, low genetic potential and diseases (Pandey, 1992; Bagust, 1994 and 1999; Permin, 1997; Permin and Bisgaard, 1999), are responsible for the low output. Despite the low production, scavenging chickens still accounts for a major part of all meat produced in many developing countries, where poultry is an important component of rural, peri-urban, and urban households. As such, poultry plays a big role in rural as well as the national economy (FAO, 2000; Mlodzi and Minga, 2003).

Commercial chicken production systems are concentrated near the peri-urban centers and main cities, where the infrastructure is good and markets are guaranteed. In Nigeria, the commercial chicken industry is vertically integrated, with farms dedicated to the production of multiplication birds in the form of grandparent stocks (GPS) and parent stocks

(PS), and with well-managed feed mills and extension and marketing systems. Similarly, in Tanzania and Ethiopia, the commercial system is vertically integrated, with breeders' farms that rely on the importation of GPD and PS. In Ethiopia, importation of exotic breeds is mainly undertaken by the government-run Poultry Breed Multiplication and Distribution Centers (PMDC). Some PMDCs in different regions of Ethiopia have their parent stock and hatcheries from which they multiply and distribute breeding and production birds to urban and rural areas. The PMDCs also import and produce exotic day-old-chicks (DOC). Also in Ethiopia, there are a handful of other medium-scale and large-scale commercial germplasm importers. In addition to the PMDCs, related input services are provided by the private and public sectors. The public sector importers include the agricultural research institutes such as Debre Zeit Agricultural Research Centre, while the private sector chicken farms generally are few, the largest being the Elfora Agro-Industries, Alema and Genesis (FAO, 2000; Mlodzi and Minga, 2003).

Despite being larger than the commercial system by far, and having greater potential for productivity improvement, the indigenous chicken production system in the three countries (Nigeria, Tanzania, and Ethiopia) currently receives little support from the public system. For example, in Nigeria, Tanzania, and Ethiopia, indigenous chicken producers mainly generate breeding stocks from their flocks and from the few exotic birds that survive to productive age. There are very few (1 to 2) sources of "improved" indigenous germplasm in Nigeria, Tanzania, and Ethiopia, most of which are managed by government or public institutions, hence are not operating at a sufficient scale and level of efficiency to continuously meet farmers' seed-stock needs.

In Ethiopia, the Growth and Transformation Plan of the government has put ambitious targets to double the local chicken meat proportion of the total meat consumption by 2030 as part of

a broader and integrated livestock productivity improvement program. Nigeria is the largest producer of broiler chickens in Sub-Saharan Africa, and the government is embarking on a new Poultry Transformation Program for the smallholder chicken production and marketing system to meet demand. The Nigerian government is making huge investments in the poultry value chain by establishing state-run feed mills and hatcheries to enable a timely and reliable local supply of affordable day-old chicks for smallholders. In Tanzania, past livestock policies did not focus on the local chicken. However, this has changed now, and the chicken sector is increasingly attracting public and private investments, with the Central Corridor of the country having been identified as a potential area for local chicken production (http://www.africacgg.net - June 2nd 2017).

2.1.2 Some past interventions to smallholder chicken production

Many development projects in smallholder family chicken production in the last few decades (1970-2013) have 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). Genetic improvements have also been attempted by initiatives of government and development agencies, mostly in the form of crossbreeding programs that involved the introduction of exotic commercial cocks with indigenous hens. However, most of these have neither been systematic nor long-term enough

to have an impact. Nevertheless, more recently, there have been a small number of examples where selection programs have targeted genetically improving, multiplying, and distributing improved indigenous chickens in a limited number of African countries. Most of the improved tropically-adapted germplasm such as the Shika Brown at the National Animal Production Research Institute (NAPRI) of Nigeria, or the improved local germplasm such as Funaab-Alpha at the Federal University of Agriculture in Abeokuta, Nigeria are managed by public institutions, with limited opportunities for poor farmers to access the various improved birds. Consequently, this approach even though it seems successful, have failed to spur the anticipated growth and development of the smallholder production sectors in Nigeria, Tanzania, and Ethiopia (http://www.africacgg.net - June 2nd 2017)

2.1.3 Current Interventions- the African Chicken Genetic Gains (ACGG)

While much of the work discussed above has resulted in a limited amount of impact, the African Chicken Genetic Gains (ACGG) is an International Livestock Research Institute (ILRI) led Bill & Melinda Gates Foundation (BMGF) funded five-year (2015–2019) project that seeks to increase access of poor smallholder farmers in Tanzania, Ethiopia, and Nigeria to more productive and agro-ecologically adaptable chicken breeds. The African Chicken Genetic Gains (ACGG) works in-line with the agricultural research for development (R4D) strategy thereby has its major target on empowering women, functioning community innovation platforms, robust public-private partnerships, and capacity development to tackle poverty and improve food security (http://www.africacgg.net - June 2nd 2017). The ACGG program seeks to improve chicken genetics and the delivery of adapted chickens to support economic conditions, productivity growth, increased household animal protein intake, and the empowerment of women farmers in rural communities. The African Chicken Genetic Gains

has come to leverage the existing system with the belief that the improved germplasms from these programs have potential to contribute to significant productivity gains, impact to smallholder, and opportunity for women in the various countries. Therefore, they aim to leverage the existing history of work while implementing innovative approaches to the development and supply of genetics to the various national chicken value chains. They seek to adopt the following five unique pillars in their innovative approach:

1. High-productive genotypes that is well-adapted to low-input production systems: While the chicken value chain has received heavy investment, little of this has been targeted at improving genetics for smallholders. Therefore, the ACGG work will build off of and support the improvement of existing animal health and management infrastructure to target genetics because they believe that genetics is key to increased productivity and can be utilized as a "pull factor," positively influencing investment and entrepreneurship in the other aspects of the value chain such as improved health and feeding interventions (http://www.africacgg.net - June 2nd 2017).
2. Farmer preferred breeds of chickens: The principle driver of product development will be smallholder and local consumer preferences. This platform will inform the innovation of genetic technologies with farmer preferences and responses generated through rigorous on-farm and field testing (http://www.africacgg.net - June 2nd 2017).
3. Innovation platforms for developing solutions across the value chain: An innovation platform will be formed and platform meetings facilitated, drawing membership from the key actors in-country chicken value chain. The platform will be informed by the results of need-driven applied research, the focus of which will be to generate and mobilize new knowledge in chicken science, genomics, and genetics, to deliver increased productivity in small scale, intensifying chicken systems in sub-Saharan Africa (http://www.africacgg.net - June 2nd 2017).

4. Co-operate partnership for improvement, multiplication, and delivery: This chicken platform is and will be strategically designed and implemented to foster public-private partnerships. The public-private partnership will be critical to the success of the program. For example, the testing and innovation of the germplasm lines will be done in a manner that will enable the business case to be made for the farmer-preferred lines. They view the uptake of the lines by the private sector to be critical for the adoption, maintenance, multiplication, and delivery of the genetic technologies, and therefore, the development and maintenance of ongoing relationships with the private sector which is critical to ensuring the quality, sustainability, and impact of their research platform, through the respective national and regional innovation platform activities (http://www.africacgg.net - June 2nd 2017).
5. Women at the center to ensure success*:* Because women are key actors in smallholder chicken value chains (i.e. own, manage flocks and trade in the chicken and chicken products), the program will place women at the center of its activities-right from the identification of the constraints, definition of breeding objectives, testing of the improved lines for suitability as well as participation in the innovation platform meetings. During the data collections to monitor relative performances of various chicken test-lines, and at least 50% of the enumerators will be women. The platform will be designed to test and respond to the unique concerns and preferences of women as farmers and consumers. To effectively do this significant time of both gender specialists and socio-economists' time is planned and budgeted for in this program, with a primary aim being to assess the gender-segregated potentials, roles gender-segregated benefits and impacts of the introduction and adoption of the improved chicken lines at household levels and beyond(http://www.africacgg.net - June 2nd 2017).

Beyond targeting Ethiopia, Nigeria, Tanzania – the outcome of this program would still maintain the potential to impact millions of rural lives and peri-urban households in other countries with large backyard chicken production (http://www.africacgg.net - June 2nd 2017).

2.1.4 Improved Tropically Adapted Chicken Breeds (iTABs)

2.1.4.1 Shika Brown

The Shika-brown commercial layers evolved as a result of many years of breeding and selection work at the National Animal Production Research Institute (NAPRI), Zaria, Nigeria. The birds were the products of many crosses of specialized lines of the foundation stock, experimented and proven for good performance in all the six geo-political zones of Nigeria (Kallah, 1999). Shika brown is an egg type strain of chicken which is the first developed livestock species to be registered and released in Nigeria. Shika brown, having satisfied all the necessary registration conditions was registered and released in the year 2000. Its productive attributes combines both the higher adaptability to local environmental conditions with excellent production performance and efficient utilization of locally accessible feedstuffs. This is to say that the chicken is well adapted to tropical climates and offers the most effective economic returns and is less vulnerable to common poultry diseases, hence minimum investment on medication is needed. The breed is early maturing, maintains high persistency of production, with an average hen-house production of about 80% during peak production. The generally low values of mortality (less than 7%) indicate the "hardiness" of the breed and high adaptability. The key features are 1. Hardiness and resistant to common tropical diseases. 2. High persistence of lay (12 weeks above 80%) 290 eggs/laying period and 140-150gm feed/bird/day and 3. The average egg weight is 60g and body weight at end of lay is 1.8kg (NAPRI, 2016).

2.1.4.2 Kuroiler

The Kuroiler is a commercial dual-purpose hybrid chicken introduced in the early 1990s, from India derived through crossing either colored broiler males with Rhode Island Red females, or, White Leghorn males crossed with female Rhode Island. The breed was created by Vinod Kapur of Kegg Farms Private LTD, and the name is a portmanteau of Kegg and Broiler (Victorial and Paul, 2012, Saving the world, 2009). The breed has a reputation for its low maintenance requirement and the ability to thrive on household and agricultural wastes. Based on the research data collected under extensive management systems in Uganda, Kuroiler breed is capable of producing about 150-200 eggs annually. The meat yield per bird of Kuroiler is also greater; males approximately 3.5kg (7.7lb) and the female about 2.5kg (5.5lb) whereas the native bird weighs 2.5kg (5.5lb) and the female 1.2kg (2.6lb). Owing to its distinctive genetic attributes, the Kuroiler chicken tends to possess strong immunity to diseases. The Kuroiler chicken is a potential bio-converter of agricultural, household and natural waste abundant in villages into human protein food and substantial incomes for the rural households (Getachew *et al*., 2016).

2.1.4.3 Sasso

Sasso is an internationally popular breed originating from France. Sasso offers proven, hardy, easy to manage and versatile bird to Indian farmers. Farmers can keep them in different production systems – from intensive production, down to earth simple sheds on a deep litter with basic equipment, in the backyard, in orchards, in coconut and rubber plantations, in forests areas, in the hills and even in the drylands and in the hot desert. The major target in Sasso breeding Program is the high production of hatching eggs (HE) from the parent breeder (HE >220) and a slow-growing, robust, easy to manage, multi-colored broiler which can be grown under different rearing systems - from indoor and intensive to Free Range and village

family-based production. The feed is less dense, less expensive, lower in energy and protein, and allows the use of higher levels of cheaper and unconventional farm by-products. Due to a slower growth rate, Sasso meat is more firm and has that rich chicken flavor, juicy and tasty like the meat of traditional Indian country chicken and it commands a higher market price. Besides, Sasso delivers better profit to the farmers and great taste to the consumers (http://www.sasso.fr/ouverture.pdf,php?...Sasso June 2nd 2017).

2.1.4.4 Fulani

The Fulani chicken is a heavy ecotype chicken found in the dry Savannahs (Guinea and Sahel Savannah), Mountainous regions and cattle kraals of the Northern part of Nigeria. It can weigh about 0.9- 2.5kg at maturity. The purity of the Fulani chicken is preserved by the isolated family group lifestyle of the Fulani keepers which largely hinders their interbreeding with other native chicken. Although the origin is uncertain, some authors (Ogundipo, 1990; Tiamiyu, 1999) believed; that Fulani-ecotype chicken is a crossbred between indigenous fowls and Rhode Island Red chicken used in the previous cockerels exchange program. Atteh (1999) has confirmed that the chicken is more superior to all other ecotypes within Nigeria in terms of live weight.

2.1.4.5 Funaab Alpha

Funaab Alpha is a dual-purpose breed developed by the poultry breeding and research team of the Federal University of Agriculture, Abeokuta (FUNAAB) in Nigeria; led by Professor Olufumilayo Adebambo. Funaab alpha was developed from a random crossing of different Nigerian chicken from the Southern part of Nigeria (http://unaab.edu.ng/2017/06/funaab-don-develop-improved-chicken - July 15th 2017). The breed was developed as the need for a

more adaptive indigenous breed of chicken in Nigeria became paramount in 1994. 500 birds were collected all over South Western Nigeria. Among the 500 birds were the normal feathered, frizzle feathered and naked neck indigenous birds (Adebambo, 2015). The evaluation method employed was biometric data collection, genetic screening for survivability, reproductive performance and elimination of broodiness. The improvement program was consistent for a period of ten generations of recurrent selection with the primary purpose of developing an improved Nigerian indigenous chicken, the Funaab-Alpha chicken (Saleh *et al*., 2010). Several species of Funaab-Alpha have been developed; the Funaab-Alpha layer white, male and female Funaab-Alpha black, Alpha gold cock, Alpha brown female, Alpha blue cock and female, cock and female Alpha naked neck, Alpha white cock and female, Alpha barred cock and female, and Alpha frizzle (Adebambo, 2015).

Studies suggested that Funaab-Alpha has a high-performance value compared with the exotic breeds on semen evaluation (Peters *et al*., 2008); egg production and linear measurements (Akanni *et al*., 2009; Omeje and Okafor, 2008 and Sonaiya, 2015). Therefore, the Alpha chicken has been adopted by the African Chicken Genetic Gains- Nigerian (ACGG-NG) project both for the on-station testing of 2015 and the on-farm testing in over 2700 rural households across 5 agro-ecological zones in Nigeria (Adebambo, 2015). However, the expectant favorable outcome of this project based on the farmers' preferences would suggest that the Alpha chicken be selected and/or adopted for commercialization (Adebambo, 2015).

2.1.5 Economics importance of the Improved Tropically Adapted Chicken Breeds (iTABs)

Adetayo and Babafunso (2001) reported that indigenous chicken, under intensive conditions produce on average of 80-90 eggs per hen in a period of 280 days with an egg mean weight of 36.8g. The tropically-adapted exotic or selected/improved indigenous lines and line

combinations are able to produce nearly 200 eggs/bird /year and grow fast (reaching 2.0-3.0 kg live weight at 8 to 10 weeks of age). The improved indigenous line also has a high feed conversion ratio of 2:1 to 3:1 using locally available feed resources. The adoption of the improved tropically adapted chickens into the tropical farming system has the capacity of multiple functions which include: fighting malnutrition; better price; minimum land, labor, and capital; low skilled-labor as well as a regular source of income (http://www.africacgg.net - June 2nd 2017).

2.2 Genetic differentiation of population

Genetic differentiation can be defined as the accumulation of differences in the allele frequencies between a completely or partially isolated population due to evolutionary forces such as selection or genetic drift (Mc Graw- Hill Dictionary, 2003). However, Genetic differentiation of populations can be as a result of the uneven (nonrandom) spatial distribution of genetic variation in a species (Hartl and Clark, 1997), reflecting a departure from panmixia (a condition in which the population is a single entity with complete random mating). Such a condition can be broken by various factors, for example, selection makes certain individuals to have mating advantages over the others, or a reduction in gene flow allows subpopulations in two geographical localities to be influenced independently by genetic drift. This will ultimately result in the differentiation of allele frequencies between these subpopulations, leading to population genetic structure.

However, in a large population of given specie, some individuals could have more similarities in their genetic make-up than others. The proportion of these genetically similar individuals among sub-population within the given large population can be taken as a population genetic structure. The condition was created as a result of a reduction in the rate of reproduction between subpopulation of a large population due to occurrence of some physical

and behavioural impediments (Arun, 2013). Consequently, this could lead to a decrease in the effective population size of a population, thereby, exposing the population to the effect of random genetic drift. Another consequence of population genetic structure can be seen in its ability to expose a subpopulation to a high risk of specific selection effects and environmental interactions caused by localized changes in allele frequencies (Arun, 2013). It can also create an artificial signal of a population bottleneck (Wakeley, 2000; Chikhi *et al*., 2010). Understanding population genetic structure in the study of evolution using genetic markers, is a pre-requisite for fitting in into the different biological field of study (Pritchard *et al*., 2000). Sewall Wright (Wright, 1951) was among the first to recognize the importance of subdivision of individuals within a subpopulation with equivalent genetic make-up. Hence, he was the first to emphasize the ecological importance of subpopulation structure and how it could have direct consequences for the spread of mutations and adaptation.

Several models of population evolution have been hypothesized and tested (Arun, 2013). Among the models are; those that acknowledge panmixia taking account of random mating and gene transfer to the next generation. Secondly, are those that partially acknowledge panmixia taking into consideration the effects of gene flow. Based on the first model, researchers have observed post-glacial distributions of species from an ancestral panmixia, into current subpopulations (Nason *et al*., 2002). Starkey *et al*. (2003), also, revealed the ancestry of populations which have been isolated by geographical barriers to gene flow.

Xu and Shete, (2005), disclosed that genetic subpopulation structure, based on several studies, could have a direct implication on a downstream genetic analysis. For instance, population structure in genome-wide association studies (GWAS) could lead to an increase in the generation of false impression in identifying associations between loci. Several studies also, have identified this issue and attempted to address it in different ways (Xu and Shete, 2005; Price *et al*., 2006; Marchini *et al*., 2004), but central to all these methods is the

identification of subpopulation structure. Because of the ambiguousness in the understanding of the condition or the term genetic differentiation (population genetic structure), many scholars have mistaken it with several other models of evolution such as demography, selection, mutations, non-random allelic association, and recurrent migration among others. Hence a simplified definition of population genetic differentiation can be explained as quantifying the overall genetic variation in individuals of the same species primarily in terms of their allele or genotype frequency distribution across one or more genetic loci (Weir and Hill, 2002). Nevertheless, not quite often can population genetic structuring be inferred, there are also cases when its result can be ignored. This is otherwise known as the "Wahlund Effect". This situation usually affects the Hardy-Weinberg equilibrium principles, thereby leading to heterozygote deficiency in the absence of Hardy-Weinberg Equilibrium. Consequently, the Wahlund Effect could lead to an unfavorable conditions known as linkage (and association), bottlenecks, and cryptic relatedness.

In addition to the negative effects of population substructure, it also have the ability to impact the analysis of interspecies pairwise genetic relatedness (which is a known tool in GWAS and other methods mentioned above). Nevertheless, genetic relatedness can be estimated in terms of the probability that two randomly sampled genes at a given genetic locus are identical by descent (IBD) (Weir *et al.*, 2006). Descent as regards to population bottleneck could either be recent or deep. Evolution of individuals from the immediate past generations could be termed "a recent relatedness" whereas individuals evolving from an ancient admixture event with a characteristic gene flow and subsequent incorporation of genes from different genetic subpopulations may be referred to as "a deep relatedness".

2.2.1 Genetic differentiation Methods

There are many methods of population genetic differentiation. These includes- Wright's F-statistics, model-based clustering methods, non-model-based clustering, to name a few. In this study, I detail the most commonly used of these methods based on Wright's F-statistics.

2.2.1.1 F-statistics and AMOVA

F-statistics is one of the oldest tools for estimating population genetic differentiation, and is based on the assumption that a present subpopulation were the product of an ancestral descendant with respect to the principles of Hardy- Weinberg equilibrium (HWE), and linkage disequilibrium (Wright, 1951). F-statistics usually view a subpopulation as having undergone similar process of evolution as at the time of divergence. Many authors of population genetics have defined F-statistics in different ways but most importantly are its definition as regards to genetic relationship/correlation and/or inbreeding coefficient. However, genetic differentiation can take place within individuals, between individuals of the same subpopulation, between individuals of different subpopulations, boldly explained as;

- F-statistics due to common ancestry, or FIT (profoundly termed F), and explains the relationship between individuals within a given population,
- F-statistics due to related ancestry, FST (profoundly termed θ), and explains the relationship between individuals of the same subpopulation,
- FIS (profoundly termed f), and explains the relationship between individuals within subpopulations.

Details of these definitions can be seen in the work of (Nei, 1973; Weir and Cockerham, 1984; Whitlock, 2011). However, (Hartl and Clark, 1997) present the most general definition of F as the ratio of reduction in heterozygosity, that deviates from Hardy-Weinberg Equilibrium (HWE) principle in a given population.

Nevertheless, some authors such as Nei (1973), Weir and Cockerham (1984), Jost (2008), Hamrick and Godt, (1997)' proposed a different view of the general definition, taking into account, the population sample sizes, equal weighting for alleles (i.e. substituting allele frequencies with weighted allele frequencies based on their relative rarity) etc. Meanwhile, the effective population size and population history remains the major determinants of F statistics, (Weir and Cockerham, 1984). In order wards, Holsinger and Weir (2009) reported that F statistics values can range from 0 to 1. 0 values imply that there is no differentiation, whereas 1 implies total differentiation. Practically, an FST value of 0:00 - 0:05 could mean low differentiation; 0:05 - 0:15, moderate differentiation; while FST > 0:15 implies high levels of differentiation (Hartl and Clark, 1997).

The Analysis of Molecular Variance (AMOVA) was designed by Excoffier *et al*. (1992) who composed most of its literature so as to give more insight on the level of genetic distances and F-statistics. AMOVA could provide an alternative insight for understanding the estimate correlation 'F' (Φ) statistics as discussed above. It can also serve as a basis for testing hypothesis for different patterns of subpopulation structure. The main target in the adoption of AMOVA was to obtain an insight for grouping total variance in allele frequencies (across multiple loci) within and among individuals (within populations, among populations, within subpopulations, and among subpopulations). All of these therefore were to ensure an appropriate definition and understanding of genetic distances between haplotypic data.

2.2.2 Inference of the Magnitude of Gene Flow on Genetic Differentiation

Gene flow is a fundamental micro evolutionary force that can determine the potential for genetic differentiation among populations and for local adaptation and also influences the geographical spread of novel adaptations (Keyghobadi *et al*., 2005). It can be estimated by the use of GST. The level of gene flow is a very important population parameter. For

practical reasons, the concern is usually on the absolute magnitude of gene flow, i.e. the number of individuals immigrated to and interbred in a population per generation, that is, 'Nem'. One distinctive advantage of the FST statistics (GST) is that it incorporates the inference of the magnitude of gene flow (Nem) among populations under certain conditions. Recently, extensive studies have been carried out to uncover more insight into the usefulness of FST and GST as regards to the inference of gene flow. The equilibrium value of GST is largely not sensitive to the violation of the assumption of the mutation model, such as the infinite allele's assumption (Leng and Zhang, 2011). Dobzhansky and Wright (1941), initially suggested that for an island model of population structure that deviates from the normal assumptions of no mutation, and small migration rate (m), there exists the following approximation among the fixation index FST, the effective population size Ne and m under equilibrium,

$$F_{ST} \approx \frac{1}{4Nem+1} \quad (1)$$

Wright (1931), obtained this formula differently. Therefore, if the equilibrium value of FST can be estimated from empirical genetic data, Nem can then be calculated.

Nei (1975) also derived a rather complex formula for GST under the island model at equilibrium. However, this can be reduced to

$$G_{ST} \approx \frac{1}{1+4Nem\,\frac{K(m+\mu)}{K-1}} \quad (2)$$

Where, m represents the mutation rate per generation and K is the number of subpopulations (Takahata and Nei, 1984). When the number of subpopulation K is sufficiently large, m << 1

and m << m, the right side of the above formula is approximately equivalent to that of equation (2). If the mutation rate is not negligible, the formula becomes

$$G_{ST} \approx \frac{1}{4Nem + 4Ne\mu + 1} \quad (3)$$

(Cockerham and Tachida, 1987; Cockerham and Weir, 1993).

Although there is no direct relationship between F'ST (G'ST) and m, Meirmans and Hedrick (2011), suggested that an estimate of the number of migrants that is unaffected by HS can be obtained through a combination of FST and F'ST (equally applicable to G'ST), $\frac{1}{Nem} = \frac{1-F'st}{4Fst}$ (4)

Simulation studies revealed that the equilibrium value of Jost Dest (D) is highly sensitive to the assumed mutation model (Leng and Zhang, 2011). Because of this property, the utility of D in population parameter estimation is rather limited.

2.2.3 Demographic characterization

Demographic information is key steps in the strategizing breeding plan for the Farm Animal Genetic Resources (FAnGR) management. There are about three factors for the measurement of risk status factors. First, is the risk status relative to population size and structure; where effective population size (Ne) becomes the major determinant of risk status (FAO, 1992; Gandini *et al*., 2004). Effective population size comprises the entire population (including males and females). One major consequence of evaluating risk status using effective population size is its ability to enhance or encourage inbreeding which in turn would lead to reduction of within population genetic diversity. Secondly, is the risk status associated with the present and future population trends. Illustratively, (Groeneveld, *et al.*, 2010), suggested

that a rapid downward movement could imply an increase in the level of risk status. Thirdly, is the risk status relative to the geographic distribution of the population. This factor is of the fact that the smaller the population, the higher the potential risk, especially, with respect to disease encroachment. Therefore, there is need for integration of large demographic information at the breed level across countries. This will help to standardize breed priorities for national conservation in terms of acceptability and popularity. More popular breeds (that is breeds present in more than one country) are known as 'transboundary breeds' whereas, less popular breeds (that is breeds present in only one country) are called 'local breeds' (FAO, 2007a). However, 'transboundary breeds' can be 'regional' or 'international' depending on their level of dispersal to different countries. For an effective conservation of farm animal genetic resources (especially those at risk), adequate and regular monitoring of demographic data is highly recommended at least ones in each animals' specified generation "about eight years for horses and donkeys, five years for bovine, buffalo, sheep and goats, three years for pigs and two years for poultry species, (Groeneveld, *et al*., 2010). The monitoring process may include;

- A regular dates of animal at risk for at least ones in a generation of each animal as stated above.
- Regular updates on the reproductive technology being adopted, etc.

