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Genetic Characterization of Three Strains of *Gallus domesticus* Linnaeus, 1758 in Nigeria using Random Amplified Polymorphic DNA (RAPD) and Microsatellite DNA Markers

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ABSTRACT

This study was carried out to characterize three strains of Nigerian indigenous chickens, *Gallus domesticus* using molecular markers; Microsatellite (SSR) and Random Amplified Polymorphic DNA (RAPD). This was with a view to determining the amount of genetic diversity in the chicken populations as well as differentiating the strains genetically which has implications for their conservation and effective management. A total of thirty chickens; ten each belonging to normal feather, frizzle feather and naked neck strain were characterized by five RAPD and five microsatellite DNA primers. Indices of genetic diversity and differentiation within and among the populations were estimated using Genalex software. Result from both the RAPD and microsatellite data suggest that the three chicken strains had relatively low genetic diversity as indicated in their Ho values which ranged from 0.301 to 0.523. Dendrogram based on the microsatellite data showed a clear separation among the studied chicken strains. This might be due to the fact that these local chicken strains originated from different background and bred for different purposes. Our result suggests that the studied *G. domesticus* strains needs conservation intervention in order to preserve the uniqueness of their genetic identity and also monitor their long-term health and persistence.
KEYWORDS: Genetic Diversity, Conservation, Strain, Differentiation, Heterozygosity

INTRODUCTION

The indigenous chicken (*Gallus domesticus*) which belongs to the family Phasianidae, order Galliformes is widely believed to have evolved from the wild red jungle fowl, *Gallus gallus* from south-east Asia (1). *G. domesticus* is distributed widely in Nigeria and are raised by several households especially in rural areas; constituting about 80% of the 120 million poultry type raised in Nigeria (2). Indigenous chickens are strong and hardy; they adapt easily to changes in environment and they have the ability to fend for their own food by exploiting different food source in their immediate environment ranging from plants to grains, cooked food and even small arthropods and lizards. They also have the ability to hatch on their own and they possess appreciable immunity against endemic diseases. *G. domesticus* flesh is a choice meat for majority of Nigerians because of its sweet taste, high nutrition and leanness. It is also considered a special delicacy for occasions and its sales generate huge profit which serves as a good source of income at both subsistence and commercial scale (3).

Previous descriptions of local chickens in Nigeria are based on morphological characterization of various chicken types such as normal feather, naked neck, frizzle feather, featherless wing and rose comb (4). Multivariate analysis was used to morphologically characterize three strains namely: frizzle feather, normal feather and naked neck (5). Meanwhile, in recent past; the use of molecular markers has been adopted to genetically characterize several strains of the indigenous chicken which include normal and frizzled feathered strain, naked neck strain, dwarf type and those with colour variants such as black, white, brown, mottled etc. (6). The indigenous chickens have also been characterized based on location as various ecotypes of the indigenous chicken in different ecological zones of Nigeria have been reported (7). Similarly, two ecotypes from rain forest and savannah zones of Nigeria were reported and characterized as Yoruba and Fulani ecotypes respectively (8). Three different strains characterized in South-Eastern states of Nigeria were identified as Nsukka, Owerri and Awgu types (9). The normal feathered strain of *G. domesticus* has been reported as the most common, having the highest abundance in different agro ecological zones. The naked neck and frizzled feathered strains are scarce and may eventually become endangered if conservation efforts are not adequately channeled towards them. This can be achieved by estimating and monitoring their genetic diversity while

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attempting to know how genetically differentiated the strains are. Genetic diversity is the variation in DNA sequences from individual members of a given species (10). It is necessary for the long-term health of populations and persistence of species as it helps individual organisms to adapt and cope with environmental changes. When a population of a species is sufficiently diverse, some individuals would possess alleles that would be able to withstand pressure, survive, reproduce and contribute to the gene pool of the next generation, thus preventing extinction of such a population. Loss of genetic diversity is known to reduce the potential of populations to evolve (11). Genetic diversity among individuals leads to differentiation at the population level, species level and other higher taxonomic groups (12).

Random Amplified Polymorphic DNA (RAPD) marker and microsatellite markers are suitable genetic markers that have been widely used in characterizing strains/populations of different species of chickens. The sustainable management, utilization and conservation of domestic animals require its genetic characterization. The genetic characterization of *G. domesticus* strains is of utmost importance in the bid to improve their production and the conservation of their genetic diversity in order to forestall their extinction. This study therefore seeks to determine the genetic diversity and differentiation pattern among three strains (Frizzle feathered, FRF; Normal feathered, NF and Naked neck, NNK) of *Gallus domesticus* using RAPD and microsatellite markers.

