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Original Research Paper

Analysis of Genetic diversity, Polymorphism and Relationship between Some Nigerian Muscovy Duck (*Caraina Moshata*) Populations Using Mitochondrial Cytochrome b genes

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Abstract

Muscovy ducks (*Cairina moschata*) are one of the poultry species that contributes significantly to protein consumption around the world as they complement eggs and meat supply from chicken in terms of availability and affordability, especially among the resource poor farmers in developing nations. In Nigeria, Muscovy duck is an integral part of poultry sector that needs more attention for higher productivity because of their adaptability and hardy nature. The genetic diversity, polymorphism and relationship between some Muscovy duck populations in Nigeria were analyzed using mitochondrial cytochrome b gene (Mt CYTB). The results showed a total of 40 polymorphic sites consisting of 19 singletons variables with 42 mutations. The 72 cytochrome b sequences obtained were assigned to 17 distinct haplotypes with low diversity (0.439) among the populations studied. Phylogenetic analysis showed close clustering of monophyletic clade within some populations across the locations with the exception of a few populations (BON 10, BON 20 and ADE 11) that show a distant relationship of polyphyletic clustering. These findings inferred that the genetic diversity within the Muscovy ducks from the study area is low, there were variants forms of specific DNA sequence, and only few populations of the Muscovy ducks are from more than one common ancestor concerning mitochondrial cytochrome b gene. Therefore, selective mating of Nigerian Muscovy ducks and the use of other types of markers for diversity study are recommended for rapid genetic gain in any breeding program designed for their improvement.

Key words: Genetic diversity, Muscovy duck, Mitochondrial cytochrome b gene, Polymorphism

Introduction

Poultry production in Nigeria is dominated by small scale poultry farming because out of 150.682 million poultry population in the country, only 25% were commercially farmed, 15% were semi-commercial, while backyards poultry farming covered 60%. In Nigeria, most households and resource poor farmers depend on Muscovy duck as major source of meat and eggs because they are locally adapted, require low cost of feeding and housing, are generally kept in the backyard, they are hardy, resistant to environmental stress, highly prolific, resistance to common poultry diseases and less exigent to feed quality Yakubu (2011).

Muscovy ducks were domesticated hundreds of years ago, yet there is paucity of information on their genetic resource and diversity. Over the years, there has been a great concern universally over the loss of biodiversity amongst domestic animals because most of the Nigerian livestock heritage lies in the genetic diversity of native breeds and there exists only limited information on their population diversity, genetic preservation and biodiversity which are very important to their improvement because their outcomes are useful for animal breeder in any effort designed to develop the livestock genome data banks (Crawford and

Gavora, 1993; Sola-Ojo *et al*., 2021). Characterization and conservation of Muscovy duck genetic resources in Nigeria are expedient in order to guide breeders in developing breeds that will be able to adapt to future climatic requirements, production systems, available feed resources, environmental issues, laws and regulations, as well as disease prevalence, this will help in food supply, food security with high cultural and historical values that are of utmost importance to humans (Tromso, 2010).

The assessment of genetic variability at the DNA level has been enabled by the recent advances in molecular technology. Different classes of molecular markers such as restriction fragment length polymorphism (RFLP) random amplified polymorphic DNA (RAPD) markers, amplified fragment length polymorphisms (AFLP), mitochondrial DNA (mtDNA) markers, microsatellites and minisatellites, as well as single nucleotide polymorphisms (SNP) markers have been used in genetic diversity studies (Weigend *et al.,* 2013).

The use of mitochondrial DNA (mtDNA) has been long considered to be the effective genetic marker in assessment of the genetic structure among populations and species, and it also reveals the phylogenetic evolution among species because of its classical relationship with maternal inheritance, less recombinant, rapid evolution, less selection pressure and great genetic variance. This molecule marker has been extensively used to study animal phylogenetic evolution and genetic diversity by several authors (Saccone et al., 2000, Li et al., 2008, Leekaew et al., 2010, Adeola et al., 2015). Past studies have assessed the genetic polymorphism in ducks including; Dolmatova et al. (2000) where the authors revealed the possibility of using RAPD markers for the detection of differences among lines of White Pekin ducks, El-Gendy et al. (2005) also studied the effect of elucidating the genetic variation within and between duck populations and estimated the phylogenetic relationships among five duck breeds of two species namely; Muscovy Sudani of *Cairina* species, and White Pekin, Damietti as well as Khaki Campbell of Anas species using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR), while Ogah *et al*., (2017) compared Nigerian Muscovy duck with some selected duck breeds using mtDNA D-loop region and reported high variability among the Muscovy duck with no maternal linkage with other duck species. A clear geographical differentiation and maternal genetic evolutionary history of Muscovy duck populations in India have also been reported by Kameshpandian et al. (2016). More recently, studies based on mitochondrial DNA (mtDNA) have attempted to assess genetic diversity and population structure of Muscovy duck populations from Nigeria (Adeola et al., 2020 and Sola-Ojo et al. 2021).