Meanwhile, a lot of factors could be limiting the frequency at which demographic data are collected, monitored and updated. These factors are prevalent mostly in the developing countries, and such factors may include;

1. Unreliability of data due to inadequacy in the method of collection

2. Cost of collecting data

3. Data limitations relative to the geographic distribution of breeds (Aberle, *et al*., 2004).

4. Inadequacy in the development of methods for representing samples of national animal population.

5. Lack of measures for capturing the genetic dilution due to crossbreeding (FAO, 2007b).

Often times, local populations of breeds are seen migrating gradually and merging with the neighboring populations thus making it uneasy to access demography relative to population history or recent interactions between breeds. Good enough, a well coordinated and accurate combination of results from studies can provide insight for understanding such relationships.

2.2.3.1 Mismatch Distribution

This is the distribution of the number of pairwise differences between haplotypes, from which parameters of a demographic or spatial population expansion can be estimated. It shows whether there is a population expansion or decline. When the chart constructed is ragged, it shows a constant population but if smooth having a peak it shows an increasing population (Brinkman and Leipe, 2001).

2.3 Mitochondrial Genetic Differentiation

Mitochondria are organelles found within the cell. They are the sites for energy production during aerobic respiration. Each mammalian mitochondrion contains its peculiar DNA, in the form of a covalently closed circular double-stranded DNA molecule that contains 13 protein-coding genes, 2 ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes (Gray, 1989). The inheritance of mtDNA differs from that of nuclear DNA in that it has a direct lineage to the ancestral mother. The reason for this maternal inheritance pattern is that when an egg is fertilized, the cells of the resulting embryo contain the mtDNA and cytoplasm of the egg from the mother, not of the sperm from the father. However, the reason for the popular

adoption of mtDNA markers is that it is found in great abundance in the cell cytoplasm and hence easily amplified (Galtier *et al.,* 2009).

Furthermore, across a wide range of animal species, the mitochondrial gene content is strongly conserved, has little duplication, has no intron, and has very short intragenic regions (Gissi *et al*., 2008). The mtDNA also has specific biological properties, i.e. its direct link to its ancestral maternal inheritance and its involvement in metabolic functions that make it an appropriate marker of molecular biodiversity. Lastly, it is worth noting that mtDNA is of little use in investigating the recent loss of genetic variation and any individual-level events such as identity, individual dispersal, and mating systems. Males will carry the mtDNA of their dam, but their offspring will carry the mtDNA of their mother, and not of their father. Thus, only daughters will pass the mtDNA on to future generations. Numerous published studies have reached conclusions about population history, patterns of gene flow, genetic structure, and species limits, based on mtDNA sequence variation (Zink and Barrowclough, 2008). MtDNA has been used to study the phylogeographic structure of avian species (Ceccobelli *et al*., 2013), to infer regions of domestication and to identify the number of maternal lineages and their geographic origins (FAO, 2007a). The huge abundance of mtDNA makes it an excellent candidate for archaeological studies that are often old or degraded samples.

2.3.1 Mutations

Mutation describes an alteration in the DNA sequences of an organism. It occurs as a result of chemical changes at the S-phase of the cell during the process of DNA replication. During DNA replication, some repairable and irreparable chemical changes take place at the S phase of the cell cycle thereby resulting to variations in the nucleotide sequences of an organism. Some of these variations tend to be normal while some tend to be abnormal. Most of the

abnormal variations produce permanent changes in the nucleotide sequence, hence are termed mutations (Condit *et al*., 2002). These variations are responsible for the slight phenotypic differences observed between organisms of the same species. Apart from mutations, other factors such as direct gene transfer (sexual reproduction), out-breeding, deploidy as well as horizontal gene transfer can act to bring about genetic variation in the nucleotide sequence of the DNA. Meanwhile, among these agents of variations, mutation tends to be the primary source of variation in the DNA (Klug *et al*., 2006). Mutation can be beneficial, neutral or harmful and can bring about lethal gene (death of the cell (Nacman *et al*., 2000). Two main groups of mutation exist; these are somatic or body cell mutation and germ-line mutations. Somatic mutations are those mutations that can be acquired and usually occur in the cells of the body. They are mostly caused by environmental factors resulting in copying error during DNA replication, hence they are non inheritable and as such are not considered as important factor of evolution. On the other hand, germline mutations are those mutations that occur in the ova or sperm cell of sexually reproducing organisms. They are mostly inheritable and are otherwise termed 'hereditary mutations' (Nacman *et al*., 2000). Furthermore, germline mutations or hereditary mutations can be grouped into two; De Vivo mutations and De Novo mutations. De Vivo mutations are those germline mutations with no known family records while De Novo mutations are characterized by a known family history. Sun *et al*., (2009), reported that mutation size varies from a single nucleotide base also known as point mutation to a chromosomal mutation. On the other hand, Stamatoyannopoulos *et al*., (2009), reported that mutation rate can be measured in terms of evolutionary divergence and nucleotide diversity. However, mutation can be triggered by both internal and external conditions. Internal conditions could arise from error in the copying or repairing process during DNA replication and repairing respectively (Klug *et al*., 2006). Nevertheless, environmental conditions that can lead to mutation might range from excessive exposure of cell to

ultraviolet rays, radiations, virus and chemicals (Nelson *et al*., 2005). Additionally, mutation in the genome is classified into two namely;

- Transitions mutations involving a condition in which a purine base changes to another purine base, or pyrimidine base changes to pyrimidine.
- Transversion mutation which involves a change in the nucleotide sequence from purine base to pyrimidine and vice-versa.

The functional and phenotypic effects of mutations and, consequently, the strength of negative selection vary widely among nucleotide sites in any genome. At the opposite ends of the continuum, mutations at some sites are effectively neutral, while mutations at some other sites are lethal. Nucleotide sites can be subdivided, according to their molecular function, into classes with different typical strengths of negative selection. Generally, rapidly evolving segments of intergenic regions and introns, as well as most of synonymous coding sites, are controlled by only weak selection or even by no selection at all. Slowly evolving segments of intergenic regions and introns, as well as UTRs and non-synonymous coding sites, are under much stronger selection (Bird, *et al*., 2006). However, even within such functional classes, the strength of negative selection varies widely among individual sites (Yampolsky, *et al*., 2006). Mutation and selection are generally thought to be independent evolutionary forces (Kimura, 1983). In other words, the rate with which a mutation occurs is routinely assumed to be independent of the effect of this mutation on fitness. Inferences of the strength of selection on specific genes and sites within genes usually rely on this assumption. Although selection for reduced mutability is stronger at sites where mutations are more deleterious (Kondrashov, 1995) it is hard to imagine adaptive fine-tuning of mutation rates at the level of individual nucleotide sites. Thus, one might expect selective constraint and mutability to vary more or less independently across individual sites. However, another phenomenon may lead to a

seemingly counterintuitive association between stronger negative selection and higher mutation rates. Sites that are under weak or no selection are free to evolve and to get rid of hypermutable contexts. In contrast, negative selection will preserve such contexts at functionally important sites, provided that they confer a higher fitness. In particular, non-synonymous (Subramanian and Kumar 2006) and even synonymous (Subramanian and Kumar 2003) coding sites of mammalian genomes are enriched, relative to what is expected at a neutral mutational equilibrium, by CpG contexts, leading to a substantially higher mutation rate within coding exons than within introns.

2.3.2 Polymorphism

As explained above, two types of variations that occur in the nucleotide sequence of a DNA can be normal and abnormal variations. Usually, normal variations cause temporary changes in the sequence but abnormal involves abnormal changes amongst individuals of a randomly mating populations (Condit *et al*., 2002). These changes therefore create multiple forms of individuals that are highly adapted to varying environments at a given place and are usually governed by natural selection. This condition however, is known as polymorphism, and individuals involved are said to be polymorphic individuals or are called polymorphs. Therefore, polymorphism can be defined as the occurrence of two or more alternate forms of individuals of given species; or it can be defined as the variation in the DNA sequences among individuals (Joshi *et al*., 2004).

In a population with prevalent occurrence of random mating, a condition known as transient polymorphism can occur. This involves uniform spread of genes between individuals leading to gene homogenization. Balance polymorphism is another polymorphic condition that can be seen in a population of non-randomly mating individuals. Balance polymorphism usually

involves a logical spreading of gene such that two or more forms of individuals maintain a unique variance at a given time. Polymorphism is mainly maintained by natural selection hence it differs extensively among different species of animals as reported by Charlesworth and Charlesworth, (2016). Information on polymorphism can be generated from the analysis of a mutant allele at a given locus using a DNA sequence data or restriction map data. Stallen *et al*., (2000), compared the stability and extent of polymorphism using DNA markers and protein markers. He observed that DNA markers are more reliable as they tend to generate more authentic polymorphic data. However, information on polymorphism can serve as a basic tool for a strategic development and exploration in the genetic characterization of population. Several polymorphisms exist but for the context of this study, I will detail only the single nucleotide polymorphisms (SNPs).

2.3.3 Single Nucleotide Polymorphism (SNP)

As mentioned earlier, mutation can vary in size ranging from Single nucleotide polymorphism (SNP) to chromosomal mutation. Single nucleotide polymorphism (SNP) describes a point mutation that occurs in the genome of an organism. They account for most of the variations, about 90% in the whole genome and take place in every 100 – 300 bases along the three billion bases in the human genome (Twyman and Primrose, 2003). They can appear randomly in the form of base substitution, insertion, or deletion. They have great contributions in the genetic diversity study (Buckley *et al*., 2005; Mart *et al*., 1999). Most SNPs that occurred in the genome usually are seen within the protein coding regions/genes, and are thus responsible for several changes of biological functions of a protein as their work is usually to initiate the process of molecular evolution. SNPs have widely been studied in the past hence its knowledge offers a great insight in the understanding of molecular evolution, genetic diseases and and abnormalities that affect chickens across populations, thereby

broadening the study and understanding of genes. However, Moreno-Loshuertos *et al*., (2006) reported that the differences in reactive oxygen species production were correlated with mtGenome haplotypes in mouse. Analysis of Johnson *et al*., (2001) and Jenuth *et al*., (1997) SNPs and haplotypes, revealed that variations in the dihydrouridine loop of tRNA arginine were associated with the production of hydrogen peroxide, a free radical, which influences respiratory performance. Therefore, the novel SNPs and haplotypes as mentioned earlier can provide useful insight into biochemical and molecular studies of economically important phenotypes in chicken. Essentially, SNPs are of different types partitioned into two groups. The first group comprises of those SNPs that occur in the coding genes while the second group comprises of those that occur in the non coding genes within a whole genome. Some of these SNPs can cause a change in amino acids, which may affect important traits in the chicken. However, SNPs occurring in the non-coding region could practically influence economic traits because the D-loop contains the origin of replication of the H-strand and the promoters for L- and H-strand transcriptions. In humans and model species such as mice, common mtDNA polymorphisms have been associated with important disease traits as well as other characteristics including climatic adaptation and longevity (Wallace, 2005).

2.3.3.1 SNPs occurring in the coding regions

There are essentially two types of SNPs associated with the coding sequences. These are synonymous and non synonymous SNPs. These SNPs are the key drive to evolution and biological functions of a protein hence they are usually found along the coding sequences of a matured mRNA. Their effects usually result in the production of a ‘target protein’; a missense or truncated protein based on the amino acid produced.

2.3.3.1.1 Synonymous SNPs

In the process of DNA replication, a nucleotide base substitution may occur within a codon in the genome thereby producing the same amino acid as the original amino acid. This resulting amino acid following the base substitution however does not alter the production of the target protein. This is otherwise referred to as synonymous substitution. Synonymous substitutions usually occur at the first and the third position within a codon (Nei *et al*., 2000). Illustratively, the codon 'CCT' may code for leucine, hence, any change taking place at the third position of the CCT codon by either the A, G and C bases still has the capacity of producing the amino acid 'leucine'. For example, the codon CTT that codes for Leucine, a change at the third position by any of the remaining three bases (A, G and C) would still give rise to the same amino acid. This happens because several codons may encode a single amino acid owing to the fact that the genetic code possesses a degenerating nature.

2.3.3.1.2 Nonsynonymous SNPs

This is the opposite of synonymous SNPs. It involves a nucleotide base substitution within a codon that results in the production of amino acid that differs from the original amino acid thereby altering the production of the target protein. This resulting protein can be functional (though may differ in their functions) or non-functional. Non synonymous SNPs can be of two categories. Missense SNPs and Non sense SNPs. Missense SNPs involves the alteration of the original amino acid and hence the alteration of the target protein; take for example, in a hemoglobin chain, GAG encodes for an amino acid 'Glutamine' but a replacement of the A (Adenine) by a T (thymine) at the second nucleotide position of the sixth codon position would instead produce an amino acid 'Valine' (GTG). On the other hand, Nonsense SNPs involves the alteration of the amino acid which would eventually produce a premature stop codon thereby giving rise to a truncated protein.

2.4 Measures of Genetic Differentiation

2.4.1 Genetic Diversity

As deserving conservation, Genetic diversity is one of the three forms of biodiversity recognized by the World Conservation Union (IUCN). Conservation of genetic diversity within populations is based on two arguments: the necessity of genetic diversity for evolution to occur, and the expected relationship between heterozygosity and population fitness. Therefore, 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

2.4.1.1 Nucleotide Diversity

Nucleotides are the structural components or building blocks of DNA and RNA consisting of base pairs, a sugar molecule, and a phosphate group. Nucleotide diversity (π) is a concept in molecular genetics that is used to estimate the level of variation in a population. It is estimated as the mean differences in the nucleotide sites between randomly selected DNA sequences in a population (Nei and Li, 1979). It works for other important genetic diversity tools to examine between and within-population variation (Kilian *et al*., 2000; Yu *et al*., 2004).

2.4.1.2 Haplotype Diversity

Haplotypes are a group of genes in an organism that is inherited together. Several definitions have been used to describe the term haplotype. Firstly, it is described as a haploid genotype, a group of closely linked genes on a chromosome that is inherited together. This implies that

they are likely to be conserved as a sequence that survives the descent of many generations of reproduction. Secondly, haplotypes are single nucleotide polymorphisms (SNPs) on one chromosome that are statistically associated (International Hap Map Project, 2003). Haplotype diversity (Hd) can also be referred to as gene or allelic diversity or expected heterozygosity in a diploid state. It is the likelihood that two random sequences are different (Rozas, 2009). Haplotype diversity is generally due to mutation processes in the genome (Stumpf, 2004). It is used in linkage studies and tracing of ancestral lineage among species (Xu *et al*., 2002).

2.5 Phylogeny

Phylogeny is the evolutionary history of entities and it is used to assess ancestral history and relationships (Harrison and Langdale, 2006). The relationship is usually presented as a tree with nodes joining the branches. Analysis of phylogeny is used in tracing the origin and evolution of species, prediction of physiological, biochemical and structural features of sequences (Chambers *et al*., 2000). The tree can be rooted or unrooted. A rooted phylogenetic tree is one in which all the objects on it share a known common ancestor. To root the tree, a different group (out-group) of species to the group under study is usually added (Harrison and Langdale, 2006). In an unrooted phylogenetic tree, all the objects are related to descendant but there is insufficient information to specify the common ancestor. Three types of relationships have been reported (monophyly, paraphyly, and polyphyly). All descendants form a single ancestor including the ancestor from the monophyletic group. The removal of a single lineage from a monophyletic group leads to the formation of a paraphyletic group. In contrast, the polyphyletic relationship is the result of convergent evolution (Kitching *et al*., 1998). A phylogenetic tree can be constructed using different features and characters of species such as morphology, Random Amplified Polymorphism DNA (RAPD) information

as well as sequence fragment of the DNA. When using sequence data, it is very important to determine whether to construct the tree from the DNA or protein level. Closely related species are constructed using DNA sequence data while distant groups are preferably constructed using amino acid sequences (Michu *et al*., 2007)., also observed maternal genetic variation of (0.90) among regions, (4.06) among breeds/within regions and (95.04) within breeds.

2.5.1 Phylogenetic Methods

Different methods of phylogenetic analysis can be performed once the data are aligned (Holder and Lewis, 2003). These methods are generally divided into two categories; the distance-based methods (neighbor-joining, Fitch-Margolis, Unweighted Pair Group with arithmetic mean and minimum evolution) which calculate genetic distance from multiple sequence alignment (amount of differences between two aligned sequences in building a tree) thus, are simplest to implement but do not invoke an evolutionary model. Another is the disrete data-based methods (maximum likelihood, maximum parsimony, and Bayesian method), which implies an implicit model of evolution. This method searches each alignment column to select regions with information for constructing a tree (Michu *et al*., 2007).

2.5.1.1 UPGMA (Unweighted Pair Group Method with Arithmetic Mean)

This is a simple agglomerative or bottom-up data clustering method used in bioinformatics for the creation of phylogenetic trees. UPGMA assumes a constant rate of evolution (molecular clock hypothesis) and is not well regarded for inferring phylogenetic trees unless this assumption has been tested and justified for the data set being used. UPGMA was initially designed for use in protein electrophoresis studies but is currently most often used to produce guide trees for sophisticated phylogenetic reconstruction algorithms. The algorithm

examines the structure present in a pairwise distance matrix to then construct a rooted tree (dendrogram) (Felsenstein, 2000)

2.5.1.2 Neighbor - joining

It constructs a tree by successive clustering of lineages, setting branch length as the lineages join. The tree is not rearranged thereafter. The tree does not assume an evolutionary clock so that it is in effect an unrooted tree. It should be somewhat similar to the tree obtained by Fitch-Margolis (FM). The program cannot evaluate the user tree nor can it prevent branch length from becoming negative. However, the algorithm is far faster than Fitch-Margolis (FM). This makes it particularly effective in their place for large studies or for bootstrap or jackknife resampling studies which requires runs on multiple data sets (Felsenstein, 2000).

2.5.1.3 Fitch-Margolis (FM)

This method uses a weighted least squares method for clustering based on genetic distance. Closely related sequences are given more weight in the tree construction process to correct for the increased inaccuracy in measuring distances between distinctly related sequences. This method increases the strength of a phylogenetic tree by ensuring that the squared deviations of all observed distances are minimized (Felsenstein, 2000).

2.5.1.4 Maximum evolution (ME)

This method is similar to Fitch-Margolis. It minimizes all the squared deviations of observed distances (Felsenstein, 2000). It does not utilize all the pairwise distances and the path lengths in constructing the tree, but it uses information from the external nodes to build the internal nodes while the internal branch length is optimized based on these observed points (Brinkman and Leipe, 2001).

2.5.1.5 Maximum parsimony (MP)

This method makes use of information from all shared characters among aligned sequences without reducing any information provided by the informative sites of the original dataset while providing optimal information about the origin of the sequences (Michu *et al*., 2007). The method works on the principle of generating a phylogenetic tree with less evolutionary changes.

2.5.1.6 Maximum likelihood (ML)

This method works by searching for a phylogenetic model that can accurately explain the evolutionary relationships of any given dataset (Brinkman and Leipe, 2001). In this method, information from each base position in aligned sequences is the focal point in constructing the tree. Here, the tree is constructed based on the assumption that the observed variation at any site is specific in generating a given tree.

2.5.1.7 Tajima's D and Fu's F

These are the test of selective neutrality of a random sample of DNA sequences or Restriction Fragment Length Polymorphism haplotypes under the infinite site model. It is a statistical test created by and named after the Japanese researcher Fumio Tajima. The purpose of the test is to distinguish between a DNA sequence evolving randomly ("neutrally") and one evolving under a non-random process, including directional selection or balancing selection, demographic expansion or contraction, genetic hitchhiking, or introgression. A randomly evolving DNA sequence contains mutations with no effect on the fitness and survival of an organism. The randomly evolving mutations are called "neutral", while mutations under selection are "non-neutral". For example, you would expect to find a mutation that causes prenatal death or severe disease to be under selection. The strength of genetic drift depends

on the population size. If a population is at a constant size with a constant mutation rate, the population will reach equilibrium of gene frequencies. This equilibrium has important properties, including the number of segregating sites *S*, and the number of nucleotide differences between pairs sampled (these are called pairwise differences). To standardize the pairwise differences, the mean or 'average' number of pairwise differences is used. This is simply the sum of the pairwise differences divided by the number of pairs and is signified by π (Felsenstein, 2000). Therefore, The D test was based on the difference between the number of segregating sites and the average number of nucleotide differences whereas the Fu's F test was based on the haplotypes (gene) frequency distribution conditional to the value of θ (Ewes, 1922). A negative value of D signifies an excess of low-frequency polymorphism indicating population expansion (Tajima, 1989) while a positive value signifies low and high polymorphism evidenced when population size decreases. Similarly, a negative Fu's F statistics value signifies the presence of genetic drift in a population (Fu, 1997). The P-value provided for both Tajima's D and Fu's F test was based on a coalescent simulation algorithm (10, 0000 permutations were run). The P values represent the probability that simulated estimates are less than the observed value for D test or less than or equal to the observed value for Fu's F test. Rejection of these tests may be caused by the violation of any of the assumptions in the null hypothesis (neutrality, constant size, panmixia, no recombination). The significant departure of these tests might be due to an excess of new mutations as a result of evolutionary forces, such as selective sweeps or population growth.

2.5.1.8 Bayesian method

The Bayesian method is the best used to construct a phylogenetic tree of a large dataset by using a statistical model. The statistical model ensures that all uncertainties associated with the datasets are fully resolved to generate a reliable phylogenetic tree (Huelsenbeck *et al*.,

2000). This method encourages the use of complex models that are associated with a substitution process of DNA.

2.5.1.9 Bootstrapping

Bootstrapping is a simple test used for testing the accuracy of the phylogenetic tree. This test was incorporated into phylogenetic analysis due to its simplicity and speed in assessing the reliability of a constructed evolutionary tree (Felsenstein, 2000). It assesses the dataset for a given tree to determine whether such a tree is constructed based on the information provided by the dataset or not. The idea is to randomly choose sub-samples from the original dataset to construct a tree and thereafter, each part of the main tree is tested to determine the frequency of being produced by the sub-sample. The bootstrap support is said to be 100% if a particular group is present in every subsample tree (Baldulf, 2003). Bootstrap of 70% or higher indicates reliable clustering (Hillis and Bull, 1993).

2.6 Reviews on the Measures of Genetic Differentiation Studies

2.6.1 Genetic Diversity of Chicken based on *Mitochondrial DNA Marker*

Adebambo *et al.* (2010) revealed the phylogeographic structure in Nigerian village chickens by mitochondrial DNA along with phenotypic and genetic information. They generated 35 haplotypes which were grouped to a single clade and 97.8% of the total maternal variation occurs within populations. Reference sequences from Asia indicated the Indian subcontinent to be the likely main center of origin of Nigerian village chicken.

Nwacharo *et al.* (2011) employed mitochondrial DNA to reveal multiple introduction of domestic chicken in East Africa. They identified 41 haplotypes from 37 polymorphic site. Five haplotypes appeared to be the most frequently observed with haplotype diversity of

0.857 among Kenya, 0.374 among Ethiopia, 0.413 among Sudan and 0.322 among Ugandu village chicken population. They also observed that haplogroup A and D are the major haplogroup in East Africa related to geographically distinct Asian mitochondrial DNA haplogroups primarily South Asia for haplogroup D and East and South Asia for haplogroup A as reported in Liu *et al*., (2006). Haplogroup A was exclusively found in Kenya whereas haplogroup D was found across Kenya, Ethiopia, Sudan and Uganda.

Muchadeyi *et al.* (2008) sought to assess a 455-bp fragment of the mtDNA D-loop diversity and phylogeographic structure of chickens from five agro-ecological zones of Zimbabwe which resulted into 34 haplotypes. They observed haplotype diversity range of 0.64, 0.66, 0.61, 0.73 and 0.69 among the five Zimbabwean ecotypes. Results obtained revealed two distinct maternal lineages evenly distributed among the chicken ecotypes derived from five agro-ecological zones of Zimbabwean with a Southeast Asian background. The second maternal lineage, probably from the Indian subcontinent, was common to the five Zimbabwean chicken ecotypes, Sudanese and Northwest European chickens as well as purebred broiler and layer chicken lines. A third maternal lineage excluded Zimbabwean and other African chickens and clustered with haplotypes presumably originating from South China.

Cuc *et al*. (2011) also reported a haplotype diversity of range of 0.615 to 0.942 within Vietnamese chicken population of Ho and Tau vang breeds respectively. They obtained the least (4) number of haplotypes simultanously within two Vietnamese chichen populations and the highest (37) number of haplotype with a very low number of polymorphic sites ranging from 8 to 43 of the same breeds respectively.

2.6.2 Genetic Distance and AMOVA amongst chicken breeds using *Mitochondrial DNA marker*

Sulandari *et al.* (2008) investigated to unravel the molecular characterization of Indonesian indigenous chickens by the hypervariable I (HVI) segment of mitochondrial DNA (mtDNA) displacement (D)-loop sequences. Subsequent genetic diversity and relationship study of Indonesian indigenous chickens identified sixty-nine haplotypes from 54 polymorphic sites with polymorphism between nucleotides 167 and 397 contributing to 94.5% of the sequence variation. Phylogenetic analysis indicated that Indonesian indigenous chickens can be grouped into five distinct clades (clade I, II, IIIc, IIId, and IV) of the previously identified seven clades in Asian indigenous chickens. There was no breed-specific clade. One of the haplotypes (represented by PLC 4) was shared by all populations, suggesting that these populations may share the same maternal ancestor showing a high mitochondrial D-loop diversity and indicated multiple maternal origins for Indonesian indigenous chickens. They discovered a farthest genetic distance of (0.88) between Arab Gold with Gaok chicken, and a closest genetic distance (0.002) between Sentul and Merawang and also between Kedu and Kapas. They further discovered a close genetic distance of 0.003 between Kedu and White Kedu. Therefore they speculated that Information of the genetic distance is needed for conducting the crossing of chicken families with far genetic distance, to form a final stock of superior indigenous chicken in order to gain high hybreed vigor (effect *heterosys* positive). However, their AMOVA result shows that genetic variation among chicken individuals within chicken population is 67.65%, while genetic variation among chicken breed population is 32.15%. They maintained that genetic variation value of 32.15% indicates low genetic differentiation resulting from the geographical groups suggests that Indonesian indigenous chickens have not been subdivided across the regions hence this implies that breeding females may have been exchanged.

Pariset *et al*. (2011) employed mtDNA and nuclear SNPs to investigate the genetic diversity of sheep breeds of three countries of the Mediterranean basin: Albania, Greece, and Italy. Their AMOVA revealed that mitochondrial diversity is mainly distributed within breeds (95.04%) and only in part among regions (0.90%) while low variability (4.06%) was dictated among breeds/within regions, which suggested the presence of weak phylogeographic structure in sheep. Therefore they concluded that Sheep generally do not have a strong geographic structure and show a high genetic variability within breeds.