Studying Muscovy duck genetic diversity would assist the breeder in making the right decision in the selection and breeding of Muscovy duck for continuous supply of animal protein, thus bridging the gap in the shortage of animal protein intake currently experienced by the teeming population globally. Therefore, this study was designed to evaluate the Nigerian Muscovy duck population genetically with the aim of estimating the diversity and relationship between individuals from different locations in Nigeria using cytochrome b genes.

Materials and methods

Experimental Birds

A total of 72 Muscovy ducks were sampled from 15 different locations in Kaduna, Kwara, Niger, and Oyo states in Nigeria as shown in Figure 1.0. The details and coordinates of the locations are shown in Table 1.0.

Blood Collection for Genomic DNA Extraction

A blood sample was collected from each bird carefully through wing venipuncture. Total genomic DNA was extracted from ethanol preserved blood samples using a standard phenol-chloroform method (Sambrook et al., 1989). The quality and quantity of the genomic DNA was checked by the use of nanodrop

spectrophotometer.

Figure 1. Proportion of individuals sampled from each region with colors indicating the sampling in Kaduna, Kwara, Niger and Oyo States of Nigeria: *Blue-ABO; Black-ADE; purple-BART; Red-BDS; Brown-BON; Orange-IDO; White-ILE; Grey-KEY; Light Blue-KIS; Yellow-MOU; Pink-NBS; Lime-NIG; Light Red-OKE; Green-PAT; Light Pink-SAM.*

Table: 1. Muscovy ducks ID, Sampling locations, and coordinates of the Sampling Sites.

Muscovy Duck ID	Sampling locations	State	Coordinates of sites
ABO	ABOTO OJA	KWARA	8^0 9' 0" N; 4^0 33' 0" E
ADE	ADETA	KWARA	8.4912^0 N; 4.5109^0 E
BART	KOSUBOSU	KWARA	9^0 35' 0" N; 3^0 15' 0" E
BDS	BODE SAADU	KWARA	8.9390^0 N; 4.7823 ^{0}E
BON	BANI	KWARA	9.6062^0 N; 3.9419 ^{0}E
IDO	IDOFIAN	KWARA	8.3753^0 N; 4.7135 ^{0}E
ILE	ILESHA BARUBA	KWARA	8^0 55' 0" N; 3^0 25' 0" E
KEY	OKE OYI	KWARA	8.5791^0 N; 4.7148 ^{0}E
KIS	KISHI	OYO	9.05^0 N; 3.51 ^{0}E
MOU	MOKWA	NIGER	8.2928^0 N; 5.0547 ^{0}E
NBS	NEW BUSSA	NIGER	9.8829^0 N; 4.5109 ^{0}E
NIG	MINNA	NIGER	9.5836^0 N; 6.5463 ^{0}E
OKE	OKUTA	KWARA	9^0 13' 0" N; 3^0 10' 59" E
PAT	PATIGI	KWARA	8.7211^0 N; 5.7563^0 E
SAM	SAMINAKA	KADUNA	10.4123^0 N; 8.6875 ^{0}E

Polymerase chain reaction and DNA sequencing

The 940bp mtDNA cytochrome b gene fragment was amplified using two different sets of primers: DUKCYB1L (5'-ATCTTTCGCCCTATCCATCC-3') and DUKCYB2R (5'-TTTGGTTTACAAGACCAATGTTTT-3'), or P105 (5'-GCCTCCTG CTAGCCATACAC-3') and P106 (5'-TACGGCGGGAAAGAGAA ATA-3'). PCR amplification was carried out on a thermocycler with a 25ul volume reaction mixture, which included 50 ng of genomic DNA, 12.5ul of master mix (Promega, Fitchburg, WI) and 2ul of (10pmol) of each primer. The PCR was perfumed at 95^0C for 5 minutes, followed by 30 cycles of 30 seconds at 94^0C, 1 minute at respective annealing temperature 54^0C, and 1 minute at 72^0C. The amplified PCR products as sequenced using the Big Dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) on the ABI 3500 Genetic Analyzer.

Data analysis

The cytochrome b gene sequence obtained was manually edited and aligned and an unrooted neighbor-joining (NJ) typology was constructed drawn using Clustal W implemented in MEGA 7.0.18. (Tiamura et al., 2016). Genetic diversity indices such as the number of haplotypes, haplotype diversity, polymorphic sites and nucleotide diversity were analyzed using Dna SP. v. 6.12.03 (Rozas, 2009). The sequences were further subjected to Multiple Sequence Alignment MSA viewer using Clustal x 2.0 and Jalview 1.8.3 (Larkin etal.,2007). A median-joining network (Bandelt et al., 1999) was constructed with NETWORK 10.2 (http://www.fluxus-engineering.com).

Results

Total Sequence, Polymorphism, Haplotype and Nucleotide diversity

Seventy-two (72) sequences of 940bp were obtained (57, and 13 sequences from Kwara and Niger states, respectively while one was obtained from Kaduna and Oyo State populations. There were 40 variables sites of approximately 6.43 of the full length with no alignment gaps and 699 monomorphic sites. The results show that the Haplotype diversity was 0.439, while the Nucleotide diversity per site (Pi) was 0.00259, with a total number of 42 mutations and 21 parsimonies informative site. The polymorphic sites consist of substitutions with 19 singletons variables of two, three and four variants with a value of 17, 12 and 0 respectively as shown in Table 2. The variant in the sequences as shown by Jalview indicated a few polymorphisms (substitutions) in the aligned sequences for NIG 50, 51 and IDO 27 Muscovy ducks' identity from 0 to 120bp (Figure 2).

Table 2. Total Sequence Variations and Haplotype diversity between the population of Muscovy Ducks Sampled

S/N	Parameters	Analysis Results
1	Number of Sequences	72
2	Site with Alignment gaps or missing gaps	0
3	Invariable (Monomorphic Sites)	699
4	Variable (Polymorphic Sites)	40
5	Nucleotide diversity per site (Pi)	0.00259
6	Haplotype diversity	0.439
7	N Haplotype	17
8	Total Number of Mutations	42
9	Singleton Variable sites	19
10	Singleton Variable sites (two variants)	17
11	Singleton Variable sites (three variants)	2
12	Singleton Variable sites (four variants)	0
13	Parsimonies Informative Sites	21

Relationship and Phylogenetic analysis

The Median Joining Network of the 72 sequences obtained for the Muscovy duck samples showed the connectivity between some populations clustering together at the epicenter, with few Muscovy ducks that are far apart and in a distant relationship (Figure 3), while the phylogenetic tree also shows some monophyletic clade relationship with BON 10, 20 and ADE 11 in a distant polyphyletic clade (Figure 4).