2.6.3 Phylogeny and maternal origin/inheritance of chicken based D-Loop (Control Region) of Mitochondrial Genome

Ohno, (1997) elaborated on the one ancestor per generation rule and three other rules of mitochondrial inheritance. He deduced that mammals possess atleast a species-specific mechanism that trigaers the elimination of sperm-derived mitochondrial DNA from a fertilized egg. The outcome of this event is termed the "one female ancestor per generation" rule and three other rules of mitochondrial inheritance. The second rule stated that sublineages of a given mitochondrial line can be generated only during the parallel descents from ancestral sisters. The third rule states that in a static population in which the production of female progeny per mated pair per generation has been a norm, and that several ancient mitochondrial lineages harking back to the female founders of the speciation may persist side by side. Finally the fourth rule was of the view that two or more individuals not related to each other in the recent past may share the identitical or nearly identical mitochondrial genome derived from the common female ancestor or ancestral sisters of many generations ago. This is to say that a set of full or maternal half-sisters should have inherited similar mitochondrial genome from their mothers, but each sister's female descendants invariably

establish an independent lineage which in time would accumulate its own characteristic mutations to become a distinct sublineage.

Zhu, (1958) suggested that mutation was the main reason different breeds with different features share a common lineage and that more than 30 subspecies or variants of domestic chicken were formed by gradual mutations. Working with different chicken breeds in 1921, Joes discovered a Leghorn with fuzz which was similar to silky chicken. Then Joes mated the strange Leghorn with normal Leghorn and silky chicken, respectively, and analyzed the inheritance and differentiation of the feather in their offspring. It was found that both the Leghorn with fuzz and silky chicken had essentially the same mutation. But how could different breeds share the same maternal lineage? Chinese researchers presumed that the primary reason for the domestication of chicken was to make available, a source of meat and for religious purposes. Later on the cockfighting breed was bred for recreation and lastly, egg-type breeds were developed (Cheng *et al.*, 2000).

Mason (1987) speculated that domesticated chicken was first used as recreational breeds, such as gamecock, then for various religious purposes, and eventually as the source of meat and egg. The differences between these two viewpoints were attributed to different citations. The former mainly referred to Chinese archaeological and other related documents, while the latter mainly referred to Indian archaeological and other related documents. Although the viewpoints were not consistent with each other, both of them implied that various types of chicken breeds might have originated from common ancestors.

Revay *et al.* (2010) studied the origin of Hungarian indigenous chicken breeds using mitochondrial DNA information. Sequences of Hungarian chickens were compared with the

D-loop chicken sequences annotated in the GenBank. Eleven haplotypes were observed from 17 variable sites. They assigned Hungarian domestic chicken into three clades and probably two maternal lineages and likely originated from the Indian subcontinent, while the minor part of our sequences presumably derives from South East Asia, China, and Japan.

Oka *et al.* (2007) analyzed mtDNA D-loop region sequences of Japanese native chickens to clarify their phylogenetic relationships, possible maternal origin and routes of introduction into Japan. The results revealed a less than an average nucleotide diversity of certain chicken breeds, thus contradicting the conventional literature that some breeds of chicken were introduced into Japan. His result therefore confirms the originality of Southeast Asia chicken, thus, suggesting that Japanese native chickens have multiple origins.

Cuc *et al.* (2011) reported multiple maternal lineages of Vietnamese local chickens inferred by mitochondrial DNA (mtDNA) D-loop sequence polymorphism to assess the genetic diversity of nine Vietnamese local chicken breeds along with two Chinese breeds kept in Vietnam. A 455-bp fragment of the mtDNA D-loop region sequence analysis identified 37 haplotypes of Vietnamese local haplotypes. They suggested that the Vietnamese domestic chickens have originated from multiple maternal lineages, presumably from Yunnan and adjacent areas in China, South and Southwest China and/or surrounding regions (i.e. Vietnam, Burma, Thailand, and India).

Bjørnstad *et al.* (2009) reported on behalf of Chicken Diversity Consortium (Africa) mitochondrial DNA D-loop analysis of southwestern Nigerian chicken and identified seventeen haplotypes for Nigerian indigenous chicken population, 1 for Giriraja and 1 for Anak titan from 23 polymorphic sites. Phylogenetic analysis showed that Nigerian

indigenous and Anak titan chicken were all grouped under clade IV, while the Indian Giriraja was under clade IIIc. They suggested single multiple maternal origins for the southwestern Nigerian domestic chicken.

Fumihito *et al.* (1996) studied a single origin and distinct dispersal patterns of domestic fowls. He analyzed mtDNA sequences of the D-loop regions that belong to red jungle fowl (*Gallus gallus*) comprising three subspecies (*Gallus gallus*, *Gallus spadiceus*, and *Gallus bankiva*) and domestic breeds (*Gallus gallus domesticus*) along with green jungle fowl (*Gallus varius*), Lafayette's jungle fowl (*Gallus Lafayette*), and one grey jungle fowl (*Gallus sonneratii*). The phylogenetic tree indicated that a continental population of *G. g. gallus* was the real matriarchic origin of all the domestic poultries examined in this study. It is also of particular interest that there were no discernible differences among *G. gallus* subspecies; *G. g. bankiva* was a notable exception. *G. g. bankiva*, on the other hand, was a distinct entity, thus deserving its subspecies status. It implied that a continental population of *G. g. gallus* sufficed as the monophyletic ancestor of all domestic breeds.

Nishibori *et al.* (2005) first demonstrated the molecular evidence for the hybridization of species in the genus *Gallus* except for *the Gallus varius* using complete sequences of mitochondrial DNA (mtDNA) and two portions of the nuclear DNA for the species in the genus *Gallus*. The phylogenetic analyses based on mtDNA sequences revealed that two grey jungle fowls (GyJF) were clustered in a clade with RJFs and one GyJF was located in a remote position close to Ceylon jungle fowl (CJF). The analyses based on the nuclear sequences revealed that alleles of GyJFs were alternatively clustered with those of CJF and

with those of RJFs thus indicating inter-species hybridizations between GyJF and RJF/chicken and between GyJF and CJF.

Liu *et al.* (2006) studied multiple maternal origins of chickens out of the Asian jungles and assessed their origins and phylogeographic history by analyzing the mitochondrial DNA hypervariable segment I (HVS-I) for domestic chickens (*Gallus gallus domesticus*) across Eurasia as well as wild red jungle fowls (*Gallus gallus*) from Southeast Asia and China. Phylogenetic analyses revealed nine highly divergent mtDNA clades (A–I) in which seven clades contained both the red jungle fowls and domestic chickens. There was no breed-specific clade in the chickens. The clades A, B, and E are distributed ubiquitously in Eurasia, while the other clades were restricted to South and Southeast Asia. Clade C was mainly distributed in Japan and Southeast China, while clades F and G were exclusive to Yunnan, China. The geographic distribution of clade D was closely related to the distribution of the pastime of cockfighting. Statistical tests detect population expansion within each subclade. These distinct distribution patterns and expansion signatures suggest that divergent clades originated from divergent regions of China and/or surrounding areas (i.e., Vietnam, Burma, and Thailand), and the Indian subcontinent, respectively, which supported the theory of multiple origins in South and Southeast Asia.

Silva *et al.* (2009) carried out mitochondrial DNA-based analysis of genetic variation and relatedness among Sri Lankan indigenous chickens and the Ceylon Jungle fowl (*Gallus Lafayette*). The control region of the mitochondrial DNA sequence for single nucleotide polymorphisms (SNPs) and other variants detected and validated 44 SNPs, which formed 42 haplotypes and six haplogroups in indigenous chicken. The variation among individuals within regions accounted for 92% of the total molecular variation among birds. The Sri

Lankan indigenous chickens were more closely related to red and grey jungle fowls than to CJF, suggesting multiple origins.

Mtileni *et al.* (2011) investigated the diversity and origin of South African conserved and field chicken populations based on 460 bp of the mitochondrial DNA (mtDNA) D-loop sequence. Sequence analysis revealed 48 polymorphic sites that defined 13 haplotypes in the South African chicken populations. Genetic diversity between the 4 South African conserved and 2 field chicken populations constituted 12.34% of the total genetic variation, whereas within-population diversity constituted 87.66% of the total variation. All 6 South African chickens were equally represented in the major clade, E, out of 5 analyzed clades, which is presumed to be of Indian subcontinent maternal origin and may have its roots in Southeast Asia showing multiple maternal lineages. Conservation flocks and field chicken populations shared the major haplotypes A, D, and E, which were presumed to be of Chinese, Southeast Asian, and Indian sub-continental origin.

Crawford, (1990), suggested that the Red jungle fowl (*Gallus gallus)* is the primary ancestor of domestic chicken and is widely distributed in East Asia, spreading through China, Eastern India, Burma, some parts of Indo-China, on the Island of Sumatra, Java and Bali.

Miao *et al*. (2013) revealed new complexities of history in chicken domestication. They carried out a genetic diversity survey of domestic chicken (G. domesticus) and trace their history of domestication. They investigated a total of 4938 mitochondrial DNA (mtDNA) fragments including 2843 previously published and 2095 *de novo* units from 2044 domestic chickens and 51 red jungle fowl (*Gallus gallus*). They used additional 50 representative samples for total mtDNA genome sequencing so as to obtain the highest possible level of

molecular resolution. They investigated a fine-gained mtDNA phylogeny by defining haplogroups A–I and W–Z. They discovered that haplogroups A–G were the commonest frequently shared by domestic chickens and red junglefowl while haplogroups H–I and W–Z were rare and were specific to domestic chickens and red junglefowl, respectively. Therefore, they re-evaluated the global mtDNA profiles of chickens, by examining the geographic distribution for each of the major haplogroups.

Mobegi and Chicken Diversity Consortium, (2006) used mitochondrial DNA (mtDNA) displacement (D)-loop sequences to study genetic differentiation, genetic diversity and phylogenetic relationship between domestic chicken populations and low breeds of Africa. He sampled 398 individuals belonging to 28 populations from 12 African countries. He analyzed the sequences of the first 397 nucleotides, 52 haplotypes were identified form 50 polymorphic sites with between 167 and 397 polymorphism contributing to 96% of the sequence variations. Genetic variations within populations and between populations accounted for 64.8% and 35.2% of the total genetic variation, respectively. He, therefore, concluded that there is high mt D-loop diversity in African chicken, which indicated multiple maternal origins for African domestic chicken.

2.6.4 Mutation association of SNPs based on *Mitochondrial DNA Marker*

Guan *et al.* (2012) carried out the first whole mitochondrial DNA sequence and haplotype variation analysis in the chicken (*Gallus gallus*) using experimental and in silicon, tools to identify nucleotide variants in coding and non-coding (D-loop) regions of 53 birds, including 33 broilers and 20 White Leghorn chickens. A total of 113 single-nucleotide polymorphisms (SNPs) were identified from the sequence analysis which comprised of 35 and 78 in the noncoding and coding regions, respectively. Among the 78 SNPs in the coding region, 19

were non-synonymous, 41 synonymous, with 70 of the 78 SNPs or 91% being transitions of either C–T (41) or G–A (29) substitutions. Similarly, they observed a significantly lower number of transversions in the noncoding region: only one or 3% of the 35 SNPs observed in the D-loop was a G–C substitution. The experimentally identified SNPs in the non-coding region formed 11 haplotypes, whereas the 14 SNPs in the coding region formed 6 haplotypes. Therefore they concluded that the SNPs described in their work provide a foundation for which the role of the mitochondrial in metabolic disorders could be further investigated.

Fumihito *et al.* (1994) studied the non-coding control region of the mitochondrial DNA of various gallinaceous birds concerning its restriction fragment length polymorphism (RFLP) and sequences of the first 400 bases. They reported the close ally *Gallus varius* (green jungle fowl); the red jungle fowl *Gallus gallus* were genetically very diverse species. Furthermore, the divergence increased to 27.83% if each transversion is regarded as an equivalent of 10 transitions. Their result indicated a single domestication event in the area inhabited by this subspecies of the red jungle fowl as the origin of all domestic breeds.

Steffen *et al*. (2008) suggested that mutations occur in some sites in the genome more frequently than in others. They deduced that, mutations in some sites have greater consequences than in others. The effect of mutations might not be independent of the frequency with which mutations occur. Indeed, sites where mutations happen frequently will be preserved if the effects of these mutations are severe or will otherwise be allowed to mutate if there are no consequences for the organism. They compared both human–chimpanzee differences and sequence variation among humans in protein coding genes and found that highly mutable nucleotide sites, such as the dinucleotide CpG (regions of DNA where a cytosine nucleotide is followed by guanine nucleotide in the linear sequences of

bases along the $5^1 - 3^1$), are on average more important and more frequently preserved by natural selection. Therefore they infer that using this information, together with other features such as sequence conservation, opens a new perspective to predict the effect of human mutations and ofcourse other animals, including their potential involvement in diseases.

Fitch (1967) noted that the nucleotide substtution pattern in cytochrome c is non-random. He suggested that if random, transversions (purine-pyrimidine changes) should be observed twice as often as transition (purine-purine or pyrimidine – pyrimidine changes) solely due to the accessible mutations, then there would be alteration in the rate at which natural selection disfavours transversions. He observed that transitions are more common than transversion. Three out of his four data set revealed that transversions are significantly more detrimental than transitions at a subset of relative sites preferences. This he attribuited to the fact that transversion are more likely to cause substitutions that radically alter biological properties of the original amino acid.

2.6.5 Genetic population structure using Wrights F-Statistica and Inference of gene flow based on mitochondrial DNA marker

Men Q *et al*. (2004) reported a high genetic diversity and strong genetic differentiation in a population of Dendrolimus kikuchii Matsumura (*Lepidoptera*; *Lasiocampidae*). He investigated the genetic diversity and structure of 182 individuals of the D.kikuchii sampled throughout its main distribution areas in China using three mitochondrial genes (COI, COII, and Cytb). They employed the use of Analysis of Molecular Variance (AMOVA) using ARLEQIUN version 3.5 bases on the combination of the three gene sequences as well as pairwise fixation indices (F_{ST}). Referring to the criterion for genetic differentiation by Wright (1978), they defined genetic differentiation as low for Fst < 0.05, moderate for 0.05, <FST

<0.15, high for 0.15<FST<0.25and very high for Fst > 0.25. They tested isolation by distance Fst/ (1- Fst). Their results show a high level of genetic structuring of the D.kikuchii in the sampled areas. Therefore, they concluded that the high level of population genetic structuring is related to the weak flight capacity of the D.kikuchii, variation in its life history and the geographic distance among populations. Distribution of pairwise differences (mismatch distributions) obtained with COI, COII Cytb and combined gene data indicated the population of D.kikuchi in Southern China did not experience population expansion.

Hassan *et al*. (2007) evaluated the genetic differentiation within and between chicken populations by estimating genetic diversity (H_T and H_S) and genetic subdivision (Gst) for each locus across all populations. The average total genetic diversity (H_T), genetic diversity within a population (Hs) and coefficient of genetic differentiation (Gst) across all loci were estimated at 0.342 0.019V, 0.241 V 0.007 and 0.296 respectively. They obtained the mean Gst value across all loci to be (0.194) indicating around 19.4% of total genetic variation and was attributed to being due to breed differences, while the remaining 80.6% was accounted for differences among individuals. Thus, the higher coefficient of genetic differentiation (GST) across all loci in APO VLDL-II indicated that very high variation is proportioned among populations.

Piertney *et al*. (2000) studied matrilineal genetic structure and female-mediated gene flow in red grouse (lagopus lagopus scoticus). They examined the DNA sequence variation at the hypervariable 59end of the mitochondrial control region in 247 individuals to detect genetic divergence among 14 populations of red grouse (Lagopus lagopus scoticus) in Northeastern Scotland. They observed Ten haplotypes shared among populations. Analysis of molecular variance, Nei's GST, and a Cladistic estimate of the amount of gene flow indicated a lack of

overall population differentiation. They suggested that the lack of mitochondrial DNA divergence was attributed to the fact that grouse cocks are territorial and show extreme natal philopatry while females are the dispersing sex, which resulted to discordance owing to sex-biased dispersal, with extensive female-mediated gene flow. Meanwhile, they found it difficult to reconcile how effective dispersal of females would not homogenize both mitochondrial and nuclear structure simultaneously. As a result, they employed a model that examines the spatial and temporal dynamics of di-parentally and un-parentally inherited genes to show that, under realistic ecological scenarios and with specific differences in the dispersal of males and females, the local effective size of the nuclear genome can be less than that of the mitochondrial and the patterns of structuring. However, they were able to obtain meaningful results.

Liu *et al*. (2010) attempted to test whether there are possible genetic communications between Chinese gamecocks and their neighboring native chicken breeds. Therefore they carried out a comprehensively analysis of an integrative mitochondrial DNA (mtDNA). They compared the first hyper-variable segment I sequences of the mtDNA control region (HVS-I) of 69 gamecocks belonging to 5 breeds across China and 259 native chicken belonging to 18 breeds sampled from the neighboring regions or provinces, with sequences data retrieved from GenBank and previous works. Firstly, they constructed phylogenetic trees and compared the mtDNA data of Chinese gamecocks with the neighboring native breeds using the red jungle fowls as the outgroup, in order to trace and learn more about the genetic information between Chinese gamecocks and the neighboring native chicken breeds and the followed artificial selection. Then, they analyzed the gene flow at an inter-group level and computed the corresponding population differentiation, particularly focusing on Chinese gamecocks. Analysis of Molecular Variance (AMOVA) and phylogeny demonstrated

Chinese gamecocks formed monophyletic groups that had been differentiated significantly with other native chicken breeds, though some of the clusters appeared to be an admixture of gamecocks and domesticated chicken. All the analysis demonstrated significant differentiation within chicken populations sampled and presented a gene flow mode of geographical distribution ($p<0.05$, AMOVA). They found most of the chicken breeds have been an admixture with possible gene flow among them, except for three gamecock breeds and three native chicken breeds, Chahua, Gushi and Tibetan chicken, whose races are relatively pure.

2.6.6 Demographic insight of chicken populations based on mitochondrial DNA

Teinlek *et al*. (2018) in their study of Genetic diversity analysis of Thai indigenous chickens based on complete sequences of mitochondrial DNA D-loop region, estimated neutrality test based on Tajima' D between four Thai indigenous chicken varieties, including Pra-dhu-hang-dam (PD), Leung-hang-khao (LK), Chee (CH), and Dang (DA). The values with their respective P-values were aall statistically non significant and ranged from 1.31973 (0.909), 0.76207 (0.818), 1.37028 (0.931), −0.00305 (0.546) and 0.40273 (0.738) for the respective Thai indigenous chicken populations. They deduced that non-significant Tajima's D being observed in all studied populations of Thai indigenous chickens signified that they were consistent with a population at mutation-drift equilibrium as suggested by (Kimura, 1983), thereby suggesting neutral selection is involved. This could be attributed to the fact that indigenous chickens have been generally raised as free-range backyard flocks in which random mating is permitted without farmer's interest in programmed breeding. The tropical to subtropical environment is also suitable for the chickens to survive and reproduce.

Therefore the concluded that impacts of selection pressures either natural or man-made are less likely applied on these populations, which supports the neutrality found.

Eltanany, (2016) sought to reveal the demographic expansion of Egyptian indigenous chickens (EIC) using representative breeds: Sinai (North), Fayoumi (Middle) and Dandarawi (South) of Egypt as well as to deeply clarify their genetic diversity, possible matrilineal origin and dispersal routes. He observed negative Fu's F across populations except with the Sinai (North) which showed positive Fu's F. he also observed negative Tajim's D across populations except with the Fayoumi (Middle) which showed positive Tajim's D. The unimodal mismatch distribution and the mean negative values of Tajima's *D* (−0.659) and Fu's *Fs* (−0.157) indicated demographic expansion among EIC and pointed to Fayoumi as the oldest EIC population.

Neutrality tests (Tajima's D and Fu's Fs) and mismatch analysis conducted by (Gu, *et al*., 2016) in their investigation of the genetic variability and genetic structure of *H. gallinarum* indicated that *H. gallinarum* experienced a population expansion in the past. Our results indicated that *H. gallinarum* experienced a rapid population expansion in the past, and there was a low genetic diversity and an absence of population structure across the population

Adebambo *et al*. (2010) also reported that the D-loop region in Nigerian chicken showed a departure from equilibrium (significant negative values for both Tajima's D and Fu's F) thereby suggesting the tendency for population expansion among Nigerian chicken population.

Waples, (1998) deduced that numerous statistical tests are available for the analysis of population structure. However, exclusive focus on the results of statistical tests can be misleading. He was of the view that the significant level of a statiscal test provides little

information by itself, therefore it becomes necessary to consider the richness of data and the power of the test. He concluded that statistical test will be sensitive to these factors if researchers could be able to maximize the signal: noise ratio by putting up intensive sampling effort. In addition to this, he stated that even relatively minor departures from random sampling assumption can cause misleading rejection of the null hypothesis of large amount of data are collected. Therefore, he recommended that researchers should not rely solely on statistical tests to guide decisions about identification and management of stocks among species including chicken.

2.7 Softwares used in Genetic Differentiation Analysis

2.7.1 DnaSP software

The DnaSP, an acronym for DNA sequence polymorphism is a molecular genetics tool that is employed by molecular biologists and geneticists to analyze sequence polymorphisms within and between population using the non-coding, synonymous or non-synonymous sites (Librado and Rozas, 2009). This software can handle large datasets with thousands of nucleotides. The software cans easily export and import data from other programs such as multiple alignment and phylogenetic tree analysis programs. The DNA polymorphisms unveil by DnaSP is increasingly used to study the evolutionary changes in genomes of populations (Begun *et al.*, 2007).

2.7.2 Mega software

Mega (molecular evolutionary genetic analysis) software is used to analyze DNA and protein sequences to provide good insight into their evolutionary history (Tamura *et al.,* 2013). The data must be formatted in the basic ASCII file format. Multiple tasks can be performed in MEGA such as sequence alignment, estimation of evolutionary distance between sequenced

data, building a tree from sequence data, bootstrapping, testing for selection, building trees from distance data and computation of sequence statistics.

2.7.3 Fintch TV

FintchTV is free software for viewing chromatogram files and viewing and editing sequencing data. It provides a popular way to easily view and edit your DNA sequence chromatogram data. It can display an entire trace in a scalable multi-pane view and enables the editing of base cells and save them to a new chromatogram file. It also enhances the ability to search sequence data using regular expressions or simple base queries, view raw data from AB1 files, and export data in FASTA format (www.geospiza.com/finchtv)

2.7.4 Network software

Network software is used to generate a phylogenetic tree as well as constructing the network of a sequence dataset. Using this software, it is possible to estimate the age of an ancestor in a phylogenetic tree. Here, the sequences will first be aligned before the network profile is constructed. The software is recommended for a good publication quality as well as good graphic presentations.

2.7.5 Arlequim software

Arlequin software is a functional software used in the analysis of population dataset such as estimation of genetic diversity parameters, computation of allele and haplotype frequencies, test of departure from linkage equilibrium, selective neutrality and demographic equilibrium, estimation of parameters from past population expansion and analysis of population structure using analysis of molecular variance (AMOVA) framework. Arlequin can work with DNA sequences and microsatellite data types (Excoffier *et al.,* 2005). The graphical interface is

designed to allow users to rapidly select the different analyses they want to perform on their data. The statistical tests implemented in Arlequin have been chosen such as to minimize hidden assumptions and to be as powerful as possible. Thus, they often take the form of either permutation tests or exact tests, with some exceptions (Felsenstein, 2000).

CHAPTER THREE

Materials and Methods

3.1 Location and Sample collection

The samples listed in Table 3.1 and figure 3.1 was collected from six populations of Improved tropically adapted chicken Breeds (iTABs): [(Shika Brown (SB), sample size n = 14; Fulani Ecotype (Fi), n = 13; Sasso (Sa), n = 13; Kuroiler (Ku), n=13; Funaab- ALPHA (FU), n=12 and Noiler (No), n=12] in Imo State. Imo State is located in the humid region of south eastern Nigeria and lies between Latitude 4º45' N and 7º 15'N; Longitude 6º50' E and 7º25'E. The agro-ecological distribution of the area is characterized by an average annual rainfall of 174.4mm, average annual humidity of 73.42%, and means minimum temperature of 23.5º C and mean maximum temperature of 32.1º (Okorie, 2015). The chicken populations used were sourced from six (6) households each in three (3) African Chicken Genetic Gains (ACGG) Project site in Imo State (Figure 3.1). All chickens were housed and cared for according to the guidelines established by the African Chicken Genetic Gains committee (http://www.africacgg.net - 2nd June, 2017).

3.2 Blood sample collection

A total of 77 (14 + 13 + 13 + 13 + 12 + 12) whole blood samples of approximately 2 ml were collected from the six populations of the improved tropically adapted chicken breeds (iTABs) as described below in table 3.2. Blood samples were collected from the wing vein with a 23 gauge needle and 2 ml sterile syringes, into 2 ml tube containing ethylenediaminetetraacetic acid (EDTA), stored at –20ºc before transferring to the laboratory for analysis.

Table 3.1 Study samples and descriptions

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

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) and Okorie (2015).