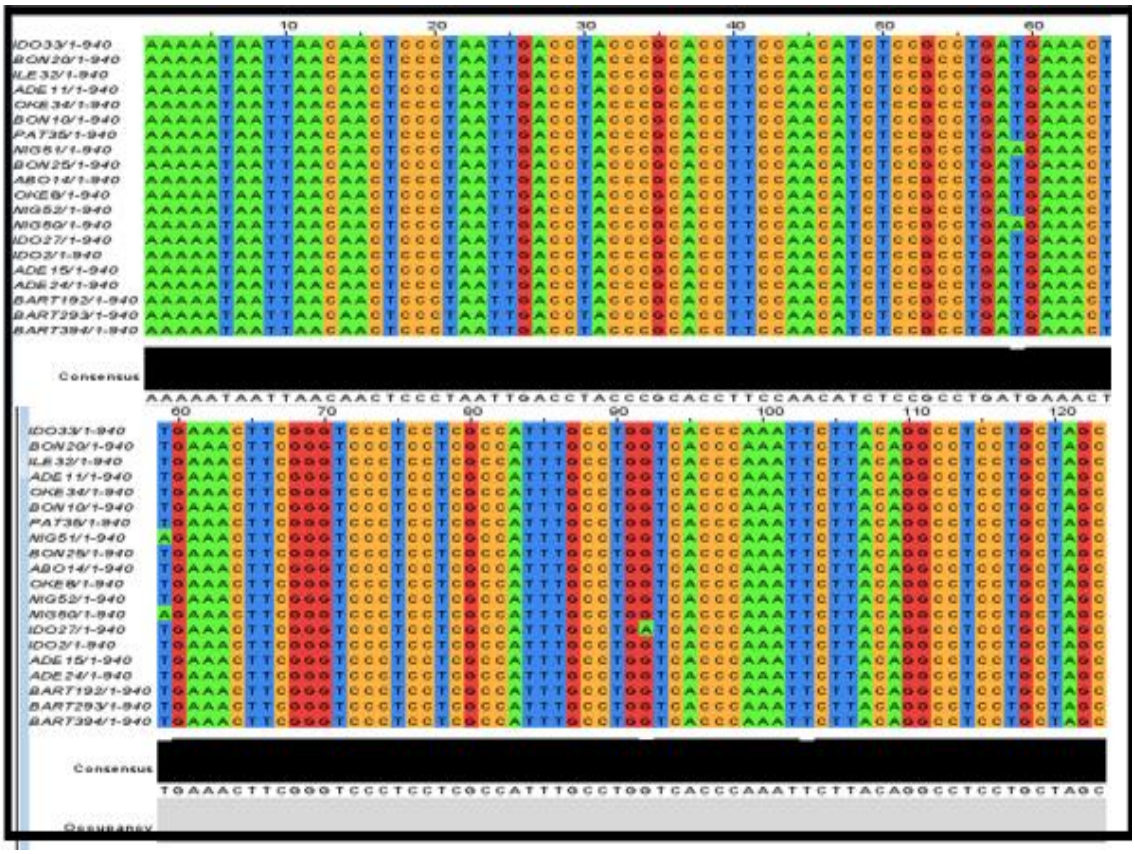

Figure 2. Polymorphism in Muscovy Duck sequenced from 0 to 120bp as shown by Multiple sequence alignment viewer.

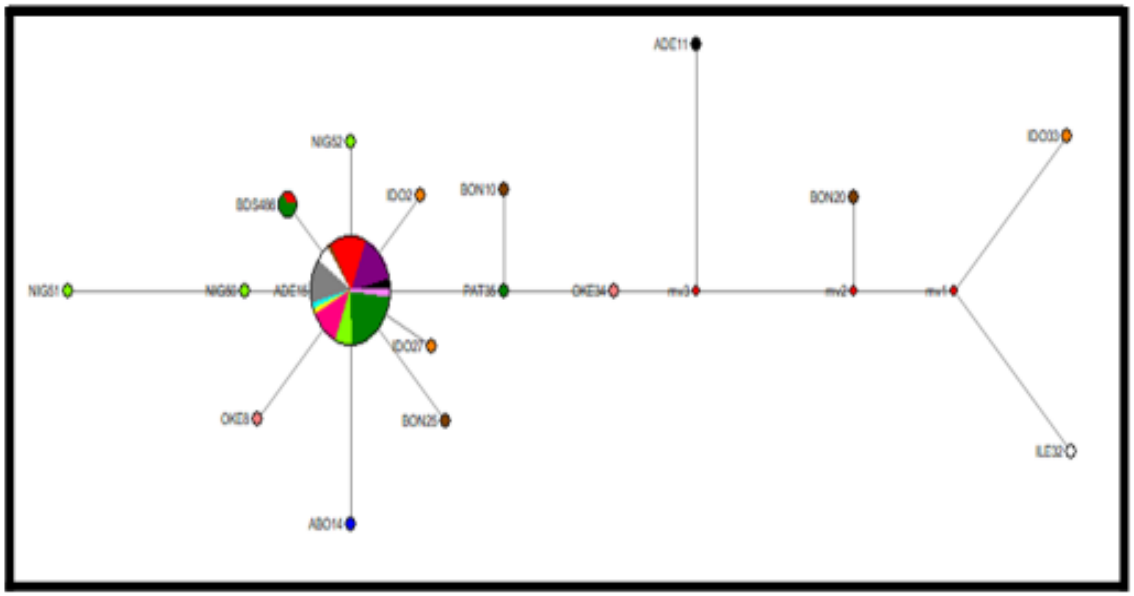

Figure 3. Median-joining network of 72 Nigerian Muscovy duck samples constructed by using NETWORK 10.2. Colors indicate the geographical distribution of the sampling locations across States in Kaduna, Kwara, Niger and Oyo. *Blue – ABO (ABOTO); Black – ADE (ADETA); Purple – BART (KOSUBOSU); Red – BDS (BODESAADU); Brown – BON (BANI); Orange – IDO (IDOFIAN); White – ILE (ILESHA BARUBA); Grey – KEY (OKEOYI); Light Blue – KIS (KISHI); Yellow – MOU (MOKWA); Pink – NBS (NEW BUSA); Lime – NIG (MINNA); Light Red – OKE (OKUTA); Green – PAT (PATIGI); Light Pink – SAM (SAMINAKA).*

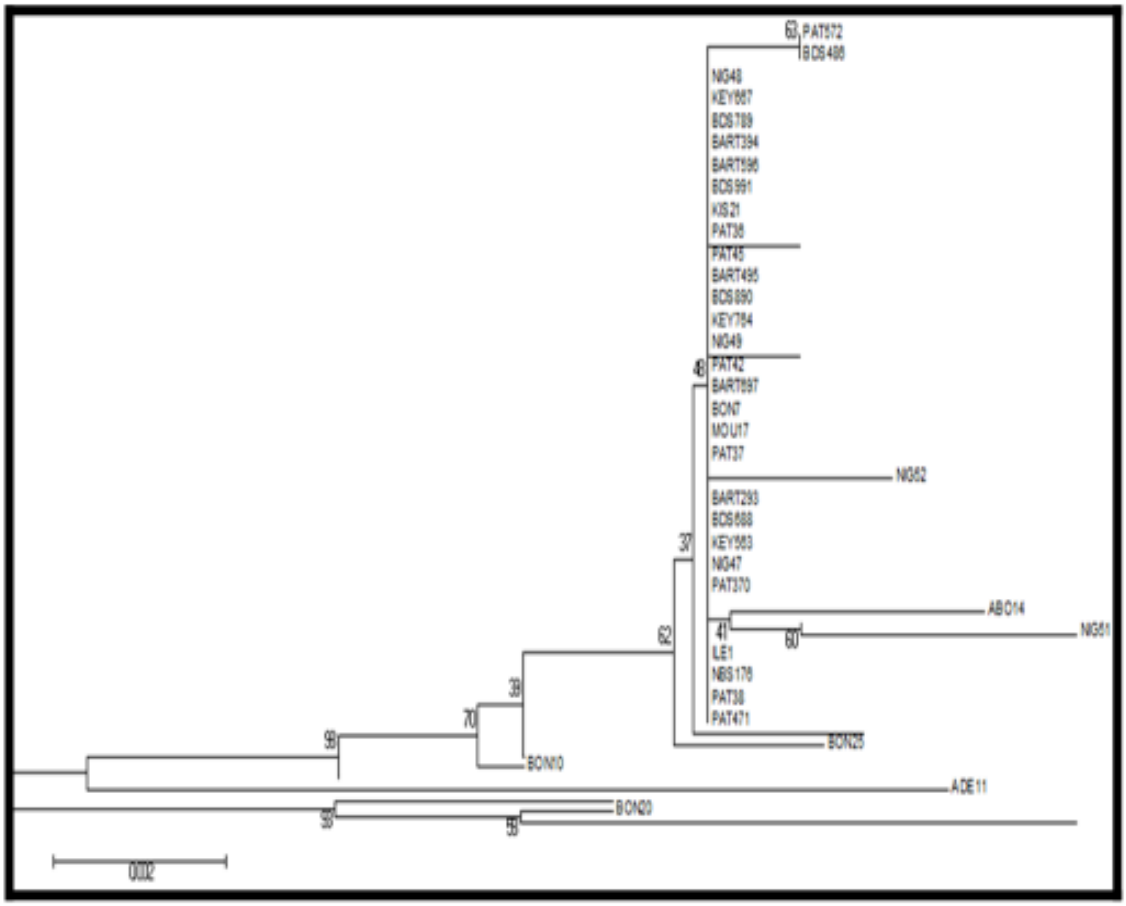

Figure 4: Phylogenetic relationship among 72 Nigerian Muscovy duck samples.