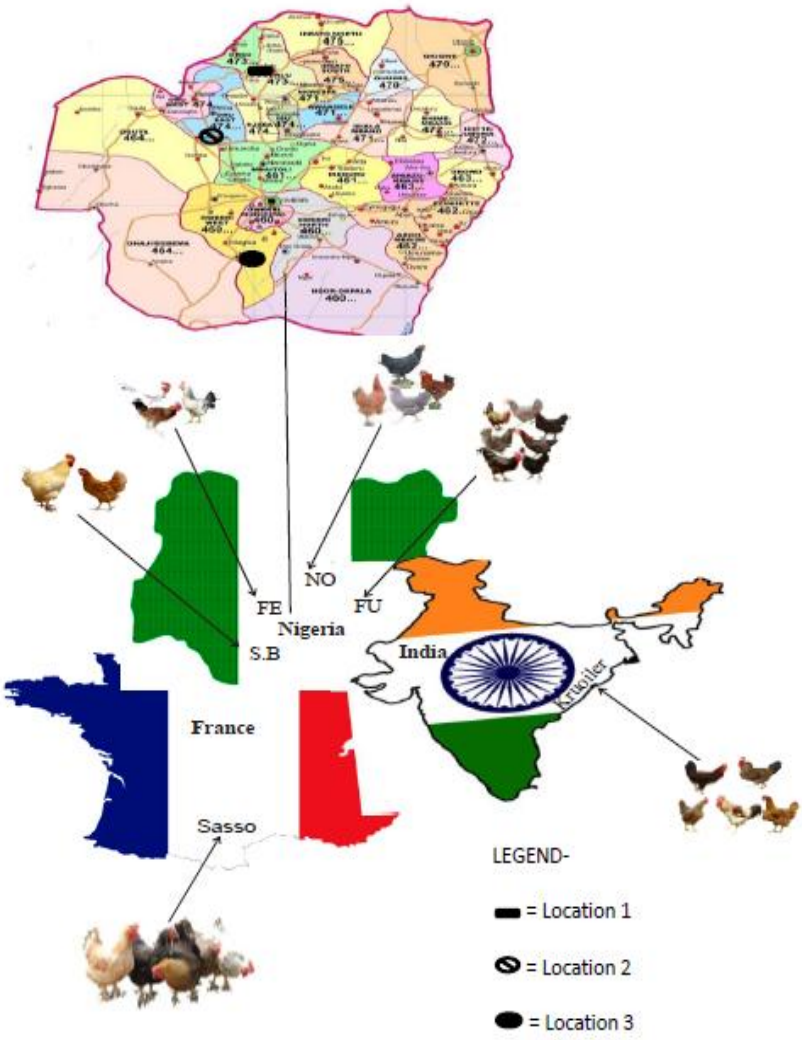

Fig. 3.1: Map of Imo State showing the study location and breed origin of the samples used. Sources: mirian's on farm image caption.
http://www.mapsofindia.com/India ; 2nd June, 2017
http://www.wikipedia.org/wiki/template.nigeria_states_map 2nd June, 2017
http://www.francetoday.com/france-map 2nd June, 2017
http://www.en.wikipedia.org/wiki/imo_state_map 2nd June, 2017

3.3 DNA Extraction

Extraction of genomic DNA and purification from the blood samples was carried out in the Biotechnology Laboratory Unit of Animal Breeding and Genetics Department, Federal University of Agriculture Abeokuta (FUNAAB) Ogun State. DNA was extracted using Quick-DNA^{TM} Miniprep Plus Kit (Zymo Research, USA). It is useful for easy extraction and preparation of high-quality DNA from biological fluids and cell protocol. Quick- DNA^{TM} is compatible with commonly used anticoagulants (i.e. EDTA, heparins, and citrate). The isolated DNA is ideal for PCR, endonuclease digestion, bisulphate conversion. The following were the procedure used for the genomic DNA extraction as contained in the Quick-DNA^{TM} Miniprep Plus Kit.

- Blood cell was pelleted via pipetting up and down while the supernatant was discarded.
- 200 µl blood cell was resuspended in 1-5x10^6 DNA elution buffer.
- 1,060 µl stored buffer was added to each 20 mg tube of proteinase K stored at - 20°C
- Up to 200 µl sample was added to a micro centrifuge tube followed by 200 µl Biofluid and cell buffer (Red) and 20 µl Proteinase K
- The mixture was spinned for thorough mixing and then incubated for at 55°c for 10minutes.
- 1 volume (420 µl) genomic binding buffer was added to 420 µl digested sample and was mixed thoroughly.
- The mixture was transferred to a zymo-spin ^{TM} 11-cxl column in a collection tube, centrifuged (<= 12,000x g) for 1 minute while the collection tube with flow-through was discarded
- 400 µl DNA pre-wash buffers were added to the column in a new collection tube. It was recentrifuged for 1minute while the collection tube was emptied.

- 700 µl gDNA wash buffer was added, recentrifuged for 1 minute while the collection tube was discarded as well.
- 200 µl of gDNA wash buffer was again added and centrifuged, while the collection tube, as well as the flow-through, was again discarded.
- Finally, the purified DNA was transferred to a clean micro centrifuge tube. And >=50 µl DNA elution buffer was added, incubated for 5 minutes and then centrifuged at top speed (12,000xg) for 1minute to elude the DNA. The eluted DNA was then stored at -20°C for further analysis.

3.4 Polymerase chain reaction

PCR amplification was carried out on Five hundred and ninety-two base pairs of the mtDNA D-loop region, at STAB VIDA Laboratory Quinta de forre, Portugal. The primers used were primers from Mobegi *et al*. (2005): L16750 forward (5'- AGGACTACGGCTTGAAAAGC -3'), and H547 Reverse (3'- ATGTGCCTGACCGAGGAACCAG -5'). However, Desjardins and Morais, (1990) and Komiyama *et al*., (2003), reported the accession number NC_001323 and AB098668 respectively, for the forward and reverse primers. PCR reactions were carried out at 96℃ for 15 mins, followed by 35 cycles consisting of 30-sec denaturation at 95℃, 30-sec annealing at 56℃ and 30 mins extension at 70℃, with a final extension at 70℃ for 5 mins using a Gene Amp PCR System 9700 (USA). The reagents contained 5 µl genomic DNA, 1.5mM $Mgcl_2$, 0.2mMdNTP, 1 µl of 10x PCR buffer, 0.4 µM of each primer and two units of STAB VIDA proprietary Surf Hot Taq Polymerase in a 25 µl reaction volume. The PCR products were then electrophoresed (120 V, 20 min) on 1.5% agarose gels (Figure 3.2), and purified before sequencing using exofast protocol according to the manufacturer.

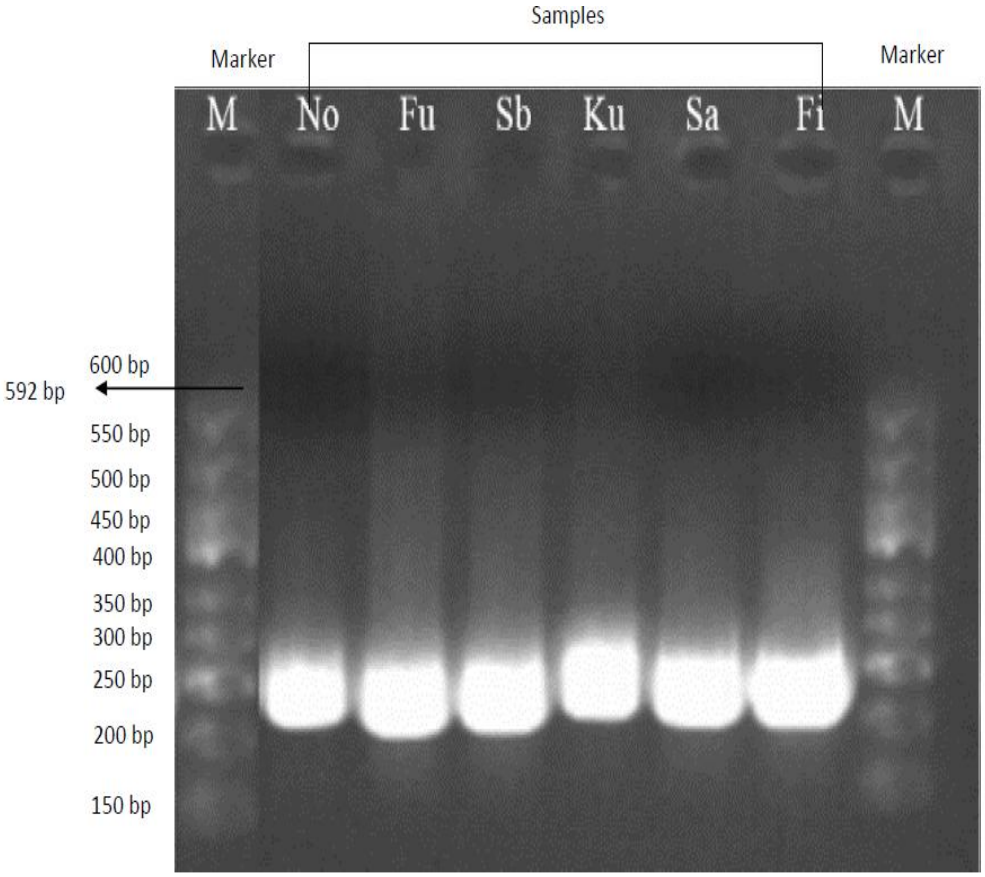

Figure 3.2**:** PCR Gel electrophoresis result of mtDNA D-loop region showing a 592-bp amplicon size for the iTABS: Noiler (No), FUNAAB Alpha (Fu), Shika Brown (Sb), Kuroiler (Ku), Sasso (Sa), and Fulani (Fi), M= 200-bp molecular –weight size marker.

3.5 Sequencing of D-loop (control) regions of mtDNA

Partial sequencing of the mtDNA D-loop region was done for the blood-DNA extracts using the primers; forward (5'- AGGACTACGGCTTGAAAAGC -3') and reverse (3'- TCCTGATGCCGAACTTTTCG -5'). Sequencing reaction was performed at STAB VIDA Laboratory, Quinta de forre, Portugal with G16750 x L sequencer using 20 µl reaction comprising approximately 20 ng of purified PCR product as template DNA, 8 µL of Big Dye Terminator Reaction Mix (dNTPs, ddNTPs, buffer, enzyme and $MgCl_2$), 8 µl of deionized water, 2 µl of primer programmed at 35 cycles at 95°C for 10 seconds, 60°C for four minutes.

3.6 Statistical analysis

3.6.1 Finch TV 1.4.0 software

Finch TV software version 1.4.0 (www.geospiza.com/finchtv) was used to view, assemble and edit the sequences. Thereafter undetermined bases (Ns) from both sides were trimmed off the sequences.

3.6.2 MEGA 7.0 software

Using MEGA 7.0 (Tamura *et al*., 2015), the first 315 base pairs were translated to protein sequences giving rise to 105 protein sequences. Thereafter, alignment of the sequences was done in muscle format to a reference sequence of (accession number KX987152.1; Source Mitochondrial *Gallus gallus* chicken (Huang *et al.,* 2017)) excluding all the gaps. After the alignment, the sequences were translated back to nucleotide sequences and were saved in MEGA format. Following 1000 bootstrap replicates, a Maximum Likelihood (ML) tree was constructed in bootstrap model. To classify the breeds into maternal lineage based on the D-loop region of the iTABs haplotypes, three D-loop sequences of Galliformes; *Meleagris*

gallopavo, *Cortunix japonica* and *Anas platyrhynchos* (GenBank accession numbers EF153719, AP003195, and MF069250, respectively), were included as outgroups. Eight reference sequences of Gallus species: Red Jungle Fowl, green jungle fowl, grey jungle fowl and Ceylon jungle fowl; and two sub-species of Gallus from the most common haplotypes of different clades found in Asian domestic chickens were retrieved from the Genbank database available on the National Centre for Biotechnology Information (NCBI) with the following accession numbers (Table 3.2). These were all aligned with the query sequence obtained from the improved tropically adapted chicken breeds of the studied populations to construct a maximum likelihood tree of 1000 bootstrap replication (Tamura et al., 2015). The tree was drawn to scale, with branch lengths within the same units as those of the evolutionary distances used to infer the phylogenetic tree. The topology of the tree was analyzed using Maximum Composite Likelihood method (Kumar *et al*., 2004) and is in the units of the number of base substitutions per site.

Table 3.2 Haplotypes of reference breed obtained from genbank

S/N	Breed	Code of haplotype	Accession No. in genbank	Author	Site of collection
1	Ceylon junglefowl(CJF	*G. lafayetii1*	D66893	Fumihito *et al.*,	Sri Lanka
2	Ceylon junglefowl(CJF)	*G. lafayetii3*	AP003325	Nichibon *et al.*,	Sri Lanka
3	Green junglefowl(GJF)	*G. varius1*	D82912	Fumihito *et al.*,	Indonesia
4	Green junglefowl(GJF)	*G. varius4*	AP003324	Nishibori *et al.*,	Japan
5	Red junglefowl (RJF)	*gallus4*	KP211423	Bhattacharge *et al*	India
6	Red junglefowl(RJF)	*gallus5*	AP003322	Nishibori *et al.*,	Japan
7	Grey junglefowl(GrJF)	*G. Sonerretii2*	AP003320	Nishibori *et al.*,	Japan
8	Grey junglefowl(GrJF)	*G. Sonerretii3*	AP006746	Nishibori *et al.*,	Japan
9	Bankiva I	*G. g. bankiva*	AB009430	Miyake *et al.*,	Indonesia
10	Spadiceus I	*G. g. spadiceus*	AP003321	Guan *et al.*,	USA
11	Turkey	*Meleagris gallopavo*	EF153719	Zhang *et al.*,	China
12	Quail	*Cortunix japonica*	AP003195	Nishibori *et al.*,	Japan
13	Duck(Black duck)	*Anas platyrhynchos*	MF069250	Lin *et al.*,	China

Source:https://www.ncbi.ncbi.nlm.nih.gov/nucrore/?term=MTDNA+chicken+duck+quail+turkey - 28th May 2017

3.6.3 DnaSP 6.11.01 software

DnaSP software (Librado *et al*., 2017) was used to estimate diversity indices based on DNA polymorphism in the aligned regions including nucleotide diversity (π) and haplotype diversity (Hd). The DnaSP software (Librado *et al*., 2017) was also used to estimate the coefficient of genetic differentiation GST, and estimate of geneflow (Nm) based on haplotype data, and FST (Wright and Pickton, 1998); including demographic expansion within and among the populations of study. In other to estimate the level of genetic distance between and among the chicken types, net genetic distance (Da) in nucleotide diversity (Dxy) was computed using DnaSP software (Librado *et al*., 2017).

3.6.4 Arlequin 3.5.2.2 software

Arlequin 3.5.2.2 software (Excoffier, 2005) was used to estimate the hierarchical Analysis of Molecular Variance (AMOVA) computed to give more insight on how genetic variation is distributed between individuals within populations, between groups. Analysis of molecular variance was based on conventional F-statistics of haplotype frequencies. The calculations were performed based on 1,023 permutations. Results were obtained from two groups of iTABs; Nigerian based and Introduced, assuming the introduced iTABs were unselected from other populations sampled.

3.6.5 Network 4.6.1.6 software

Network 4.6.1.6 (Tobias *et al*., 2002-2018) was used for the median-joining of both the phylogenetic tree of the populations of Improved Tropically Adapted Chicken Breeds (iTABs) used in this study and also for estimating the extent of relationship between the present Improved Tropically Adapted Chicken Breeds (iTABs) with the D-loop sequence of other chicken obtained from Genbank while Codon code Aligner version 6.06 was used to analyze the mutation of SNPs in the aligned sequences.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Genetic diversity indices of iTABs in Imo State

Results in Table 4.1 shows values for diversity indices for the study of mtDNA of the improved tropically adapted chicken breeds (iTABs) in Imo State. Alignment of the 315bp sequence fragment produced 618 nucleotide sites. Among the populations, 62 polymorphic sites were produced from 14 haplotypes (figure 4.1). The highest haplotype had 24 individuals of the iTABs found under haplotype 3, followed by 21 individuals found under haplotype 4, and 16 individuals of iTABs found under haplotype 2. The least common haplotypes had 1 individual of the iTABs each for haplotype 1, 5, 6, 7, 9, 13 and 14. Haplotype 8, 10 and 11 had 2 individuals of the iTABs each, while only 3 individuals were found under haplotype 12. The 62 polymorphic sites were distributed from site 226 to 375 nucleotide of the D-loop region as presented in figure 4.2. The complete alignment showed that there was a higher sequence variation between nucleotide 301 and 325 with (1) 4-variant, (3) 3-variant and (17) 2-variant (base substitution). The observed haplotype diversity and nucleotide diversity among the populations was 0.80±0.025 and 0.39±0.013. Sequence conservation among the populations was low (0.22) which is equivalent to 22.3%.

Within the populations, varied polymorphic sites were obtained with the lowest value in Kuroiler (174) and the highest in Sasso (189). The number of haplotypes ranged from the lowest value (H=5) in Shika Brown and Noiler to the highest value (H=10) in FUNAAB-Alpha. Haplotype diversity was high within populations. Values ranged from 0.73±0.113 in Sasso to 0.97±0.070 in FUNAAB-Alpha. Nucleotide diversity within populations ranged from 0.38±0.070 in Sasso to 0.47±0.045 in FUNAAB-Alpha. Within the populations under

study, Sasso showed the highest sequence conservation (29.2%) whereas Shika Brown exhibits the lowest sequence conservation (20.8%).

Table 4.1 Genetic diversity indices of iTABs in Imo State

	Among populations	Within population					
Diversity indices		Noiler	FUNAAB-Alpha -	Shika Brown	Kuroiler	Sasso	Fulani
Number of gene copies	77	12	12	14	13	13	13
Number of sites/loci	618	618	618	618	618	618	618
Polymorphic sites	62	180	188	184	174	189	183
Number of haplotype	14	5	10	5	6	7	8
Hap div.(Hd) SEM	0.80± 0.025	0.82± 0.025	0.97± 0.070	0.81± 0.004	0.85± 0.065	0.73± 0.113	0.90± 0.067
Nuc div.(Pi) SEM	0.39± 0.013	0.46± 0.045	0.47± 0.045	0.43± 0.053	0.45± 0.040	0.38± 0.070	0.44± 0.045
Sequence conservation	0.22 (22.3%)	0.23 (23.3%)	0.23 (23.1%)	0.21 (20.8%)	0.23 (23.1%)	0.29 (29.2%)	0.24 (23.8%)

iTABs= Improved Tropically Adapted Chicken Breeds; SEM=Standard Error of Mean; Hap div=Haplotype diversity; Nuc div = Nucleotide diversity