Discussion:

Muscovy ducks as an integral part of the local poultry sector in Nigeria are concentrated mostly in rural areas and reared by small-holder peasants, their persistent for improvement and empirical research have not helped its development, therefore information on their genetic attributes is vital and pivotal and to their conservation and improvement.

The results of this study corroborate the assertion of Yakubu *et al.,* (2011) that genetic diversity is important for duck breeding management as it reflects the relationship between the Muscovy duck population and it will guide the breeder in designing the plan for Nigerian Muscovy duck. It is also in line with the report of Kalita (2015) that genetic diversity is useful for long term breeding programs such as genetic mapping and molecular breeding marker-based as it is a combination of genes found in a population and different patterns of variation across populations in the same species. From this study, it was discovered that the genetic distance across duck populations is diverse but is of closer niche within the population with the use of mitochondrial cytochrome b gene which has been known and reported to be useful with respect to structure and function of its protein product (Esposti et al*.,* 1993). The results show the systematic diversity through the phylogenetic tree and it was in line with the report of Kirchman et al. (2000) and that of Lovejoy and de Arau´jo, (2000) and there is evident that the majority of Nigerian Muscovy ducks studied are related by descent and this could be as a result of the high rate of random mating within the population of Nigerian Muscovy duck studied.

This study also shows that the Muscovy duck sampled from the 15 locations were of 17 haplotypes with haplotype diversity of 43.9% and this corroborates with the findings of Ogah et al. (2017) where the author compared Nigerian Muscovy duck with some selected duck breeds using mtDNA D-loop region and reported few diversities between the population of Nigerian Muscovy duck. This study was further substantiated by the report of Maak et al. (2003); He et al. (2008) and Leekaew et al. (2010) which classified

some Muscovy ducks as an outgroup because of their polyphyletic relationship nature.

The results of this study also reflected the fact that the Haplotype (Hd) and Nucleotide diversity (Pi) of populations were the main indexes for evaluating the mtDNA variation and genetic diversity of any breed or population because the greater the diversity of the haplotype and nucleotide, the richer the genetic diversity of any population. A low value of nucleotide diversity and few mutations obtained for all duck populations studied is in line with the report of Wu et al. (2008) where low levels of genetic diversity of a population were reported and the authors reported few genetic mutations which were attributed to a possibility of genetic drift which allows changes of gene frequencies with non-significant value.

The phylogenetic tree obtained in this study revealed a clustered relationship in support of earlier results and this also shows that Muscovy ducks, like other domestic animals, are transported around through human activities and this creates an opportunity for intensive genetic admixture as stated by Adeola et al. (2015, 2020). In addition, since the Nigerian Muscovy duck has been managed with no planned breeding program, the impacts of selection pressures are minimized and might not have applied to their population. The distant genetic relationship observed in three populations from this study might be a result of migration or random genetic drift in their natural populations.

Conclusion

Genetic characterization is essential for the sustainable utilization and conservation of Nigerian Muscovy duck genetic resources and this study has established low genetic diversity, few polymorphisms with the majority of the Muscovy duck studied clustered within the same monophyletic clade which is an indication that Nigeria Muscovy duck is likely descent of common maternal lineage with respect to MtCYTB and are under genetic equilibrium within and between genetic variations across the studies locations.

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Conflict of interests

The authors declare no competing interests.

Availability of data and Materials

The authors declare that data collected and materials used are available.

Authors Contributions

Yusuf, Sola-Ojo and Adeniyi conceived and designed the study

Yusuf, Sola-Ojo, Adeniyi, Adesina and Okeke were involved in data collection

Sola-Ojo and Adeniyi analyzed and interpreted the data

Yusuf and Sola-Ojo wrote the manuscripts while other authors contributed in editing and approved the manuscript

Ethics Approval and Consent to participate

This work was carried out under the ethical approval of the University of Ilorin, Ilorin, Kwara State, Nigeria with approval number UERC/ASN/ 2021/2161.

Consent for Publication

Not applicable

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