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            2222222222222333333333333333333333333333333333333333333333
            2222333333334000000000111111112222444444445555566666666777777
            6789014567890123456789124567890124123456789145670134567890134 5  N
Reference   ACCCTCCATAGACAGTTCTAAACATATCAAGCCCACAGGACATACTCACTATGTTCTCCCCT
Hap_1       ............................................................  1
Hap_2       ..............C..C.......C....T.........................T...C  16
Hap_3       ...GCTA.ACC..CA.GT.CTT.CC.A....T..........CT.A.AC.......AA..A  24
Hap_4       .TT.A.TCCCC.T.C.A.C..GT.CTAACTATGAGGTTACAGGCAAAT.ATCTCATGTTTTC  21
Hap_5       ..........G......C.................................C  1
Hap_6       GTT.A.TCCCC.T.C.A.C..GT.CTAACTATGAGGTT.CAGGCAAAT.ATCTCATGTTTTC  1
Hap_7       ..............C..C..G....C....T..G.........................C  1
Hap_8       ..AGCTA.GCC..CA.GT.CTT.CC.A....T..G........C..A.AC.......AA..A  2
Hap_9       GTT.A.TCCCC.T.C.A.C..GT.CTAACTATGAGGTTACAGGCAAAT.ATCTCATGTTTTC  1
Hap_10      ................C.................................C  2
Hap_11      .TTTA.TCCCC.T.C.ATC..G..CTAACTATGAGGTTACAGGCAAAT.ATCTCATGTTT.C  2
Hap_12      ..AGCTA.ACC..CA.GT.CTT.CC.A....T..G........CT.A.AC.......AA..A  3
Hap_13      ..............C..C.......C....T.........................TT..C  1
Hap_14      C............CA.GT.CTC.C..A....T..........CT.A.AC.......AA..A  1
```

Figure 4.1: Nucleotide polymorphism observed in mtDNA D-loop region of 77 iTABs and their frequencies (N) based on haplotype data. Vertically oriented numbers indicate the site position and the sequences shown are only the variable sites. Dots (.) indicate identity with the reference sequence (genBank accession number KX987152; Source Mitochondrial *Gallus gallus* chicken), while different base letters denote substitution. The abbreviations Hap_(1-14) denote haplotype numbers.

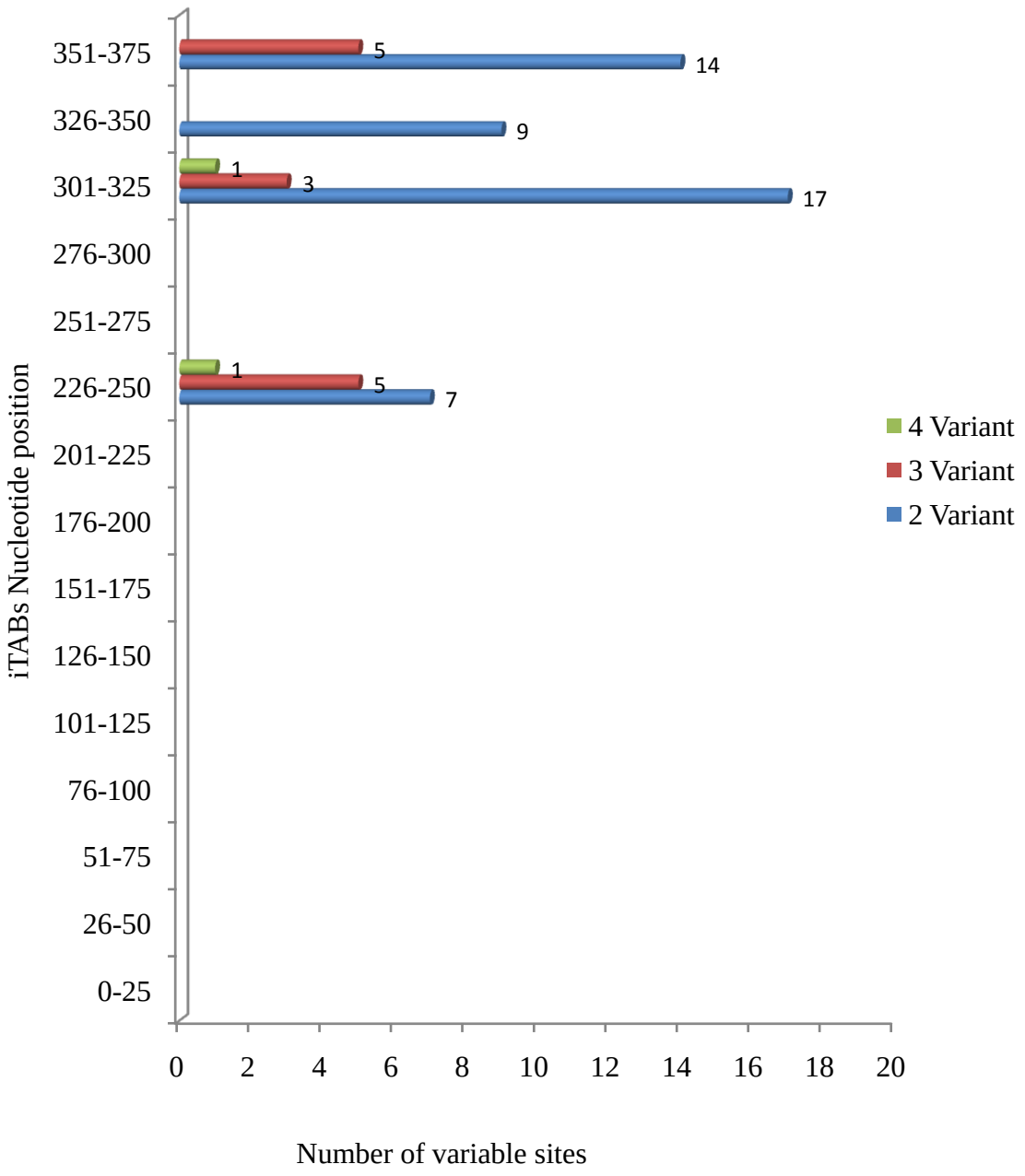

Figure 4.2. Sequence of variance distribution on D-loop segment of mtDNA of the Improved Tropically Adapted Chicken Breeds (iTABs).

4.1.2 Genetic Distance among populations

Results in Table 4.2 revealed the genetic distance estimated among populations, represented by net genetic distance (Da) in nucleotide divergence (Dxy). The observed net genetic distances (Da) ranged from the lowest value (0.385) found between Sasso and Shika Brown to the highest value (0.457) found between FUNAAB-Alpha and Sasso. Nucleotide divergence (Dxy) showed negative (-) values among all the iTABs except between Sasso and Noiler (0.035), Sasso and FUNAAB-Alpha (0.036), Sasso and Shika Brown (0.021) as well as between Sasso and Kuroiler (0.023). The highest nucleotide divergence (Dxy) 0.036 was observed between Sasso and FUNAAB-Alpha while the lowest 0.021 was between Sasso and Shika Brown

Table 4.2 Genetic distance among the six populations of iTABs in Imo State

	Noiler	FUNAAB-Alpha	Shika brown	Kuroiler	Sasso	Fulani
Noiler		-0.376	-0.005	-0.033	0.035	-0.005
FUNAAB-Alpha	0.426		-0.004	-0.032	0.036	-0.004
Shika brown	0.443	0.444		-0.012	0.021	-0.015
Kuroiler	0.425	0.426	0.430		0.023	-0.001
Sasso	0.456	0.457	0.385	0.439		-0.005
Fulani	0.447	0.449	0.422	0.446	0.405	

Below diagonal represents net genetic distance Da; above diagonal represents Nucleotide divergence (Dxy). Analysis was computed in Bootstrap model, all the values were enlarged 100 times.

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4.1.2.1 Analysis of molecular variance (AMOVA) based on F-Statistics

The mean pairwise F-Statistics estimated by AMOVA is presented in Table 4.2.1. The AMOVA analysis revealed that 97.39% of the total genetic variability occurred within individuals in the population of the iTABs, while 0.74% and 3.35% of the variations were found among groups and among populations within groups respectively. Fixation indices observed was -0.00738 among groups, 0.03321 among populations within groups and 0.02608 within populations

Table 4.2.1 Analysis of Molecular Variance (AMOVA)

SOV	Df	SS	Variance components	%Variance	Fixation Indices	P-value of fixation
Among groups	1	0.470	-0.00301Va	-0.74	-0.00738	0.522±0.015
Among populations within groups	4	2.289	0.01366 Vb	3.35	0.03321	P=0.083±0.007
Within populations	71	28.227	0.39756 Vc	97.39	0.02608	P=0.093±0.009
Total	76	30.986	0.40821			

SOV=source of variation; df= degree of freedom; SS= sum of square.

4.1.3 Phylogenetic relationship analysis

The phylogenetic relationship of the iTABs from six different populations based on the mitochondrial D-loop is presented in Figure 4.3. The phylogram divided the iTABs populations into two main clusters defined by six haplogroups which separated the samples based on the evolutionary relationship. Cluster I (ancient lineage 1) consisted of 1 haplogroup (haplogroup A) with 12 sub-lineages. Cluster 2 (ancient lineage 2) which comprises of 5 haplogroups (Haplogroup B, C, D, E, and F) was further divided into 2 main sub-clusters (sub-cluster II and III). Subcluster II is defined by 1 haplogroup (haplogroup B) with 3 sub-lineages, while Shika brown was observed to have differentiated into a separate lineage within the same haplogroup. Individuals of iTABs found in sub-cluster III are defined by 4 haplogroups (haplogroup C, D, E, and F). The first haplogroup (haplogroup C) had a separate lineage and was made up of about 11 sublineages with Fulani L2 (3) having a separate lineage from samples within this haplogroup. However, the remaining 3 haplogroups (haplogroup D, E, and F) maintained a common lineage with 4, 4 and 3 sublineages, respectively, while Sasso L3(5) and Noiler L3(5) were recently separated into different lineages each within the fourth haplogroup (haplogroup D).

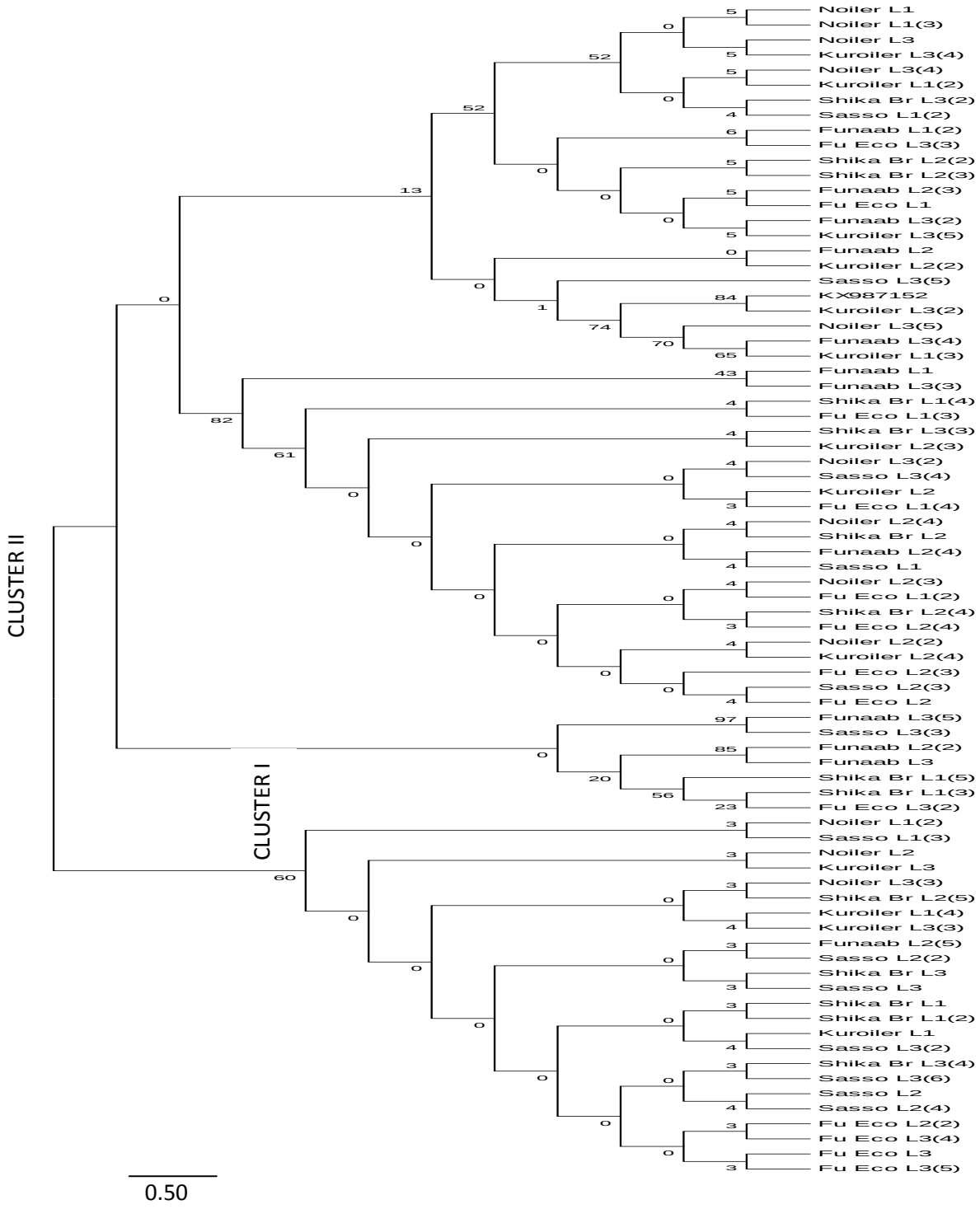

Figure 4.3. Maximum Likelihood Phylogenetic tree showing the evolutionary relationship between the six iTABs populations based on mitochondrial D-loop region. The numbers at nodes represent the percentage bootstrap values for interior branches after 1000 replications.

4.1.3.1 Network analysis of iTABs Haplotype variations

The median-joining network analysis of the 14 haplotypes is presented in (Figure 4.4). There were three major Clusters (Cluster A, B, and C). Cluster A1 centered on hap2 and comprised of all individuals in haplotypes 2, 7, 13, 10 and 5; A2 (haplotype 1) and A3 (haplotype 14). Similarly, cluster B was centered on haplotype 4 and consisted of individuals in haplotypes 4, 6, 9 and 11. Lastly, cluster C centered mainly on haplotype 3 and consisted of individuals in haplotypes 3, 8 and 12.

Noiler **FUNAAB-Alpha** **Shika brown** **Kuroiler**

Sasso **Fulani**

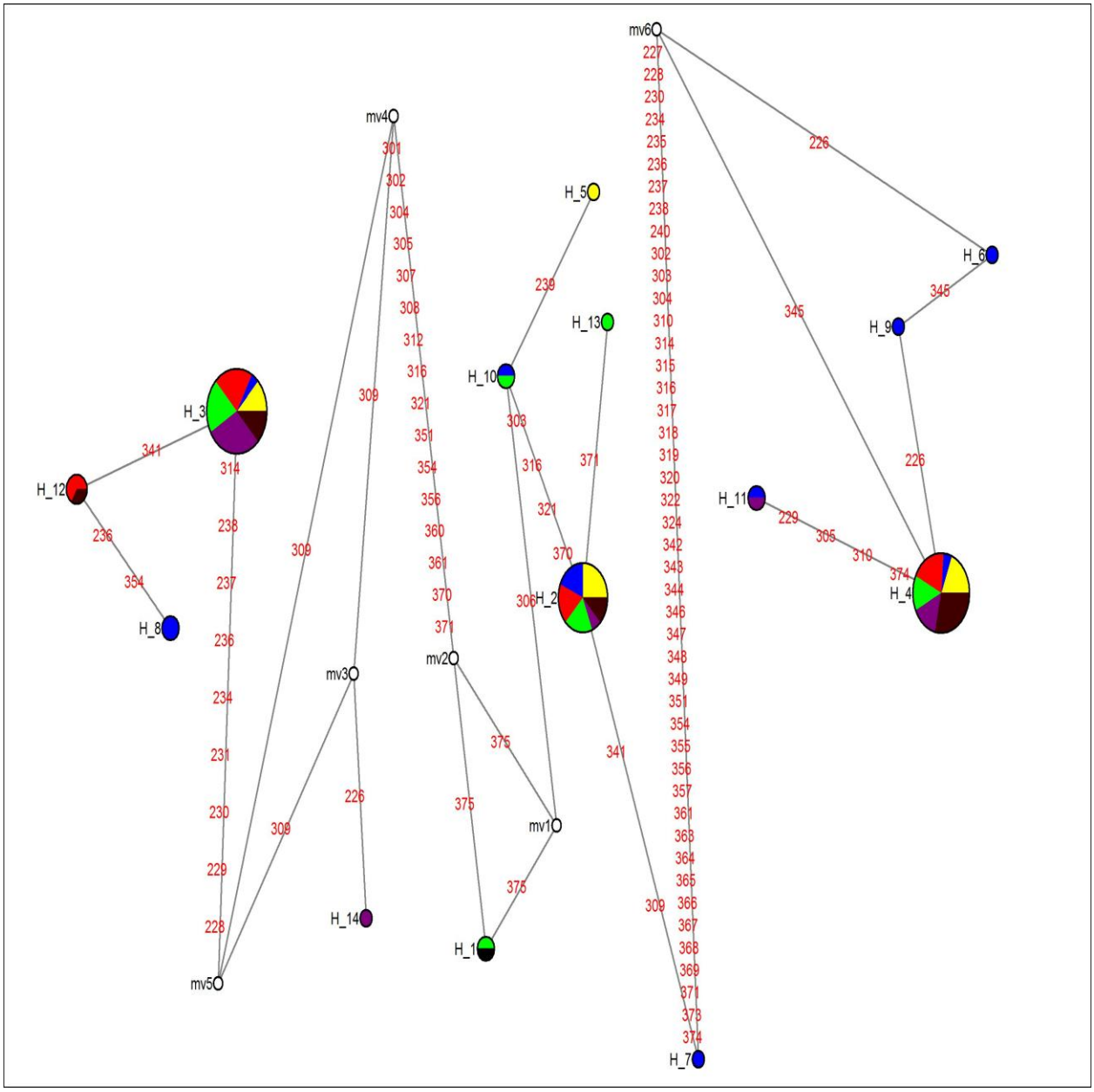

Figure 4.4 Media joining network profile of mtDNA D-loop haplotypes observed in the current study. The circle size corresponds to haplotype frequency and the numbers on the line correspond to mutational positions connecting haplotypes. Empty circles are median vectors used in connecting indirectly related haplotypes.

4.1.4 Maternal lineage/inheritance

The maximum likelihood phylogenetic tree (Fig 4.5) shows the maternal lineage of the iTABs. The phylogram split all the populations into two main clusters (clusters 1 and 2). Cluster 1 is made up of the out groups used in this study. Cluster 2 is further divided into two sub-cluster (sub-cluster 1 and 2). Subcluster 1 contained two *G. gallus*, two *G. sonneratti*, one *G. lafayet*te of the reference sequences and one outgroup *M.gallopavo*. The studied populations of iTABs clustered mainly in sub-cluster 2 with one *G.spadiceus*, two *G. varius*, one *G.lafayetti* and a *G. spadiceus* of the reference sequences. About 10 haplogroups (haplogroup A, B, C, D, E, F, G, H, I and W) were identified within sub-cluster 1 and 2. The first two haplogroups (haplogroup A and B) were found under subcluster 1 and thus defines a clade (clade I), while the remaining haplogroups fell under sub-cluster 2. The studied iTABs were distributed under haplogroup C, D, E, F, H, and I. Haplogroup C occurred at the frequency of (29.87%) had 29 individuals of iTABs found under haplotype 3, 8 and 12. Haplogroup D (at frequency of 1.30%), had one individual of iTABs and of course one breed found under haplotype 14. Haplogroup E (23.38%,) was shared by 18 individuals of iTABs found under haplotype 7, 2 and 13.

Haplogroup F (5.20%) was shared among 4 individuals of iTABs with the reference sequence found under haplotype 5, 10 and 1. Haplogroup H occurred at the frequency of (37.66%) and was shared among 23 individuals of iTABs found under haplotype 6, 9 and 4 while haplogroup I (2.60%) had just two individuals of iTABs and of course two breeds found under haplotype 11. Among the six haplogroups identified, haplogroup C, E and H comprised of mostly the iTABs used in this study; therefore grouped the iTABs into 3 clades (clade II, III and IV). Meanwhile one of the *G. varius* of the reference sequences fell under haplogroup G. *G. bankiva* and *G. Lafayette* shared haplogroup I with the individuals of iTABs found in haplotype 11 while *G.spadiceus* and the second *G. varius* were found under haplogroup W.

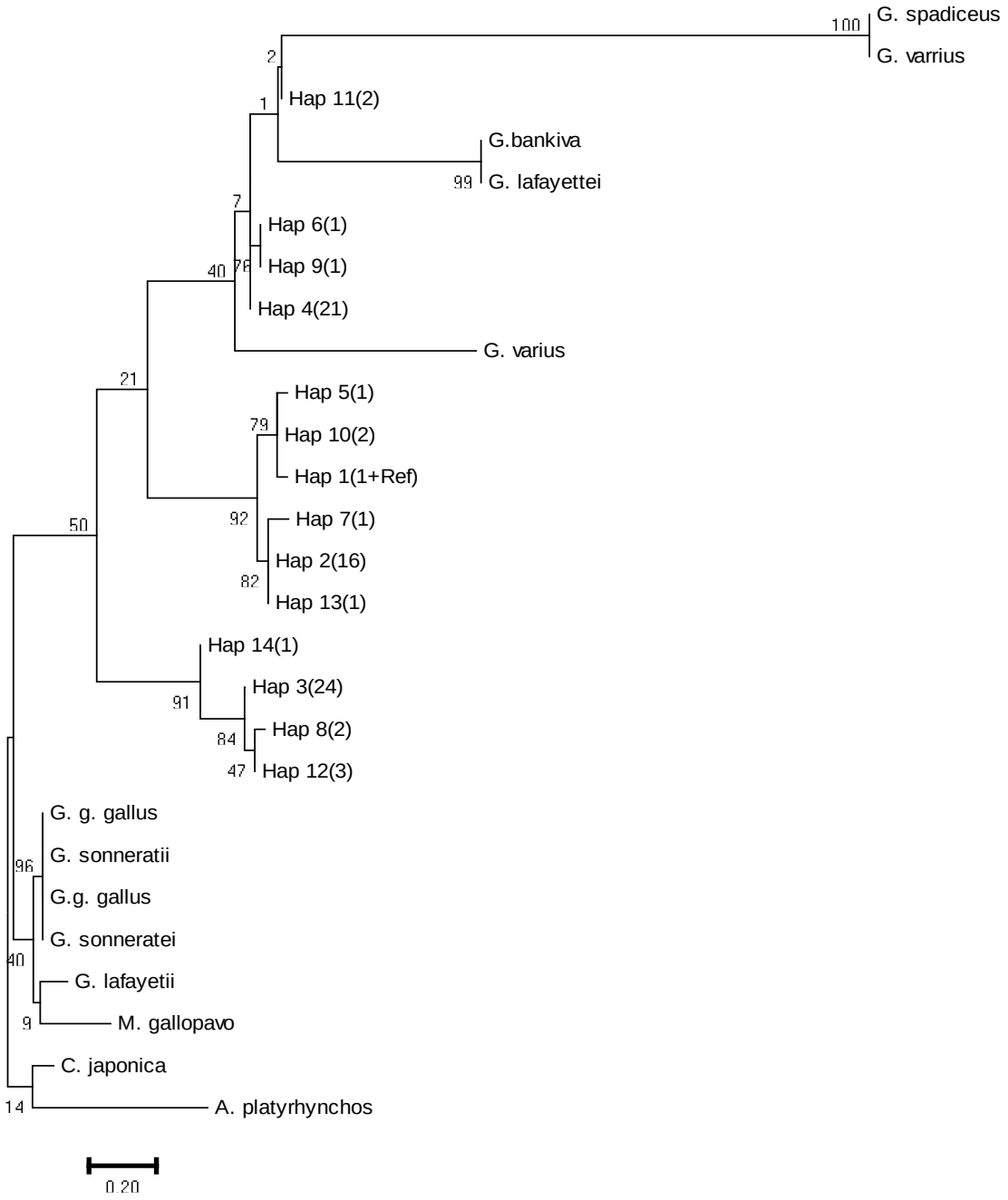

Figure 4.5: Maximum likelihood tree reconstructed using MEGA 7.0 software from 14 haplotypes identified in 77 sequences of iTABs. Three fowls from Galliformes order retrieved from Genbank; Meleagris gallopavo, Cortunix japonica and Anas platyrhynchos (GenBank accession numbers EF153719, AP003195 and MF069250, respectively), were included as out-groups and ten reference sequences from the most common haplotypes of different clades of wild and domestic chicken were also included in the tree for reference purposes. The numbers at nodes represent the percentage bootstrap values for interior branches after 1000 replications.

4.1.4.1 Network analysis of Haplotype variations between iTABs and other chicken populations

The Media Joining Network profile in figure 4.6, revealed a total of twenty (20) haplotypes. Five (5) main clades were identified from the 20 haplotypes. Clade I (connecting clade) which originated from hap_16 was surrounded by individuals from hap_ 19, 14, 17 and 20 (with a distance of 7, 7, 4 and 15 mutations) respectively. Clade II which comprised of mostly iTABs consisted of hap_8, 11, 3(with only 1 mutation distance from one another) and hap_12(with a distance of 4, 5 and 6 mutations from hap_3, 11 and 8), respectively, thus making individuals of hap_3 the central ancestor. The studied iTABs also clustered in clade III and comprises mainly of hap_2, 7, 9, 1 and 5; with individuals of hap_2 having the central lineage. Distances between the respective haplotypes in this clade ranged from 2, 3, 5 and 4 mutations, from the central haplotype (hap_2). Clade IVa consisted of individual iTABs in hap_4 and 6 with a reference sequence of gallus (*G. varius*) found in haplotype 18, while the central lineage is found in hap_4. The observed distance between hap_6 and 18 from the central haplotype (hap_4) is 1 and 16, respectively. A recent clade (clade IVb) was observed to have occurred just after about 3 mutation distances from clade IVa and was shared between individuals of iTABs in hap_10 and two populations from reference sequences (*G. bankiva and G. lafayettii*) found in hap_15. The genetic relationship between haplotypes in this clade was observed after about a distance of 16 mutations. However, the last clade observed was clade V which also comprised of two individuals of the Gallus reference sequence. *G. spadiceus and G. varius* from hap_13 were observed to have shared a distance relationship between iTABs in clade III, IVa and IVb in the ratio of (21:19:20), respectively.

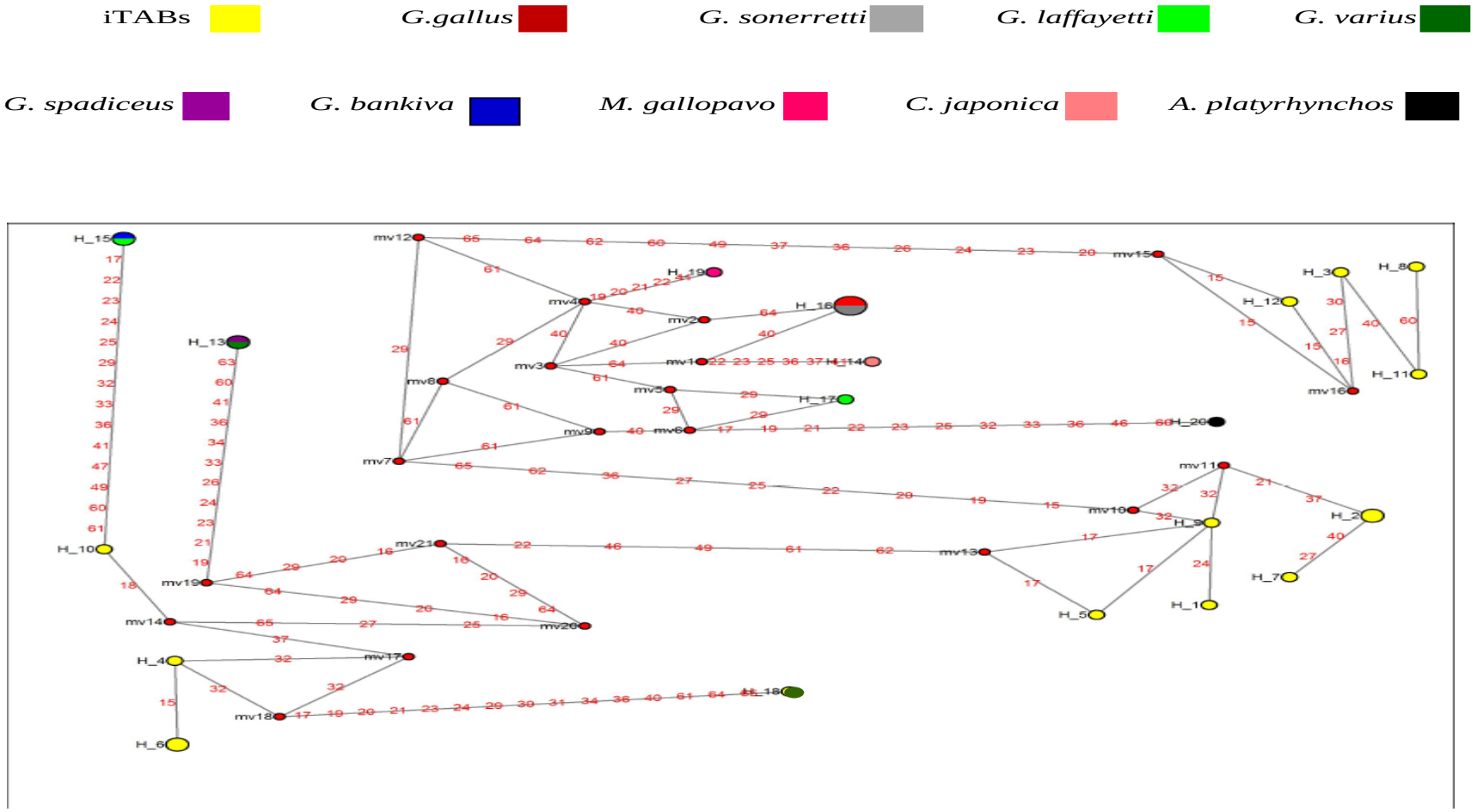

Figure 4.6 Media joining network profile of mtDNA D-loop haplotypes observed in the current study. The circle sizes correspond to haplotype frequency and the numbers on the line correspond to mutational positions connecting haplotypes. White circles are median vectors used in connecting indirectly related haplotypes.

4.1.5 Mutation Analysis of SNP

Mutation analyses of all SNPs within each population of the iTABs are presented in Table 4.3A-E. There were 157 SNPs (mutated bases) at 41 positions from a total of 23,242 bases analyzed. The 157 mutated bases occurred in the ratio of 8:2:42:4:52:49 for the population of Noiler, Fulani, FUNAAB-Alpha, Shika Brown, Kuroiler and Sasso respectively. Among the 157 SNPs observed, 26 were synonymous whereas 131 were non-synonymous. The 26 synonymous SNPs were in the ratio of 3:0:7:3:11:2 for the population of Noiler, Fulani, FUNAAB-Alpha, Shika Brown, Kuroiler and Sasso respectively. The 131 non-synonymous SNP across the populations' occurred in the ratio of 5:2:35:1:41:47 for the population of Noiler, Fulani, FUNAAB-Alpha, Shika Brown, Kuroiler and Sasso respectively. All SNPs in Noiler, Fulani, Shika brown and Sasso were associated with transition mutation. Transition mutations were prevalent in FUNAAB-Alpha and Kuroiler with only 1 and 4 transversion mutations being found in each of the populations, respectively. Multiple mutation of SNP (Transversion and Transition) was observed in Kuroiler with the predominant amino acid substitution of Tyrosine and Serine. The transversion that occurred in Funaab-ALPHA was associated with non-synonymous SNP whereas, in Kuroiler, the 4 transversions occurred in the ratio of 3 non-synonymous to 1 synonymous, and 1 simultaneous synonymous and non-synonymous for the multiple mutations of SNP observed in Kuroiler. All the Amino acids substitutions in Noiler and Fulani populations occurred just once. The predominant amino acid substitution in FUNAAB-Alpha was Proline (13 times), Leucine (3) in Shika Brown. Iso-leucine substitution, Proline substitution, and Serine substitution were predominant and occurred in equal numbers (11 times) each in Kuroiler. Phenyl substitution was highest in Sasso (13 times). In all, only two sites in Kuroiler had coding regions therefore producing no SNPs.

Table 4.3 A Mutation analysis of single nucleotide polymorphism (SNPs) in mitochondrial D-Loop within populations of Improved Tropically Adapted Chicken Breeds (iTABs) in Imo state.

No d- loop					Fi d-loop				
S/N	SNP	Amino acid change	d_S/d_N	Mutation types	S/N	SNP	Amino acid change	d_S/d_N	Mutation types
1	27A>G	Arg9Arg	d_S	Trans	1	127A>G	Thr43Ala	d_N	Trans
2	31C>T	Leu11Phe	d_N	Trans	2	322C>T	Pro108Ser	d_N	Trans
3	44C>T	Thr15Ile	d_N	Trans					
4	49T>C	Ser17Pro	d_N	Trans					
5	56A>G	Asn19Ser	d_N	Trans					
6	98T>C	Phe33Ser	d_N	Trans					
7	118C>T	Leu40Leu	d_S	Trans					
8	234T>C	Ala78Ala	d_S	Trans					

NO (Noiler); Fi (Fulani); d_N (non-synonymous); d_S (synonymous); Trans (Transition); Transv (Transversion)

Table 4.3B Mutation analysis of single nucleotide polymorphism (SNPs) in mitochondrial D-Loop within populations of Improved Tropically Adapted Chicken Breeds (iTABs) in Imo state.

FU d-loop					FU d-loop (cont.)				
S/N	SNP	Amino acid Chang	d_S/d_N	Mutation types	S/N	SNP	Amino acid change	d_S/d_N	Mutation types
1	28C>T	Leu10Phe	d_N	Trans	22	46C>T	Pro16Ser	d_N	Trans
2	28C>T	Leu10Phe	d_N	Trans	23	66A>G	Leu22Leu	d_S	Trans
3	34G>A	Ala12Thr	d_N	Trans	24	66A>G	Leu22Leu	d_S	Trans
4	34G>A	Ala12Thr	d_N	Trans	25	66A>G	Leu22Leu	d_S	Trans
5	34G>A	Ala12Thr	d_N	Trans	26	66A>G	Leu22Leu	d_S	Trans
6	34G>A	Ala12Thr	d_N	Trans	27	95T>C	Phe32Ser	d_N	Trans
7	34G>A	Ala12Thr	d_N	Trans	28	95T>C	Phe32Ser	d_N	Trans
8	34G>A	Ala12Thr	d_N	Trans	29	115C>T	Leu39Leu	d_S	Trans
9	34G>A	Ala12Thr	d_N	Trans	30	127A>G	Thr43Ala	d_N	Trans
10	34G>A	Ala12Thr	d_N	Trans	31	127A>G	Thr43Ala	d_N	Trans
11	41C>T	Thr14Ile	d_N	Trans	32	127A>G	Thr43Ala	d_N	Trans
12	41C>T	Thr14Ile	d_N	Trans	33	127A>G	Thr43Ala	d_N	Trans
13	46C>T	Pro16Ser	d_N	Trans	34	140T>C	Phe47Ser	d_N	Trans
14	46C>T	Pro16Ser	d_N	Trans	35	140T>C	Phe47Ser	d_N	Trans
15	46C>T	Pro16Ser	d_N	Trans	36	140T>C	Phe47Ser	d_N	Trans
16	46C>T	Pro16Ser	d_N	Trans	37	140T>C	Phe47Ser	d_N	Trans
17	46C>T	Pro16Ser	d_N	Trans	38	231T>C	Ala77Ala	d_S	Trans
18	46C>T	Pro16Ser	d_N	Trans	39	231T>C	Ala77Ala	d_S	Trans
19	46C>T	Pro16Ser	d_N	Trans	40	271C>A	Pro91Thr	d_N	Transv
20	46C>T	Pro16Ser	d_N	Trans	41	322C>T	Pro108Ser	d_N	Trans
21	46C>T	Pro16Ser	d_N	Trans	42	322C>T	Pro108Ser	d_N	Trans

Fu (Funaab-Alpha); d_N (non-synonymous); d_S (synonymous); Trans(Transition); Transv (Transversion)

Table 4.3C: Mutation analysis of single nucleotide polymorphism (SNPs) in mitochondrial D-Loop within populations of Improved Tropically Adapted Chicken Breeds (iTABs) in Imo state.

SB D-loop

S/N	SNP	Amino acid change	d_S/d_N	Mutation types
1	100C>T	Pro34Ser	d_N	Trans
2	129A>G	Leu43Leu	d_S	Trans
3	129A>G	Leu43Leu	d_S	Trans
4	129A>G	Leu43Leu	d_S	Trans

KU D-loop

S/N	SNP	Amino acid change	d_S/d_N	Mutation types
1	28C>T	Leu10Phe	d_N	Trans
2	28C>T	Leu10Phe	d_N	Trans
3	31C>T	Gln11STP	d_N	Trans
4	41T>C	Ile14Thr	d_N	Trans
5	41T>C	Ile14Thr	d_N	Trans
6	41T>C	Ile14Thr	d_N	Trans
7	41T>C	Ile14Thr	d_N	Trans
8	41T>C	Ile14Thr	d_N	Trans
9	41T>C	Ile14Thr	d_N	Trans
10	41T>C	Ile14Thr	d_N	Trans
11	41T>C	Ile14Thr	d_N	Trans
12	41T>C	Ile14Thr	d_N	Trans
13	41T>C	Ile14Thr	d_N	Trans
14	41T>C	Ile14Thr	d_N	Trans
15	46C>T	Pro16Ser	d_N	Trans
16	46C>T	Pro16Ser	d_N	Trans

KU D-loop(Cont.)

S/N	SNP	Amino acid change	d_S/d_N	Mutation types
17	46C>T	Pro16Ser	d_N	Trans
18	46C>T	Pro16Ser	d_N	Trans
19	46C>T	Pro16Ser	d_N	Trans
20	46C>T	Pro16Ser	d_N	Trans
21	46C>T	Pro16Ser	d_N	Trans
22	46C>T	Pro16Ser	d_N	Trans
23	46C>T	Pro16Ser	d_N	Trans
24	46C>T	Pro16Ser	d_N	Trans
25	46C>T	Pro16Ser	d_N	Trans
26	95C>T	Ser32Phe	d_N	Trans
27	95C>T	Ser32Phe	d_N	Trans
28	95C>T	Ser32Phe	d_N	Trans
29	95C>T	Ser32Phe	d_N	Trans
30	95C>T	Ser32Phe	d_N	Trans
31	95C>T	Ser32Phe	d_N	Trans
32	95C>T	Ser32Phe	d_N	Trans
33	95C>T	Ser32Phe	d_N	Trans
34	95C>T	Ser32Phe	d_N	Trans
35	95C>T	Ser32Phe	d_N	Trans
36	95C>T	Ser32Phe	d_N	Trans
37	96insT	coding region		
38	100C>T	Gln34STP	d_N	Trans
39	115C>T	Leu39Leu	d_S	Trans

SB (Shika brown); KU (Kuroiler); d_N (non-synonymous); d_S (synonymous); Trans(Transition); Transv (Transversion).

Table 4.3D Mutation analysis of single nucleotide polymorphism (SNPs) in mitochondrial D-Loop within populations of Improved Tropically Adapted Chicken Breeds (iTABs) in Imo state.

KU D-loop(cont.)					Sa D-loop				
S/N	SNP	Amino acid change	d_S/d_N	Mutation types	S/N	SNP	Amino acid change	d_S/d_N	Mutation types
40	115C>T	Leu39Leu	d_S	Trans	1	28T>C	Phe10Leu	d_N	Trans
41	115C>T	Leu39Leu	d_S	Trans	2	28T>C	Phe10Leu	d_N	Trans
42	115C>T	Leu39Leu	d_S	Trans	3	28T>C	Phe10Leu	d_N	Trans
43	115C>T	Leu39Leu	d_S	Trans	4	28T>C	Phe10Leu	d_N	Trans
44	115C>T	Leu39Leu	d_S	Trans	5	28T>C	Phe10Leu	d_N	Trans
45	115C>T	Leu39Leu	d_S	Trans	6	28T>C	Phe10Leu	d_N	Trans
46	115C>T	Leu39Leu	d_S	Trans	7	28T>C	Phe10Leu	d_N	Trans
47	213G>T	Glu71Asp	d_N	Transv	8	28T>C	Phe10Leu	d_N	Trans
48	231T>C	Ala77Ala	d_S	Trans	9	28T>C	Phe10Leu	d_N	Trans
49	231T>C	Ala77Ala	d_S	Trans	10	28T>C	Phe10Leu	d_N	Trans
50	258G>T	Gln86His	d_N	Transv	11	28T>C	Phe10Leu	d_N	Trans
51	321G>T	Arg107Arg	d_S	Transv	12	31T>C	STP11Gln	d_N	Trans
52	323delC	coding region			13	31T>C	STP11Gln	d_N	Trans
53	332A>C	Tyr111Ser	d_N	Transv	14	31T>C	STP11Gln	d_N	Trans
54	333T>C&A	Tyr111Ser&Ser	d_N/d_S	Trans&Transv	15	31T>C	STP11Gln	d_N	Trans
					16	31T>C	STP11Gln	d_N	Trans

KU (Kuroiler); Sa (Sasso); d_N (non-synonymous); d_S (synonymous); Trans(Transition); Transv (Transversion).

Table 4.3E Mutation analysis of single nucleotide polymorphism (SNPs) in mitochondrial D-Loop within populations of Improved Tropically Adapted Chicken Breeds (iTABs) in Imo state.

Sa D-loop(cont.)				
S/N	SNP	Amino acid change	d_S/d_N	Mutation types
17	31T>C	STP11Gln	d_N	Trans
18	31T>C	STP11Gln	d_N	Trans
19	31T>C	STP11Gln	d_N	Trans
20	31T>C	STP11Gln	d_N	Trans
21	31T>C	STP11Gln	d_N	Trans
22	31T>C	STP11Gln	d_N	Trans
23	41T>C	Ile14Thr	d_N	Trans
24	41T>C	Ile14Thr	d_N	Trans
25	41T>C	Ile14Thr	d_N	Trans
26	41T>C	Ile14Thr	d_N	Trans
27	41T>C	Ile14Thr	d_N	Trans
28	41T>C	Ile14Thr	d_N	Trans
29	41T>C	Ile14Thr	d_N	Trans
30	41T>C	Ile14Thr	d_N	Trans
31	41T>C	Ile14Thr	d_N	Trans
32	41T>C	Ile14Thr	d_N	Trans
33	41T>C	Ile14Thr	d_N	Trans

Sa D-loop(cont.)				
S/N	SNP	Amino acid change	d_S/d_N	Mutation types
34	46C>T	Pro16Ser	d_N	Trans
35	46C>T	Pro16Ser	d_N	Trans
36	46C>T	Pro16Ser	d_N	Trans
37	46C>T	Pro16Ser	d_N	Trans
38	46C>T	Pro16Ser	d_N	Trans
39	46C>T	Pro16Ser	d_N	Trans
40	46C>T	Pro16Ser	d_N	Trans
41	46C>T	Pro16Ser	d_N	Trans
42	46C>T	Pro16Ser	d_N	Trans
43	46C>T	Pro16Ser	d_N	Trans
44	46C>T	Pro16Ser	d_N	Trans
45	95T>C	Phe32Ser	d_N	Trans
46	95T>C	Phe32Ser	d_N	Trans
47	100C>T	Gln34STP	d_N	Trans
48	231T>C	Ala77Ala	d_S	Trans
49	231T>C	Ala77Ala	d_S	Trans

Sa (Sasso); d_N (non-synonymous); d_S (synonymous); Trans (Transition); Transv (Transversion).

4.1.6 Estimate of gene flow within and between iTABs population

Results of the estimate gene flow and coefficient of genetic differentiation (Table 4.4), revealed distinct gene exchange (GST = 0.018, Nm = 14.01; GST= 0.057, Nm = 4.12; GST=0.02, Nm=137.04; GST=0.002, Nm=165.10; between Sasso and Noiler; Sasso and FUNAAB-Alpha; Sasso and Shika Brown as well as between Sasso and Fulani respectively. The highest gene flow (165) was observed between Sasso and Fulani while the lowest gene flow (4.12) was observed between Sasso and FUNAAB-Alpha. Positive values for coefficient of differentiation were observed between some iTABs and ranged from 0.002 - 0.057, between Sasso and Fulani, and Sasso and FUNAAB-Alpha respectively. However, moderate geneflow was found within the Nigeria-sourced populations (GST=0.003, Nm=96.14; Gst=0.019, Nm= 12.59) FUNAAB-Alpha - Fulani and FUNAAB-Alpha – Shika Brown, respectively. The coefficient of differentiation was highest (0.019) between FUNAAB-Alpha and Shika Brown and lowest between FUNAAB-Alpha and Fulani. There was no obvious genetic exchange or communication between the two foreign sourced iTABs as well as between Kuroiler and the Nigeria sourced-iTABs.

Table 4. 4 Estimate of gene flow (Nm) and coefficient of differentiation (GST) between iTABs populations

	Noiler	FUNAAB-Alpha	Shika Brown	Kuroiler	Sasso	Fulani
Noiler		-116.00	-12.95	-10.13	14.01	-33.20
FUNAAB-Alpha	-0.002		12.59	-273.08	4.12	96.14
Shika Brown	-0.020	0.019		-10.59	137.04	-11.70
Kuroiler	-0.025	-0.001	-0.024		-65.00	-20.87
Sasso	0.018	0.057	0.020	-0.004		165.10
Fulani	-0.008	0.003	-0.022	-0.012	0.002	

Note: Lower diagonal represents Coefficient of differentiation (Gst) and upper diagonal represents gene flow (Nm); $p<0.05$ meant significant difference between the six iTABs populations. For all GSTs less than or equal to zero (0), the estimator was considered undefined and not studied in the Nm analysis (noted as n/m).

4.1.7 Genetic population structure of iTABs using F-statistics

Results in Table 4.5, shows the genetic population structure between the iTABs using Fst values. Fst values revealed moderate genetic differentiation between Sasso and Kuroiler (0.097); high genetic differentiation between Shika Brown and FUNAAB-Alpha (0.113), Kuroiler and FUNAAB-Alpha (0.171) as well as between Sasso and Shika Brown (0.107). Similarly, Sasso and Noiler (0.321), Sasso and FUNAAB-Alpha (0.517) as well as Sasso and Fulani (0.207) were highly differentiated. Significant values were found only between Sasso and FUNAAB-Alpha (**0.517**).

Table 4.5 Genetic population structure of iTABs using F-statistics

	Noiler	Funaab-ALPHA	Shika Brown	Kuroiler	Sasso	Fulani
Noiler						
Funaab-ALPHA	-0.259					
Shika brown	-0.004	0.113				
Kuroiler	-0.069	0.171	-0.107			
Sasso	0.321	**0.517**	0.107	0.097		
Fulani ecotype	-0.006	-0.202	-0.055	-0.100	0.207	

F_{ST} values (below diagonal) represents F_{STs} generated by Arlequin.

4.1.8 Demographic expansion

The results of the demographic expansion in all the populations were presented in Table 4.6. Within the populations, Tajima'D shows positive non-significant values and ranges from the least 0.140 in Noiler to the highest 1.176 in Kuroiler. On the other hand, Fu's F revealed positive value within populations and non-significant in FUNAAB-Alpha 1.454 (0.20>0.10>0,05) and Sasso 0.019. Significant Fu's F was observed within Noiler (1.663) and Fulani (1.710), and highly significant within Shika Brown (1.762) and Kuroiler (1.770). Among the population of study, Tajima'D revealed a positive and highly significant value 2.708. Fu's F value among populations was also positive and highly significant 2.620.

Table 4.6 Demographic expansion indices for within and among the six populations of iTABs in Imo State

	Within Population						Among Population
Parameters	Noiler	Funaab-ALPHA	Shika Brown	Kuroiler	Sasso	Fulani	
Tajima' D	0.140 (P > 0.01)	0.913 (P>0.10)	1.102 (P>0.10)	1.176 (P>0.10)	0.198 (P>0.10)	1.088 (P>0.10)	2.708 (**P<0.01)
Fu's F	1.663 (*P<0.05)	1.454 (0.10>P>0.05)	1.762 (**P<0.02)	1.770 (**P<0.02)	0.019 (P>0.10)	1.710 (*P<0.05)	2.620 (**P<0.02)

4.2 Discussion

4.2.1 Diversity analysis of mtDNA in iTABs population in Imo State Nigeria.

Genetic Diversity within and between species in a given population is 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. The mtDNA variation among the iTABs populations revealed low mean differences at the nucleotide site, with a high mutational process at the genome level traced back to ancestral lineage (Stumpf, 2004). This gives an indication of a comparatively young species that had not much time to diverge from one another. The haplotype diversity value was found higher than those of the African chicken populations in: 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 similar to the African chicken in: Kenya 0.857 (Nwacharo *et al.,* 2011) and Zimbabwe0.730 (Muchadeyi *et al.,* 2008).

Genetic variation indices within population in this study generally had high values, higher variation observed more within the Nigeria-bred iTABs populations and Kuroiler. Giving the impression of a good potential for evolutionary change and adaptation. The exhibition of much higher variation (high mutational process in the genome) in FUNAAB-Alpha-iTABs compared with others could be attributed to extensive research for improvement on it for more than 20years (Saleh *et al*., 2017).This could also suggest that the observed allelic diversity could be traced to ancestral lineage (Xu *et al*., 2008). The values of haplotype diversity obtained in this study within populations were similar to that reported (Cuc *et al*., 2011) for Asian fowls.

These findings therefore, indicate that the level of mtDNA polymorphism within and among the populations of iTABs raised in Nigeria is very high compared with most African chicken populations but similar to a few African chicken populations and Asian chicken population, thus revealing a higher genetic diversity within the iTABs populations than among the 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. The high range of haplotype diversity observed within populations suggest existence of high moleculer differences within the iTABs thus suggesting that the present iTABs are likely to have a high adaptation to environmental changes under natural selection, an attribute of high genetic diversity within populations.

4.2.2 Genetic distance

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 Sasso and FUNAAB-Alpha and a low genetic distance between Sasso and ShikaBrown. The values of genetic distance were lower than the values reported for Indonesian Indigenous Chickens (Sulandari *et al*., 2008). The negative nucleotide divergence (Dxy) between all the breeds, except Sasso gives an indication of zero genetic distance. This means that these breeds are not genetically different as they must have shared the same allele within populations and could have had common ancestors.

On the contrary, Nucleotide divergence shows a positive value between Sasso and other iTABs with the highest divergent value found in FUNAAB-Alpha and the lowest value in Shika Brown. This gives the impression that the Improved Tropically Adapted Chicken Breeds (iTABs) must have diverged from their common ancestor. This could mean that Sasso and other iTABs have a shared ancestral gene pool with the most recent between Sasso and Shika Brown. This also suggested that Sasso is less genetically related to FUNAAB-Alpha and more genetically related to Shika Brown.

This finding is further confirmed by the outcome of the Analysis of molecular variance (AMOVA) obtained, which showed a negative and thus zero genetic structure among groups. The AMOVA 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). Genetic variation within the iTABs populations is higher than that reported for within Indonesian Indigenous Chickens population (Sulandari *et al*., 2008). Therefore our result suggested a higher maternal genetic variation within iTABs populations. On the contrary, the negative and therefore, zero percentage variation observed among groups were lower than the values reported among Indonesian Indigenous Chickens population, therefore indicates a lack of population substructuring among populations, meaning there were no mtDNA variation among the populations of iTABs. This result is in line with the report of Pariset *et al*., (2011). Therefore, suggesting that there is a high mtDNA variation within the population of iTABs.

4.2.3 Phylogenetic relationship of the iTABs in Imo State

The two main clusters identified among the populations represent two ancient lineages from which the iTABs are derived from. Our finding agrres with Ohno, (1997) that a set of full or maternal half-sisters should have inherited similar mitochondrial genome from their mothers, but each sister's female descendants invariably establish an independent lineage which in time would accumulate its own characteristic mutations to become a distinct sublineage. Owing to the effect of some evolutionary forces such as mutation, individuals of iTABs found in cluster II were said to have shared ancient lineages. However, due to some recent mutational events, they split into two sub-lineages thereby representing two distinct recent lineages from which some of the iTABs were derived. This, therefore, suggested that the present iTABs likely shared three (3) common lineages originating from two ancient ancestors. Lineage I is the farthest lineage and populations clustered here are said to have maintained their ancient relationship (common ancestor). This result is in line with (Revay *et al.* 2010), which therefore implies that over the years, their populations probably could not have been affected by any evolutionary forces. It could also be attributed to the recurrent crosses with a Rhode Island Breed during their developmental processes as described in the literature review section of this study. The six haplogroups that defined the three lineages of iTABs indicates the presence of six 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 them.

The closeness of the clustering implied that mating might have occurred between lineages, thus agreeing with (Gongora *et al.* 2008). Therefore, we conclude that the three major clusters observed on the iTABs which centered mainly on haplotype 2, 3 and 4 justifies the fact that the present iTABs could be derived from three common evolutionary lineages.

But the big question is; how could some breeds with different features share a common lineage? The clearification of this doubt is well documented in (Zhu, 1958; Cheng *et al.,* 2000 and Mason, 1987)

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

The six haplogroups (C, D, E, F, H and I) identified in this study were well represented among the 13-haplogroup setting (A-I and W-Z) according to suggestion based on a whole mitochondrial genome study (Miao *et al.,* 2013). The three most frequently occurring haplogroups (C, E, and H) justified by the haplotype distribution and network profile suggested the existence of three (3) major clades (clade II, III and IV) of the studied populations. The six haplogroups setting on the iTABs indicates the existence of a contribution of multiple maternal lineages in all breeds.

This agrees with the report of Silver, *et al*., (2009), therefore suggesting a closer history of maternal origin between the present iTABs and SriLankan indigenous chicken. Five (5) haplogroups were found representing more than one breed which could suggest that they were derived from common maternal ancestors. iTABs in clade IVa and IVb are well represented by (*G. varius*) and (*G. laffayetti and G. bankiva*) respectively, thus enforcing the role of *G. varius, G.laffayetii and G.bankiva* as part of the ancestor of domestic chicken including iTABs used in this study. Similarly, 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 is the main ancestor and *G. lafayetti, G. sonneratti* and *G. varius* are the secondary.

In contrast to this, Darwin (1868) suggested that the red jungle fowl is the only ancestor of all domestic chicken. The maternal lineage sharing has been reported among different indigenous chicken breeds of various geographical locations (Liu *at al.,* 2004; Nishibori *et al.,* 2005 and Fumihito *et al.,* 1996). Identical sequences expressed as the same haplotype of some individuals of different breeds/varieties presented in this study are in line with other reports (Liu et al., 2006). Domestic chickens are suggested to be in close genetic relationships in spite of distinctive phenotypes and physiological/reproductive performance.

The three clades identified in this study suggest that the present iTABs were likely to be dominated by 3 maternal lineages defined in haplogroup C, E, and H. Haplogroup C has been widely distributed in East, Southeast and South Asia. Haplogroup E is mainly distributed in Eurasian and South Asian domestic chickens (Liu *et al*., 2006 and Miao *et al*., 2013). The existence of iTABs in haplogroup E suggested a closer maternal lineage between iTABs and South African Chicken of (Mtileni, *et al*., 2011).

However, the maternal lineage associated with haplogroup E could have originated from the Indian subcontinent and possibly spread to South Asia (Liu *et al*., 2006). Similarly, haplogroup H has its origin in Southwest China and Japan (Miao *et al*., 2013). The haplogroup H is a rare clade with only a few members of gamecocks cited, including Chinese Chigulu and Japanese Shamo breeds (Oka *et al*., 2007 and Miao *et al*., 2013) to which iTABs are added according to this study. Nevertheless, haplogroup H is not documented as being in any of the junglefowl. This leaves the ancestor and domestication origin of this clade inconclusive, thereby requiring further investigation. Only 1.30% of the iTABs were found in haplogroup D indicating very little or no considerable contribution of this haplogroup to iTABs.

Meanwhile, haplogroup D has been composed of RJF and gamecocks from Republic of Indonesia, India, Japan and varied native chicken from Africa, South Asian geographic region and East Asia of which the origin has been presented to be in southwest China and /or surrounding regions of Vietnam, Myanmar, Thailand and India (Liu *et al*., 2006; Miao *et al*., 2013). About 5.20% of the iTABs belong to haplogroup F thus indicating only a 5% contribution of this haplogroup to iTABs. However, Oka *et al*. (2007) suggested that this haplogroup which was referred to as haplogroup F is isolated to only Okinawa Shamo and thus has no relationship with other Japanese domestic chickens. Both Southeast Asia and China have been suggested as centers for chicken domestication (Fumihito et al., 1996; Oka *et al*., 2007). Haplogroup I was well represented by only 2.60% of iTABs. Therefore, the haplotypes in this group have major distribution in Vietnam and have no contribution in the maternal origin of the iTABs. The existence of the iTABs into clade II, III and IV were well represented in the seven clades identified in Asian domestic chicken (Bjornstad *et al*., 2013).

Mobegi *et al*., (2006) observed that the majority of haplotypes in West African village chicken populations cluster in clade IV, likewise Bjonstad, *et al*., (2009) observed that southwestern Nigerian indigenous chicken and Anak titan chicken clustered in clade 4, while Muchadeyi *et al*., (2008) observed two distinct clades amongst both Zimbabwe and Malagasy chickens, also Adebambo *et al*., (2010) observed a single clade among Nigerian chicken populations, indicating a closer history of domestication between West African village chicken, Southwestern Nigerian indigenous chicken and iTABs; and different between Nigerian chicken and iTABs, thus suggesting the absence of admixture.

This could be due to a lack of intensive genetic intermixing between iTABs and the Nigerian chicken populations. The network revealed a shared ancient lineage between iTABs and the

individuals of Gallus species and a Galliforme found in clade I; the lineage being closer to iTABs haplotype in clade II. The network profile also shows that a recent common ancestor was observed between G. varius from Indonesia and iTABs (haplogroup H) found in clade IVa after about 15 mutation distance. Individuals in hap11 (Sasso and Funaab-ALPHA) share the same haplogroup with G. bankiva and G. lafayetii from Indonesia and Srilanka, respectively; and were found in clade IVb indicating a direct ancestral lineage. Clade IVa and IVb resemble haplotypes from clade D of (Liu *et al*., 2006 and Fumihito *et al*., 1992), which is common in junglefowl and gamecocks from Indonesia, India, and Japan. Therefore this distinct distribution patterns of iTABs (No, FU, SB, Ku, Sa, and Fi) suggests that divergent clades are likely to be descended from three common ancestors, the G. bankiva, G. laffayetti, and G. varius possibly of Japan, China, and the Indian subcontinent, which supports the theory of multiple origins in South and Southeast Asia.

4.2.5 Mutation analysis of Single Nucleotide Polymorphism (SNPs)

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). Mutational effect may be highly associated with its frequency, in that sites where mutations happen frequently will be preserved if the effects of these mutations are severe or will otherwise be allowed to mutate if there are no consequences for the organism (Steffen, *et al.*, 2008). The number of SNPs obtained across the six populations in this study are higher than the values previously reported on the studies of a whole mitochondrial DNA sequence and haplotype variation analysis in chicken (*Gallus gallus*) (Guan *et al*., 2012).

This could be attributed to the sequencing method employed in this study or it could be as a result of higher sample size used in this study. The high abundance of transition mutation

observed in this study is in line with the report of (Vignal *et al*., 2002; Fitch, 1967). The high abundance of non-synonymous transition mutations observed in Noiler, Fulani, Funaab-ALPHA, Kuroiler, and Sasso is a good indication of high variation along the genome of these chicken populations. It also indicates that the gene presently found in these breeds could have a greater fitness for natural selection; hence they are not under the influence of selective pressure. This is because transition is more conservative in nature hence they would more often create synonymous substitution, which encodes the same amino acid sequences as the original DNA (Fitch, 1967).

However, the low percentage of non-synonymous transversion observed in Funaab-ALPHA and Kuroiler indicates the existence of mutation that can alter the protein function along their genome, though in a very minute amount. This might probably lead to a reduction in the frequency of mutations in the gene pool of these breeds under unbalanced selection.

The high abundance of synonymous transitional mutation in Shika brown suggests that the genes of this population are more likely to be under strong selective pressure triggered by low frequent mutational event (Steffen, *et al.,* 2008). This implies that Shika Brown could be lacking a high percentage of adaptively driven substitution. Results obtained in this study are in line with the report of (Subramanian and Kumar, 2003; 2006). Therefore, we conclude that the present iTABs (Noiler, Fulani, Funaab-ALPHA, Kuroiler, and Sasso) could have evolved under purifying selection while Shika Brown could have evolved neutrally or under weak selection.

4.2.6 Estimate of gene flow within and between the iTABs

Gene flow is a fundamental micro evolutionary force that can determine the potential for genetic differentiation among populations and for local adaptation (Keyghobadi *et al*., 2005). The estimate of gene flow and coefficient of differentiation between populations partially agrees with previous findings in this study that all the iTABs populations except Kuroiler must have undergone genetic differentiation at places of equal horizon (horizontal gene transfer). This may reflect the introduction of breeds from different regions or possibly due to migration. The moderate gene exchange between the Sasso breed developed in France and the iTABs developed in Nigeria suggests closer chicken domestication between Nigeria and France. This could suggest that these populations must have undergone admixture with possible gene flow among them. This result is in line with the finding of (Liu *et al*., 2010). This, therefore, implies that there is a tendency of decreased speciation as gene flow tends to decrease variance across populations. This could also means that, Sasso breeds must have shared a common ancestor with the Nigerian iTABs.

This finding is in line with the result previously obtained for the Nucleotide divergence and net genetic distance estimates where a close genetic relationship was observed between Sasso and Nigerian iTABs (Shika Brown and FUNAAB-Alpha). The gene flow observed within the Nigeria populations' iTABs suggests a tendency of increased speciation between FUNAAB-Alpha and Fulani as well as, between FUNAAB-Alpha and Shika Brown. This could be attributed to the fact that gene flow tends to increase variance within populations. On the other hand, the absence of gene flow observed between Kuroiler and other iTABs suggests minimal effect of migration and genetic drift thus signifies the existence of reproductive isolation between Kuroiler and other iTABs.

We could therefore, infer that crossing of Sasso and Nigerian-sourced iTABs is capable of homogenizing the gene pool due to high rate of gene flow observed between them whereas, crossing of Nigerian-sourced and Kuroiler would exhibit a less tendency for homogenizing the gene pool.

4.2.7 Genetic population structure of iTABs using F-statistics

F-statistics usually view a subpopulation as having undergone similar process of evolution as at the time of divergence (Wright, 1951). Meanwhile, the effective population size and population history remains the major determinants of F statistics, (Weir and Cockerham, 1984). Practically, an FST value of 0:00 - 0:05 could mean low differentiation; 0:05 - 0:15, moderate differentiation; while FST > 0:15 implies high levels of differentiation (Hartl and Clark, 1997).

The positive and moderate to high Fst- values observed between Shika Brown and FUNAAB-Alpha, Sasso and Shika Brown, Noiler, FUNAAB-Alpha and Fulani respectively could be a good indication that these populations must have undergone differentiation probably at places of equal horizon (horizontal gene transfer).

This could also indicate the presence of population genetic structuring between these iTABs. This finding is similar to the findings of (Men Q, *et al*., 2004) who observed a strong genetic differentiation in a population of Dendrolimus kikuchii Matsumura. However, the population structuring was only significant between Sasso and FUNAAB-Alpha. The statistically significant value observed between Sasso and FUNAAB-Alpha is in line with the earlier observation that FUNAAB-Alpha and Sasso are more distantly related compared with other iTABs. This therefore suggests the presence of significant genetic subdivision between Sasso and FUNAAB-Alpha.

It also implied that Sasso 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. Nevertheless, the positive and moderate non-significant Fst- values observed between Sasso and Kuroiler, FUNAAB-Alpha and Kuroiler could indicate the presence of non-significant genetic population structure between these breeds. The obvious but non-statistically significant genetic population structure observed between the breeds could be attributed to the hypervariable nature of the mtDNA marker used or the small sample size of the studied population. The result is in consonance with the result obtained from the AMOVA in this study, where there was no evidence of population structuring due to non-significant genetic differentiation between regions (among groups). This, therefore, implies that there is no mtDNA variation between the populations except between Sasso and FUNAAB-Alpha. The statistically significant ($p<0.05$) genetic population structure between Sasso and FUNAAB-Alpha is in itself not sufficient for safe conclusions regarding population sub structuring. Though the statistical power could be high due to the highly polymorphic marker (mtDNA), but small sample size was used in this study. This is in contrast with the findings of (Peters, *et al*., 2008) that used a highly polymorphic marker (mtDNA) and large sample size.

Waples, (1998) reported that some minor allelic frequency differences that are unrelated to population structure and thus not biologically meaningful in the context can achieve statistical significance. Therefore, random sampling effect, as well as an artificial selection of these populations, could be the possible cause of non-statistically significant but highly differentiated or highly structured population observed among some of the differentiated iTABs.

4.2.8 Test of departure from neutrality model (demographic expansion)

Demographic information is key steps in strategizing breeding plan for Farm Animal Genetic Resources (FAnGR) management. (FAO, 1992; Gandini *et al*., 2004). The D test was based on the difference between the number of segregating sites and the average number of nucleotide differences whereas the Fu's F test was based on the haplotypes (gene) frequency distribution conditional to the value of θ (Ewes, 1922). A negative value of D signifies an excess of low-frequency polymorphism indicating population expansion (Tajima, 1989) while a positive value signifies low and high polymorphism evidenced when population size decreases. Similarly, a negative Fu's F statistics value signifies the presence of genetic drift in a population (Fu, 1997). The positive and non-significant value for Tajima's D observed within populations indicates that within these populations, there is a shred of evidence for high polymorphism under decreasing or low population size yet 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. Contrary to this, the positive highly 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 highly significant.

This could be attributed to the high occurrence of new mutations in the genetic makeup of these populations.

The positive non-significant Fu's F values dictated within the populations of Funaab-ALPHA and Sasso shows that within these breeds, even though there is a high frequency of haplotypes or gene variations, there is no evidence of genetic drift. These breeds must have experienced a rapid population expansion in the past, therefore the finding contrasts with (Gu, *et al*., 2016), hence these populations must have been constant at the time of this experiment.

The positive significant Fu's F observed in Noiler and Fulani, suggest that even though there might be low or moderate distribution of genes or haplotypes within these breeds, they still have high chance of acquiring new mutations due to the significant evidence of departure from equilibruim thereby suggesting the tendency for population growth within these breeds.

On the other hand, highly positive significant values for Fu's F test in Shika Brown and Kuroiler indicate the existence of moderate to low haplotype distribution within these breeds. This implies that even under the influence of genetic drift, they could exhibit significant evidence for departure from neutrality model. This could be as a result of an excess of new mutations as a result of evolutionary forces such as genetic sweeps or population growth. This result agrees with (Adebambo *et al*. 2010) suggesting a good indication that Shika Brown and Kuroiler breeds could have more advantage over other breeds for population growth. Similarly, there is significant evidence for population expansion among the six populations of improved tropically adapted chicken (iTABs) as indicated by the highly positive significant value of Fu's F test.

This result agrees with (Eltanany, 2016) and could be as a result of the high number of haplotype shared/distribution among these populations. Therefore, we conclude that under the normal assumption of neutral and constant population size with no panmixia and no recombination, there would be a significant decrease in the population size of the Improved Tropically Adapted Chicken Breeds (iTABs) in Imo state. However, deviation from these assumptions would encourage the accumulation of new mutations evidenced for population expansion or growth.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

Conclusion

The important results from this work can be summarized as follows;

1. The high haplotype diversity (73% – 97%) clearly displayed within the iTABs population gives the indication of a high level of mtDNA polymorphism. This was further expressed by the mutation of 157 base pairs (157SNPs) at 41 positions from a total of 23,242 base pairs analysed. Higher mutated base pairs were found within FUNAAB-Alpha, Sasso and Kuroiler in the following order; 42<49<52. However, the population of Funaab-ALPHA had richer gene pool compared to the other iTABs used in this study.All the above 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.
2. The high rate of non-synonymous transition mutation observed in this study for the population of (Noiler, Fulani, FUNAAB-Alpha, Kuroiler, and Sasso), gives an implication of a conservation substitution in which case the alteration is less likely to affect the protein structure and function severely.
3. Among the six populations of iTABs, Sasso and Shika Brown were more closely related giving the impression of similar source of origin and hence less genetically differentiated.
4. The present iTABs could have originated from three maternal lineages. They belong to three clades; clade II, III, and IV defined by haplogroup C, E and H thus suggesting multiple maternal origins possibly of Japan, China, and Indian subcontinents.

5. With the increasing rates of gene flow between Sasso and Nigerian iTABs, inbreeding could expose these breeds to a high risk of losing the original germplasm due to bottleneck.
6. Artificial selection of the present iTABs, could be the possible cause of high genetic differentiation observed among the "differentiated" populations.

Recommendations

This study therefore recommends as follows;

1. 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, and evaluate the rate of admixture within the 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.
2. The non-synonymous transition (d_NTs) fitness observed in the present iTABs suggest an evolutionarily informed approach to improving health and adaptive strategies. Therefore, gradual and continues improvement of phenotypes in Funaab-Alpha and Kuroiler is highly recommended to maintain the accumulation of favorable mutations that would maintain genetic diversity for future environmental changes.
3. To save these breeds from a population bottleneck, a sustainable breeding strategy should be developed alongside policies to regulate/control the flow of genes across breeds for effective breeding purposes. Sustainable improvement of these breeds should be by selective breeding that monitors genetic variations especially between Sasso and other iTABs**.**
4. It could be interesting to further explore the origin and development of iTABs belonging to haplogroup F as well as to understand more the relationship between iTABs as Southeast Asian chickens and Chinese chickens. In addition to this, more studies have to be carried out to ascertain theexistence of iTABs as belonging to Haplogroup H.

5.3 Contribution to knowledge

1. The high level of genetic diversity revealed in this study coupled with the high abundance of non-synonymous transition mutation discovered in the present iTABs could be harnessed for exploring selection and breeding within the framework of the chicken breeds to obtain many more lines or strains for conservation purposes.
2. This study also discovered a high gene flow between Sasso and Nigerian iTABs, thus suggesting the need for monitored and planned breeding between these populations to avoid population bottleneck caused by inbreeding. Meanwhile, Funaab-ALPHA and Shika Brown, Funaab-ALPHA and Fulani, Kuroiler and any of the iTABs could be crossed freely without any fear of population bottleneck.
3. This study discovered that the present iTABs belong to three clades; clade II, III, and IV defined by haplogroup C, E, and H and therefore supports the theory of multiple maternal origins of domestic chicken possibly of Japan, China, and Indian subcontinent.
4. The findings from this study support the existing reports by the Pluralism scholars that the Red Jungle fowl is only the primary ancestor of domestic chicken hence it enforces the role of G.*varius, G. lafayetti, and G, bankiva* as part probably, the secondary ancestors of domestic chicken including the present Improved Tropically Adapted Chicken Breeds.

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APPENDIX

Appendix 1: 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	0	0	0	1(0.077)	0	0
Hap_2	4(0.333)	3(0.250)	3(0.214)	3(0.231)	1(0.077)	2(0.154)
Hap_3	3(0.250)	1(0.083)	5(0.357)	4(0.308)	7(0.538)	4(0.308)
Hap_4	4(0.333)	1(0.083)	4 (0.286)	3(0.231)	3(0.231)	6(0.462)
Hap_5	1(0.083)	0	0	0	0	0
Hap_6	0	1(0.083)	0	0	0	0
Hap_7	0	1(0.083)	0	0	0	0
Hap_8	0	2(0.167)	0	0	0	0
Hap_9	0	1(0.083)	0	0	0	0
Hap_10	0	1 (0.083)	0	1(0.077)	0	0

Hap_11	0	1(0.083)	0	0	1(0.077)	0
Hap_12	0	0	2(0.143)	0	0	1(0.077)
Hap_13	0	0	0	1(0.077)	0	0
Hap_14	0	0	0	0	1(0.077)	0

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

Appendix 2: Haplotype Distribution observed in the D-loop mtDNA of the studied ITABs.

Hap_1: 2 [KX987152 Kuroiler_L3(2)]

Hap_2: 16 [Noiler_L1 Noiler_L1(3) Noiler_L3 Noiler_L3(4) Funaab_L1(2) Funaab_L2(3) Funaab_L3(2) Shika_Br_L2(2) Shika_Br_L2(3) Shika_Br_L3(2) Kuroiler_L1(2) Kuroiler_L3(4) Kuroiler_L3(5) Sasso_L1(2) Fu_Eco_L1 Fu_Eco_L3(3)]

Hap_3: 24 [Noiler_L1(2) Noiler_L2 Noiler_L3(3) Funaab_L2(5) Shika_Br_L1 Shika_Br_L1(2) Shika_Br_L2(5) Shika_Br_L3 Shika_Br_L3(4) Kuroiler_L1 Kuroiler_L1(4) Kuroiler_L3 Kuroiler_L3(3) Sasso_L1(3) Sasso_L2 Sasso_L2(2) Sasso_L2(4) Sasso_L3 Sasso_L3(2) Sasso_L3(6) Fu_Eco_L2(2) Fu_Eco_L3 Fu_Eco_L3(4) Fu_Eco_L3(5)]

Hap_4: 21 [Noiler_L2(2) Noiler_L2(3) Noiler_L2(4) Noiler_L3(2) Funaab_L2(4) Shika_Br_L1(4) Shika_Br_L2 Shika_Br_L2(4) Shika_Br_L3(3) Kuroiler_L2 Kuroiler_L2(3) Kuroiler_L2(4) Sasso_L1 Sasso_L2(3) Sasso_L3(4) Fu_Eco_L1(2) Fu_Eco_L1(3) Fu_Eco_L1(4) Fu_Eco_L2 Fu_Eco_L2(3) Fu_Eco_L2(4)]

Hap_5: 1 [Noiler_L3(5)]

Hap_6: 1 [Funaab_L1]

Hap_7: 1 [Funaab_L2]

Hap_8: 2 [Funaab_L2(2) Funaab_L3]

Hap_9: 1 [Funaab_L3(3)]

Hap_10: 2 [Funaab_L3(4) Kuroiler_L1(3)]

Hap_11: 2 [Funaab_L3(5) Sasso_L3(3)]

Hap_12: 3 [Shika_Br_L1(3) Shika_Br_L1(5) Fu_Eco_L3(2)]

Hap_13: 1 [Kuroiler_L2(2)]

Hap_14: 1 [Sasso_L3(5)]

Appendix 3: Nucleotide polymorphism observed in individual mtDNA D-loop region of the studied iTABs.

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                222222222222233333333333333333333333333333333333333333333333
                222233333333400000000011111111222244444444455555666666667777
                678901456789012345678912456789012412345678914567013456789013 45

Reference       ACCCTCCATAGACAGTTCTAAACATATCAAGCCCACAGGACATACTCACTATGTTCTCCCCT
Noiler_L1       ..............C..C.......C....T.........................T...C
Noiler_L12      ..AGCTA.ACC..CA.GT.CTT.CC.A....T...........CT.A.AC.......AA..A
Noiler_L13      ..............C..C.......C....T.........................T...C
Noiler_L2       ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Noiler_L22      ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Noiler_L23      ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Noiler_L24      ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Noiler_L3       ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Noiler_L32      ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Noiler_L33      ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Noiler_L34      ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Noiler_L35      ACCCTCCATAGGCAGTTCCAAACATATCAAGCCCACAGGACATACTCACTATGTTCTCCCCC
Funaab_L1       GTTCACTCCCCATACTACCAAGTACTAACTATGAGGTTGCAGGCAAATCATCTCATGTTTTC
Funaab_L12      ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Funaab_L2       ACCCTCCATAGACAGCTCCAAGCATACCAAGTCCGCAGGACATACTCACTATGTTCTTCCCC
Funaab_L22      ACAGCTAAGCCACCATGTTCTTCCCAACAAGTCCGCAGGACATCCTAAACATGTTCTAACCA
Funaab_L23      ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Funaab_L24      ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Funaab_L25      ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Funaab_L3       ACAGCTAAGCCACCATGTTCTTCCCAACAAGTCCGCAGGACATCCTAAACATGTTCTAACCA
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Funaab_L32   ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Funaab_L33   GTTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Funaab_L34   ACCCTCCATAGACAGTTCCAAACATATCAAGCCCACAGGACATACTCACTATGTTCTCCCCC
Funaab_L35   ATTTACTCCCCATACTATCAAGCACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTCC
Shika_Br_L1  ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Shika_Br_L12 ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Shika_Br_L13 ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCGCAGGACATCTTAAACATGTTCTAACCA
Shika_Br_L14 ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Shika_Br_L15 ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCGCAGGACATCTTAAACATGTTCTAACCA
Shika_Br_L2  ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Shika_Br_L22 ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Shika_Br_L23 ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Shika_Br_L24 ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Shika_Br_L25 ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Shika_Br_L3  ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Shika_Br_L32 ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Shika_Br_L33 ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Shika_Br_L34 ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Kuroiler_L1  ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Kuroiler_L12 ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Kuroiler_L13 ACCCTCCATAGACAGTTCCAAACATATCAAGCCCACAGGACATACTCACTATGTTCTCCCCC
Kuroiler_L14 ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Kuroiler_L2  ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Kuroiler_L22 ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTTCCC
Kuroiler_L23 ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Kuroiler_L24 ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
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Kuroiler_L3  ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Kuroiler_L32 ACCCTCCATAGACAGTTCTAAACATATCAAGCCCACAGGACATACTCACTATGTTCTCCCCT
Kuroiler_L33 ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Kuroiler_L34 ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Kuroiler_L35 ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Sasso_L1     ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Sasso_L12    ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Sasso_L13    ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Sasso_L2     ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Sasso_L22    ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Sasso_L23    ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Sasso_L24    ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Sasso_L3     ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Sasso_L32    ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Sasso_L33    ATTTACTCCCCATACTATCAAGCACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTCC
Sasso_L34    ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Sasso_L35    CCCCTCCATAGACCATGTTCTCCCTAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Sasso_L36    ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Fu_Eco_L1    ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Fu_Eco_L12   ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Fu_Eco_L13   ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Fu_Eco_L14   ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Fu_Eco_L2    ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Fu_Eco_L22   ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Fu_Eco_L23   ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
Fu_Eco_L24   ATTCACTCCCCATACTACCAAGTACTAACTATGAGGTTACAGGCAAATCATCTCATGTTTTC
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Fu_Eco_L3    ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Fu_Eco_L32   ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCGCAGGACATCTTAAACATGTTCTAACCA
Fu_Eco_L33   ACCCTCCATAGACAGCTCCAAACATACCAAGTCCACAGGACATACTCACTATGTTCTTCCCC
Fu_Eco_L34   ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
Fu_Eco_L35   ACAGCTAAACCACCATGTTCTTCCCAACAAGTCCACAGGACATCTTAAACATGTTCTAACCA
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Nucleotide polymorphism observed in mtDNA D-loop region of 77 iTABs. Vertically oriented numbers indicate the site position and the sequences shown are only the variable sites. Dots (.) indicate identity with the reference sequence (genBank accession number KX987152; Source Mitochondrial *Gallus gallus* chicken)), while different base letters denote substitution. The abbreviations L1-L3 denote location number and L12……L16 denotes number of individuals per location.

Appendix 4: Variable (polymorphic) sites in the D-loop mtDNA of the iTABs: 62 (Total number of mutations: 79)

Nature of the polymorphic sites	No	Site positions
Singleton variable sites(two variants)	1	239
Singleton variable sites (three variants)	0	
Singleton variable sites (four variants)	0	
Parsimony informative sites	61	
Parsimony informative sites (two variants)	46	227 231 235 237 238 240 301 303 305 306 307 308 310 312 314 315 317 318 319 320 321 322 324 341 342 343 344 345 346 347 348 349 351 355 356 357 360 363 364 365 366 367 368 369 373 374
Parsimony informative sites (three variants)	13	226 228 229 230 234 302 304 316 354 361 370 371 375
Parsimony informative sites (four variants) 2		236 309

Appendix 5 Pairwise genetic distance of the Improved Tropically Adapted Chicken Breeds from three locations in Imo State, Nigeria based on mitochondrial D-loop region.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
[1]																	
[2]	1.942																
[3]	1.146	1.420															
[4]	-0.000	1.942	1.146														
[5]	1.146	1.420	-0.000	1.146													
[6]	1.146	1.420	-0.000	1.146	-0.000												
[7]	1.146	1.420	-0.000	1.146	-0.000	-0.000											
[8]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146										
[9]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146									
[10]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420								
[11]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942							
[12]	0.006	1.942	1.146	0.006	1.146	1.146	1.146	0.006	1.146	1.942	0.006						
[13]	1.217	1.459	0.019	1.217	0.019	0.019	0.019	1.217	0.019	1.459	1.217	1.217					
[14]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217				
[15]	0.012	1.851	1.146	0.012	1.146	1.146	1.146	0.012	1.146	1.851	0.012	0.019	1.146	0.012			
[16]	2.054	0.012	1.420	2.054	1.420	1.420	1.420	2.054	1.420	0.012	2.054	2.054	1.459	2.054	1.942		
[17]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217	-0.000	0.012	2.054	

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
[18]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[19]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[20]	2.054	0.012	1.420	2.054	1.420	1.420	1.420	2.054	1.420	0.012	2.054	2.054	1.459	2.054	1.942	-0.000	2.054
[21]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217	-0.000	0.012	2.054	-0.000
[22]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[23]	0.006	1.942	1.146	0.006	1.146	1.146	1.146	0.006	1.146	1.942	0.006	-0.000	1.217	0.006	0.019	2.054	0.006
[24]	1.146	1.385	0.012	1.146	0.012	0.012	0.012	1.146	0.012	1.385	1.146	1.146	0.031	1.146	1.146	1.385	1.146
[25]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[26]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[27]	2.054	0.006	1.420	2.054	1.420	1.420	1.420	2.054	1.420	0.006	2.054	2.054	1.459	2.054	1.942	0.006	2.054
[28]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[29]	2.054	0.006	1.420	2.054	1.420	1.420	1.420	2.054	1.420	0.006	2.054	2.054	1.459	2.054	1.942	0.006	2.054
[30]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[31]	0.006	1.942	1.180	0.006	1.180	1.180	1.180	0.006	1.180	1.942	0.006	0.012	1.180	0.006	0.006	2.054	0.006
[32]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217	-0.000	0.012	2.054	-0.000
[33]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[34]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
[35]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[36]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217	-0.000	0.012	2.054	-0.000
[37]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[38]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[39]	2.054	0.006	1.459	2.054	1.459	1.459	1.459	2.054	1.459	0.006	2.054	2.054	1.501	2.054	1.942	0.019	2.054
[40]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217	-0.000	0.012	2.054	-0.000
[41]	0.006	1.942	1.146	0.006	1.146	1.146	1.146	0.006	1.146	1.942	0.006	-0.000	1.217	0.006	0.019	2.054	0.006
[42]	2.054	0.006	1.459	2.054	1.459	1.459	1.459	2.054	1.459	0.006	2.054	2.054	1.501	2.054	1.942	0.019	2.054
[43]	1.114	1.420	0.006	1.114	0.006	0.006	0.006	1.114	0.006	1.420	1.114	1.114	0.025	1.114	1.114	1.420	1.114
[44]	2.006	1.540	0.154	2.006	0.154	0.154	0.154	2.006	0.154	1.540	2.006	2.006	0.162	2.006	1.803	1.540	2.006
[45]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[46]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[47]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[48]	0.006	1.942	1.146	0.006	1.146	1.146	1.146	0.006	1.146	1.942	0.006	-0.000	1.217	0.006	0.019	2.054	0.006
[49]	2.054	0.006	1.459	2.054	1.459	1.459	1.459	2.054	1.459	0.006	2.054	2.054	1.501	2.054	1.942	0.019	2.054
[50]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217	-0.000	0.012	2.054	-0.000
[51]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217	-0.000	0.012	2.054	-0.000

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
[52]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[53]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217	-0.000	0.012	2.054	-0.000
[54]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[55]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[56]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[57]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[58]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[59]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[60]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[61]	1.146	1.385	0.012	1.146	0.012	0.012	0.012	1.146	0.012	1.385	1.146	1.146	0.031	1.146	1.146	1.385	1.146
[62]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[63]	1.942	0.019	1.420	1.942	1.420	1.420	1.420	1.942	1.420	0.019	1.942	1.942	1.459	1.942	1.851	0.031	1.942
[64]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[65]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217	-0.000	0.012	2.054	-0.000
[66]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[67]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[68]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
[69]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146-
[70]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[71]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[72]	1.146	1.420	-0.000	1.146	-0.000	-0.000	-0.000	1.146	-0.000	1.420	1.146	1.146	0.019	1.146	1.146	1.420	1.146
[73]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[74]	2.054	0.006	1.420	2.054	1.420	1.420	1.420	2.054	1.420	0.006	2.054	2.054	1.459	2.054	1.942	0.006	2.054
[75]	-0.000	1.942	1.146	-0.000	1.146	1.146	1.146	-0.000	1.146	1.942	-0.000	0.006	1.217	-0.000	0.012	2.054	-0.000
[76]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942
[77]	1.942	-0.000	1.420	1.942	1.420	1.420	1.420	1.942	1.420	-0.000	1.942	1.942	1.459	1.942	1.851	0.012	1.942

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
[18]																	
[19]	1.420																
[20]	1.420	0.012															
[21]	1.146	1.942	2.054														
[22]	-0.000	1.420	1.420	1.146													
[23]	1.146	1.942	2.054	0.006	1.146												
[24]	0.012	1.385	1.385	1.146	0.012	1.146											
[25]	1.420	-0.00	0.012	1.942	1.420	1.942	1.385										
[26]	1.420	-0.00	0.012	1.942	1.420	1.942	1.385	-0.00									
[27]	1.420	0.006	0.006	2.054	1.420	2.054	1.385	0.006	0.006								
[28]	-0.00	1.420	1.420	1.146	-0.00	1.146	0.012	1.420	1.420	1.420							
[29]	1.420	0.006	0.006	2.054	1.420	2.054	1.385	0.006	0.006	-0.00	1.420						
[30]	-0.00	1.420	1.420	1.146	-0.00	1.146	0.012	1.420	1.420	1.420	-0.00	1.420					
[31]	1.180	1.942	2.054	0.006	1.180	0.012	1.180	1.942	1.942	2.054	1.180	2.054	1.180				
[32]	1.146	1.942	2.054	-0.00	1.146	0.006	1.146	1.942	1.942	2.054	1.146	2.054	1.146	0.006			
[33]	-0.00	1.420	1.420	1.146	-0.00	1.146	0.012	1.420	1.420	1.420	-0.00	1.420	-0.00	1.180	1.146		
[34]	1.42	-0.000	0.012	1.942	1.420	1.942	1.385	-0.00	-0.00	0.006	1.420	0.006	1.420	1.942	1.942	1.420	

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L3

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
[35]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[36]	1.146	1.942	2.054	-0.000	1.146	0.006	1.146	1.942	1.942	2.054	1.146	2.054	1.146	0.006	-0.000	1.146	1.942
[37]	-0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420
[38]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[39]	1.459	0.006	0.019	2.054	1.459	2.054	1.420	0.006	0.006	0.012	1.459	0.012	1.459	2.054	2.054	1.459	0.006
[40]	1.146	1.942	2.054	-0.000	1.146	0.006	1.146	1.942	1.942	2.054	1.146	2.054	1.146	0.006	-0.000	1.146	1.942
[41]	1.146	1.942	2.054	0.006	1.146	-0.000	1.146	1.942	1.942	2.054	1.146	2.054	1.146	0.012	0.006	1.146	1.942
[42]	1.459	0.006	0.019	2.054	1.459	2.054	1.420	0.006	0.006	0.012	1.459	0.012	1.459	2.054	2.054	1.459	0.006
[43]	0.006	1.420	1.420	1.114	0.006	0.019	1.420	1.420	1.420	0.006	1.420	0.006	1.146	1.114	0.006	1.420	1.114
[44]	0.154	1.540	1.540	2.006	0.154	2.006	0.169	1.540	1.540	1.540	0.154	1.540	0.154	1.894	2.006	0.154	1.540
[45]	-0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420
[46]	-0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420
[47]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[48]	1.146	1.942	2.054	0.006	1.146	-0.000	1.146	1.942	1.942	2.054	1.146	2.054	1.146	0.012	0.006	1.146	1.942
[49]	1.459	0.006	0.019	2.054	1.459	2.054	1.420	0.006	0.006	0.012	1.459	0.012	1.459	2.054	2.054	1.459	0.006
[50]	1.146	1.942	2.054	-0.000	1.146	0.006	1.146	1.942	1.942	2.054	1.146	2.054	1.146	0.006	-0.000	1.146	1.942
[51]	1.146	1.942	2.054	-0.000	1.146	0.006	1.146	1.942	1.942	2.054	1.146	2.054	1.146	0.006	-0.000	1.146	1.942

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
[52]	-0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420
[53]	1.146	1.942	2.054	-0.000	1.146	0.006	1.146	1.942	1.942	2.054	1.146	2.054	1.146	0.006	-0.000	1.146	1.942
[54]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[55]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[56]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[57]	-0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420
[58]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[59]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[60]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[61]	0.012	1.385	1.385	1.146	0.012	1.146	-0.000	1.385	1.385	1.385	0.012	1.385	0.012	1.180	1.146	0.012	1.385
[62]	-0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420
[63]	1.420	0.019	0.031	1.942	1.420	1.942	1.385	0.019	0.019	0.025	1.420	0.025	1.420	1.942	1.942	1.420	0.019
[64]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[65]	1.146	1.942	2.054	-0.000	1.146	0.006	1.146	1.942	1.942	2.054	1.146	2.054	1.146	0.006	-0.000	1.146	1.942
[66]	-0.000	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420	1.420
[67]	-0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420
[68]	0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
[69]	-0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420
[70]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[71]	-0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420
[72]	-0.000	1.420	1.420	1.146	-0.000	1.146	0.012	1.420	1.420	1.420	-0.000	1.420	-0.000	1.180	1.146	-0.000	1.420
[73]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[74]	1.420	0.006	0.006	2.054	1.420	2.054	1.385	0.006	0.006	-0.000	1.420	-0.000	1.420	2.054	2.054	1.420	0.006
[75]	1.146	1.942	2.054	-0.000	1.146	0.006	1.146	1.942	1.942	2.054	1.146	2.054	1.146	0.006	-0.000	1.146	1.942
[76]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000
[77]	1.420	-0.000	0.012	1.942	1.420	1.942	1.385	-0.000	-0.000	0.006	1.420	0.006	1.420	1.942	1.942	1.420	-0.000

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
[35]																	
[36]	1.942																
[37]	1.420	1.146															
[38]	-0.000	1.942	1.420														
[39]	0.006	2.054	1.459	0.006													
[40]	1.942	-0.000	1.146	1.942	2.054												
[41]	1.942	0.006	1.146	1.942	2.054	0.006											
[42]	0.006	2.054	1.459	0.006	-0.000	2.054	2.054										
[43]	1.420	1.114	0.006	1.420	1.459	1.114	1.114	1.459									
[44]	1.540	2.006	0.154	1.540	1.579	2.006	2.006	1.57	9 0.147								
[45]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154							
[46]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154	-0.000						
[47]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420					
[48]	11.942	0.006	1.146	1.942	2.054	0.006	-0.000	2.054	1.114	2.006	1.146	1.146	1.942				
[49]	0.006	2.054	1.459	0.006	-0.000	2.054	2.054	-0.000	1.459	1.579	1.459	1.459	0.006	2.054			
[50]	1.942	-0.000	1.146	1.942	2.054	-0.000	0.006	2.054	1.114	2.006	1.146	1.146	1.942	0.006	2.054		
[51]	1.942	-0.000	1.146	1.942	2.054	-0.000	0.006	2.054	1.114	2.006	1.146	1.146	1.942	0.006	2.054	-0.000	

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
[52]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154	-0.000	-0.000	1.420	1.146	1.459	1.146	1.146
[53]	1.942	-0.000	1.146	1.942	2.054	-0.000	0.006	2.054	1.114	2.006	1.146	1.146	1.942	0.006	2.054	-0.000	-0.000
[54]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942
[55]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942
[56]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942
[57]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154	-0.000	-0.000	1.420	1.146	1.459	1.146	1.146
[58]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942
[59]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942
[60]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942
[61]	1.385	1.146	0.012	1.385	1.420	1.146	1.146	1.420	0.019	0.169	0.012	0.012	1.385	1.146	1.420	1.146	1.146
[62]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154	-0.000	-0.000	1.420	1.146	1.459	1.146	1.146
[63]	0.019	1.942	1.420	0.019	0.025	1.942	1.942	0.025	1.420	1.540	1.420	1.420	0.019	1.942	0.025	1.942	1.942
[64]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942
[65]	1.942	-0.000	1.146	1.942	2.054	-0.000	0.006	2.054	1.114	2.006	1.146	1.146	1.942	0.006	2.054	-0.000	-0.000
[66]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154	-0.000	-0.000	1.420	1.146	1.459	1.146	1.146
[67]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154	-0.000	-0.000	1.420	1.146	1.459	1.146	1.146
[68]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154	-0.000	-0.000	1.420	1.146	1.459	1.146	1.146

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
[69]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154	-0.000	-0.000	1.420	1.146	1.459	1.146	1.146
[70]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942
[71]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154	-0.000	-0.000	1.420	1.146	1.459	1.146	1.146
[72]	1.420	1.146	-0.000	1.420	1.459	1.146	1.146	1.459	0.006	0.154	-0.000	-0.000	1.420	1.146	1.459	1.146	1.146
[73]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942
[74]	0.006	2.054	1.420	0.006	0.012	2.054	2.054	0.012	1.420	1.540	1.420	1.420	0.006	2.054	0.012	2.054	2.054
[75]	1.942	-0.000	1.146	1.942	2.054	-0.000	0.006	2.054	1.114	2.006	1.146	1.146	1.942	0.006	2.054	-0.000	-0.000
[76]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942
[77]	-0.000	1.942	1.420	-0.000	0.006	1.942	1.942	0.006	1.420	1.540	1.420	1.420	-0.000	1.942	0.006	1.942	1.942

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
[52]																	
[53]	1.146																
[54]	1.420	1.942															
[55]	1.420	1.942	-0.000														
[56]	1.420	1.942	-0.000	-0.000													
[57]	-0.000	1.146	1.420	1.420	1.420												
[58]	1.420	1.942	-0.000	-0.000	-0.000	1.420											
[59]	1.420	1.942	-0.000	-0.000	-0.000	1.420	-0.000										
[60]	1.420	1.942	-0.000	-0.000	-0.000	1.420	-0.000	-0.000									
[61]	0.012	1.146	1.385	1.385	1.385	0.012	1.385	1.385	1.385								
[62]	-0.000	1.146	1.420	1.420	1.420	-0.000	1.420	1.420	1.420	0.012							
[63]	1.420	1.942	0.019	0.019	0.019	1.420	0.019	0.019	0.019	1.385	1.420						
[64]	1.420	1.942	-0.000	-0.000	-0.000	1.420	-0.000	-0.000	-0.000	1.385	1.420	0.019					
[65]	1.146	-0.000	1.942	1.942	1.942	1.146	1.942	1.942	1.942	1.146	1.146	1.942	1.942				
[66]	-0.000	1.146	1.420	1.420	1.420	-0.000	1.420	1.420	1.420	0.012	-0.000	1.420	1.420	1.146			
[67]	-0.000	1.146	1.420	1.420	1.420	-0.000	1.420	1.420	1.420	0.012	-0.000	1.420	1.420	1.146	-0.000		
[68]	-0.000	1.146	1.420	1.420	1.420	-0.000	1.420	1.420	1.420	0.012	-0.000	1.420	1.420	1.146	-0.000	-0.000	

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
[69]	-0.000	1.146	1.420	1.420	1.420	-0.000	1.420	1.420	1.420	0.012	-0.000	1.420	1.420	1.146	-0.000	-0.000	-0.000
[70]	1.420	1.942	-0.000	-0.000	-0.000	1.420	-0.000	-0.000	-0.000	1.385	1.420	0.019	-0.000	1.942	1.420	1.420	1.420
[71]	-0.000	1.146	1.420	1.420	1.420	-0.000	1.420	1.420	1.420	0.012	-0.000	1.420	1.420	1.146	-0.000	-0.000	-0.000
[72]	-0.000	1.146	1.420	1.420	1.420	-0.000	1.420	1.420	1.420	0.012	-0.000	1.420	1.420	1.146	-0.000	-0.000	-0.000
[73]	1.420	1.942	-0.000	-0.000	-0.000	1.420	-0.000	-0.000	-0.000	1.385	1.420	0.019	-0.000	1.942	1.420	1.420	1.420
[74]	1.420	2.054	0.006	0.006	0.006	1.420	0.006	0.006	0.006	1.385	1.420	0.025	0.006	2.054	1.420	1.420	1.420
[75]	1.146	-0.000	1.942	1.942	1.942	1.146	1.942	1.942	1.942	1.146	1.146	1.942	1.942	-0.000	1.146	1.146	1.146
[76]	1.420	1.942	-0.000	-0.000	-0.000	1.420	-0.000	-0.000	-0.000	1.385	1.420	0.019	-0.000	1.942	1.420	1.420	1.420
[77]	1.420	1.942	-0.000	-0.000	-0.000	1.420	-0.000	-0.000	-0.000	1.385	1.420	0.019	-0.000	1.942	1.420	1.420	1.420

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

Pairwise genetic distance of The Tropically adapted chicken from three locations in Imo State, Nigeria based on mitochondrial D-loop region

[	69	70	71	72	73	74	75	76	77	]
[69]										
[70]	1.420									
[71]	-0.000	1.420								
[72]	-0.000	1.420	-0.000							
[73]	1.420	-0.000	1.420	1.420						
[74]	1.420	0.006	1.420	1.420	0.006					
[75]	1.146	1.942	1.146	1.146	1.942	2.054				
[76]	1.420	-0.000	1.420	1.420	-0.000	0.006	1.942			
[77]	1.420	-0.000	1.420	1.420	-0.000	0.006	1.942	-0.000		

[1] #Noiler_L1, [2] #Noiler_L12, [3] #Noiler_L13, [4] #Noiler_L2, [5] #Noiler_L22, [6] #Noiler_L23, [7] #Noiler_L24, [8] #Noiler_L3, [9] #Noiler_L32, [10] #Noiler_L33, [11] #Noiler_L34, [12] #Noiler_L35, [13] #Funaab_L1, [14] #Funaab_L12, [15] #Funaab_L2, [16] #Funaab_L22, [17] #Funaab_L23, [18] #Funaab_L24, [19] #Funaab_L25, [20] #Funaab_L3,[21] #Funaab_L32, [22] #Funaab_L33, [23] #Funaab_L34, [24] #Funaab_L35, [25] #Shika_Br_L1, [26] #Shika_Br_L12, [27] #Shika_Br_L13, [28] #Shika_Br_L14, [29] #Shika_Br_L15, [30] #Shika_Br_L2, [31] #Shika_Br_L22, [32] #Shika_Br_L23, [33] #Shika_Br_L24, [34] #Shika_Br_L25, [35] #Shika_Br_L3, [36] #Shika_Br_L32, [37] #Shika_Br_L33, [38] #Shika_Br_L34, [39] #Kuroiler_L1, [40] #Kuroiler_L12, [41] #Kuroiler_L13, [42] #Kuroiler_L14, [43] #Kuroiler_L2, [44] #Kuroiler_L22, [45] #Kuroiler_L23, [46] #Kuroiler_L24, [47] #Kuroiler_L3, [48] #Kuroiler_L32, [49] #Kuroiler_L33, [50] #Kuroiler_L34, [51] #Kuroiler_L35, [52] #Sasso_L1, [53] #Sasso_L12, [54] #Sasso_L13, [55] #Sasso_L2, [56] #Sasso_L22, [57] #Sasso_L23, [58] #Sasso_L24, [59] #Sasso_L3, [60] #Sasso_L32, [61] #Sasso_L33, [62] #Sasso_L34, [63] #Sasso_L35, [64] #Sasso_L36, [65] #Fu_Eco_L1, [66] #Fu_Eco_L12, [67] #Fu_Eco_L13, [68] #Fu_Eco_L14, [69] #Fu_Eco_L2, [70] #Fu_Eco_L22, [71] #Fu_Eco_L23, [72] #Fu_Eco_L24, [73] #Fu_Eco_L3, [74] #Fu_Eco_L32, [75] #Fu_Eco_L33, [76] #Fu_Eco_L34, [77] #Fu_Eco_L35

[7] # Hap_7: 2 [Funaab_L2(2) Funaab_L3]
[8] #Hap_8: 2 [Funaab_L3(5) Sasso_L3(3)]
[9] #Hap_9: 3 [Shika_Br_L1(3) Shika_Br_L1(5) Fu_Eco_L3(2)]
[10] # Hap_10: 1 [Shika_Br_L2(2)]
[11] # Hap_11: 3 [Kuroiler_L1 Kuroiler_L1(4) Kuroiler_L3(3)]
[12] #Hap_12: 1 [Kuroiler_L2]
[13] #Hap_13: 1 [Kuroiler_L2(2)]
[14] #Hap_14: 1 [Sasso_L3(5)]

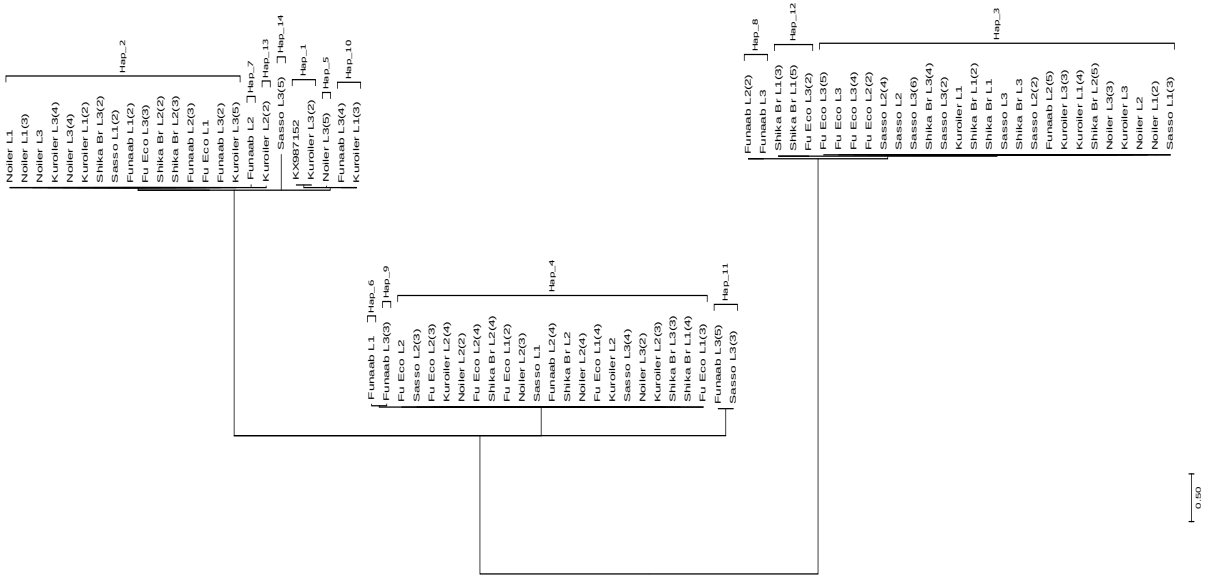
Appendix 6 Estimates of Evolutionary Divergence over Sequence Pairs between haplotypes

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14]
[1]		0.037	0.108	0.929	0.025	0.923	0.045	0.111	0.949	0.020	0.929	0.114	0.041	0.075
[2]	0.087		0.115	0.631	0.034	0.641	0.019	0.117	0.746	0.029	0.629	0.122	0.014	0.080
[3]	0.491	0.517		0.949	0.119	0.948	0.122	0.024	1.001	0.113	0.806	0.013	0.115	0.051
[4]	2.183	1.490	1.991		0.852	0.020	0.461	0.893	0.013	0.742	0.030	0.893	0.540	1.049
[5]	0.042	0.072	0.544	1.837		0.849	0.042	0.121	0.922	0.013	0.840	0.127	0.038	0.083
[6]	2.183	1.490	1.991	0.027	1.837		0.464	0.897	0.014	0.754	0.038	0.897	0.546	1.013
[7]	0.120	0.027	0.544	1.287	0.103	1.287		0.111	0.546	0.038	0.449	0.115	0.024	0.083
[8]	0.491	0.517	0.042	1.865	0.544	1.865	0.491		0.950	0.116	0.752	0.019	0.117	0.059
[9]	0.000	1.634	2.159	0.014	2.183	0.014	1.379	1.991		0.855	0.034	0.950	0.643	1.049
[10]	0.027	0.056	0.517	1.634	0.014	1.634	0.087	0.517	1.837		0.747	0.120	0.033	0.078
[11]	2.183	1.490	1.681	0.056	1.837	0.087	1.287	1.609	0.072	1.634		0.752	0.539	0.951
[12]	0.517	0.544	0.014	1.865	0.572	1.865	0.517	0.027	1.991	0.544	1.609		0.122	0.056
[13]	0.103	0.014	0.517	1.379	0.087	1.379	0.042	0.517	1.490	0.072	1.379	0.544		0.080
[14]	0.310	0.330	0.163	2.489	0.351	2.143	0.351	0.198	2.489	0.330	1.940	0.180	0.330	

[1] #Hap_1: 15 [Noiler_L1 Noiler_L2 Noiler_L3 Noiler_L3(4) Funaab_L1(2) Funaab_L2(3) Funaab_L3(2) Shika_Br_L2(3) Shika_Br_L3(2) Kuroiler_L1(2) Kuroiler_L3(4) Kuroiler_L3(5) Sasso_L1(2) Fu_Eco_L1 Fu_Eco_L3(3)];
[2] #Hap_2: 20 [Noiler_L1(2) Noiler_L3(3) Funaab_L2(5) Shika_Br_L1 Shika_Br_L1(2) Shika_Br_L2(5) Shika_Br_L3 Shika_Br_L3(4) Kuroiler_L3 Sasso_L1(3) Sasso_L2 Sasso_L2(2) Sasso_L2(4) Sasso_L3 Sasso_L3(2) Sasso_L3(6) Fu_Eco_L2(2) Fu_Eco_L3 Fu_Eco_L3(4) Fu_Eco_L3(5)];
[3] # Hap_3: 22 [Noiler_L1(3) Noiler_L2(2) Noiler_L2(3) Noiler_L2(4) Noiler_L3(2) Funaab_L2(4) Funaab_L3(3) Shika_Br_L1(4) Shika_Br_L2 Shika_Br_L2(4) Shika_Br_L3(3) Kuroiler_L2(3) Kuroiler_L2(4) Sasso_L1 Sasso_L2(3) Sasso_L3(4) Fu_Eco_L1(2) Fu_Eco_L1(3) Fu_Eco_L1(4) Fu_Eco_L2 Fu_Eco_L2(3) Fu_Eco_L2(4)]
[4] #Hap_4: 4 [Noiler_L3(5) Funaab_L3(4) Kuroiler_L1(3) Kuroiler_L3(2)]

[5] #Hap_5: 1 [Funaab_L1]
[6] #Hap_6: 1 [Funaab_L2]
[7] # Hap_7: 2 [Funaab_L2(2) Funaab_L3]
[8] #Hap_8: 2 [Funaab_L3(5) Sasso_L3(3)]
[9] #Hap_9: 3 [Shika_Br_L1(3) Shika_Br_L1(5) Fu_Eco_L3(2)]
[10] # Hap_10: 1 [Shika_Br_L2(2)]
[11] # Hap_11: 3 [Kuroiler_L1 Kuroiler_L1(4) Kuroiler_L3(3)]
[12] #Hap_12: 1 [Kuroiler_L2]
[13] #Hap_13: 1 [Kuroiler_L2(2)]
[14] #Hap_14: 1 [Sasso_L3(5)]

Appendix 7



Maximum Likelihood phylogenetic tree showing the sub-lineage relationship between the improved tropically adapted chicken breeds (iTABs) from six populations based on mitochondrial D-loop region. Number in perenthesis indicates the serial number for a breed in a particular location.

Appendix 8: Makov simulated p-values (below diagonal) and significant differences (above diagonal) of non differentiation exact tests.

	1	2	3	4	5	6
1		-	-	-	-	-
2	0.62544±0.0046		-	-	-	-
3	0.35087±0.0060	0.16767±0.0067		-	-	-
4	0.43473±0.0059	0.51864±0.0096	0.07017±0.0051		+	-
5	0.10675±0.0044	0.09073±0.0064	0.58630±0.0041	0.02206±0.0013(+)		-
6	0.64900±0.0067	0.16148±0.0064	0.91811±0.0024	0.07310±0.0046	0.43059±0.0071	

Non-differentiation: Exact P value = 0.04249 ± 0.00865 (100000 Markov steps); (significance level=0.0500)

Appendix 9 Reference sequence

```
   1 aattttattt tttaacctaa ctcccctact aagtgtaccc ccccttccc ccccaggggg
  61 ggtatactat gcataatcgt gcatacattt atataccaca tatattatgg taccggtaat
 121 atatactata tatgtactaa acccattata tgtatacggg cattaatcta tattccacat
 181 ttctcccaat gtccattcta tgcatgatcc aagacatact cattcaccct ccccatagac
 241 agttctaaac cactatcaag ccacctaact atgaatggtt acaggacata aatctcactc
 301 tcatgttctc cccctaacaa gtcacctaac tatgaatggt tacaggacat acatttaact
 361 accatgttct aacccatttg gttatgctcg ccgtatcaga tggatttatt gatcgtccac
 421 ctcacgagag atcagcaacc cctgcctgta atgtacttca tgaccagtct caggcccatt
 481 ctttcccccc acacccctcg ccctacttgc cttccaccgt acctctggtt cctcggtcag
 541 gcacatccca tgcataactc ctgaactttc tcacttttca cgaagtcatc tgtggattat
 601 cttcccctct ttagtccgtg atcgcggcat cttctctctt ctattgctgt tggttccttc
 661 tctttttggg gcttcttcac aggttgccct tcacagtgcg ggtgcggagt gctattcaag
 721 tgaagcctgg actacacctg cgttgcgtcc tatcctagtc ctctcgtgtc cctcgatgag
 781 acggtttgcg tgtatgggga atcatcttga cactgatgca ctttggatcg catttggtta
 841 tggttcttcc acccccccccg gtaaatggtg ctatttagtg aatgcttgtc ggacatattt
```

```
 901 ttatcaattt tcacttcctc tattttcttc acaaaactag gaaattcacc acaatttttt
 961 ctttgttatt ttttaatttt ttttttattt tttaaaaaca ttttttaaaa aactaaatta
1021 catacaaact accgcataaa atccctcaaa ctatacaaac gtttatcgta taatatatat
1081 acattattgt ttattctatc attattagag aaactccact accaaaacca tcattaaaac
1141 aaaaatttac atgccactta actccccctca caaacaatcg ttatttatat tgttaattag
1201 caaacacaaa acccaccttc taccactata aagcccccat agcttaaccc acaaagcatg
1261 gcactgaaga tgccaagatg gtacctacta tacctgtggg caaaagactt agtcctaacc
1321 tttctattgg tttttgctag acatatacat gcaagtatcc gcatcccagt gaaaatgccc
1381 ccaaaccttt cttcccaagc aaaaggagca ggtatcaggc acactcagca gtagcccaag
1441 acgccttgct taagccacac ccccacgggt actcagcagt aattaacctt aagcaataag
1501 tgtaaacttg acttagccat agcaacccag ggttggtaaa tcttgtgcca gccaccgcgg
1561 tcatacaaga aacccaaatc aatagctacc cggcgtaaag agtggccaca tgttatctgc
1621 accagctaag attaaaatgc aaccaagctg tcataagcct aagatccacc taaacccaac
1681 ccaaatccat cttagcctaa acgattaatt ttaacccacg aaagctagga cccaaactgg
1741 gattagatac cccactatgc ctagccctaa atctagatac ctcccatcac acatgtatcc
1801 gcctgagaac tacgagcaca aacgcttaaa actctaagga cttggcggtg ccccaaaccc
1861 acctagagga gcctgttcta taatcgataa tccacgattc acccaaccac cccttgccag
1921 cacagcctac ataccgccgt cgccagccca cctctaatga aagaacaaca gtgagctcaa
```

1981 tagcccctcg ctaataagac aggtcaaggt atagcctatg gggtgggaga aatgggctac

2041 attttctaac atagaacaaa cgaaaaagga cgtgaaaccc gcccttagaa ggaggattta

2101 gcagtaaagt gagatcatac cccctaagct cactttaaga cggctctgag gcacgtacat

2161 accgcccgtc accctcttca caagccatca acatcaataa atatatacct cccctcccgg

2221 ctaaagacga ggcaagtcgt aacaaggtaa gtgtaccgga aggtgcactt agactaccaa

2281 ggcgtagcta taacttcaaa gcattcagct tacacctgaa agataccctc aacagacaag

2341 gtcgccttga cttgcccccc ctctagcccg acaaactcgt acccttaaca taaaaaactt

2401 acctcccccc cttaaccaaa acattataaa ttgtcccagt ataggcgata gaaaagacta

2461 ccccggcgca atagaggcta actgtaccgc aagggaaaga tgaaatagca atgaaaacca

2521 taagcaaaaa acagcaaaga ccaacccttg taccttttgc atcatgattt agcaagaaca

2581 accaagcaaa gtgagctaaa gtttgccttc ccgaaaccca agcgagctac ttgcgagcag

2641 ctaaaatttg agcgaacccg tctctgttgc aaaagagtgg gatgacttgc cagtagaggt

2701 gaaaagccta ccgagctggg tgatagctgg ttacctgtca aacgaatcta agttcccccc

2761 taacccaccc cctaaagaca cccacctttg tcaaccttga gaacgttggg gttaagagca

2821 attcgatggg ggtacagctc catcgaaaaa gaacacaacc tcctccagcg gataataatc

2881 acccctcccc gcactgtggg ccttcaagca gccaccaaca aaagagtgcg tcaaagctcc

2941 ctcattaaaa aatctaaaac cctatttgac tccctcaacc aaagcaggtt aacctatgac

3001 aatagaagaa tcaatgctaa aatgagtaat ctggaaccta tcctcctacg gcgtaaactt

3061 acattaatac attattaaca gaactcaact tatacccca cactaacaag caatacgtat

3121 tcctcaatct gttaagccaa cccaggagcg cccacaggat gattaaaacc tacagaagga

3181 actcggcaaa ccaaagaccc gactgtttcc caaaaacata gccttcagct aacaacaagt

3241 attgaaggtg atgcctgccc agtgaccccc aaagttcaac ggccgcggta tcctaaccgt

3301 gcgaaggtag cgcaatcaat tgtcccgtaa attgagactt gtatgaatgg ctaaacgagg

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