MATERIALS AND METHODS

Animal Collection, DNA Extraction and Polymerase Chain Reaction (PCR)

A total of thirty *Gallus domesticus* individuals, ten from each strain viz: frizzle feathered (FRF); normal feathered (NF) and naked neck (NNK) were collected from the Teaching and Research Farm Directorate, Federal University of Agriculture (FUNAAB), Alabata and OGADEP (Ogun State Agricultural Development Project), Abeokuta, Nigeria. The three chicken strains which have been previously described by (7) were characterized by five RAPD primers and five microsatellite primers (Table 1&2). All applicable international, national and institutional ethical standards for the care and use of animals were followed. Blood samples were collected from the veins of each of the chicken in a process known as brachial venipuncture. Genomic DNA was extracted from the blood samples using Qiagen DNA easy blood and tissue kit while the quantity and quality of the DNA was measured on Nanodrop Spectrophotometer. Isolated DNA was amplified following standard PCR protocol as described by (13, 14). The PCR reaction mixture was carried out with a 25 µl final volume containing 1 µl template DNA, 2.5 µl of 10 x Buffer, 1 µl of 25 mM dNTPs, 2 µl of each pair of forward and reverse primer, 0.2 µl of (5 U/µl) Taq DNA polymerase, 2.2 µl of 25 mm/mol $Mg2+$ and 16.1 µl nuclease-free water. Amplification on the thermal cycler was run with the following program: initial denaturation at 95°C for 5 mins, denaturation at 94°C for 60 sec, annealing for 30 sec, and extension at 72°C for 60 sec and final extension at 72°C for 10 mins. The annealing temperatures of the primers used were adjusted through optimization using a temperature gradient. The PCR products were run on 2% agarose gel electrophoresis and detected under UV light with the aid of a trans-illuminator.

Table 1: List of Operon Random Primers Used.

Primer Codes	Sequence
OPA18	CAGGCCTTC
OPB08	TGCCGACTG
OPB14	AGTCAGCCAC
OPB18	AATCGGGCTG
OPB20	AGGGGTCTTG

Table 2: List of Microsatellite Primers Used for the Study

Locus	Annealing Temperature (^{0}C)	Forward and Reverse Primer Sequence (5$^/$-3$^/$)
MCW0018	55	TCCCTAGGCAAACCTGCTTAC AAGACCCCACAACTTGACTTG
MCW0029	68	CATGCAATTCAGGACCGTGCA GTGGACACCCATTTGTACCCTATG
MCW0032	55	AAGTTCCTTGTACAATTGTTA CATTACTAGTACAATCAAGATGG
MCW0036	55	CCTCATGTGAAGCATCTTTTCATA TGTCTTCAGTAGGACTGTGATAC
MCW0040	50	ACCGAAATTGAGCAGAAGTTA ACTCAAAAATGTGTAGAATATAG

Statistical Analysis

Band sizing was performed using a 100 base pair DNA ladder (Norgen PCR Sizer) loaded along side the PCR products. For microsatellite DNA analysis, DNA fragments were scored manually and the sizes were estimated using semi-log plot. The RAPD bands were scored as binary data using GelQuest software. The similarity coefficients were calculated across all pair while the binary values were transferred into NTSYS software for analysis of genetic similarities. The data generated were analysed using the GenAlEx Software, version 6.5 (15). Microsatellite data were analysed with Microsatellite Analyser (MSA) version 4.05 (16) and GenAlex 6.5 software (15, 17). The following indices of genetic diversity were assessed for each population: mean number of alleles (Na), number of effective alleles (Ne), Shannons' information index (I), observed and expected heterozygosity (Ho and He) and percentage of polymorphic loci (P). Analysis of hierarchical F statistics which includes Fis (the inbreeding coefficient within individuals relative to the subpopulation); Fit= the inbreeding coefficient within individuals relative to the total and Fst (the inbreeding coefficient within subpopulations relative to the total) were assessed over all populations for each locus. Pairwise population genetic differentiation (Fst) was used to analyze the degree of genetic differentiation between population pairs. Gene flow (Nm) was estimated from Nm = 0.25 (1-Fst)/Fst (18). Neighbour-joining method and analysis of molecular variance (19) were used to construct phylogenetic trees for the three chicken populations. Test for conformity to Hardy-Weinberg equilibrium was assessed at each locus for all the populations studied. The significance of the deviation was evaluated with a Chi-Square test following the method of (20).

RESULTS

RAPD results

A total of 35 bands were produced with 100% polymorphism across the three chicken populations (strains). The electrophoresis gel photographs for two of the primers (OPB 08 and OPB 14) are shown on Figures 1 and 2. Mean expected heterozygosity (H_E) across the three populations of *Gallus domesticus* was 0.378 $\pm$ 0.015, 0.396 $\pm$ 0.014 and 0.398 $\pm$ 0.017 for the normal feathered, frizzle feathered and naked neck populations respectively (Table 3). The mean number of alleles (A_N) across the three population, effective number of alleles (A_E) and Shannon index information (I) are shown on table 3. Analysis of molecular variance (AMOVA) showed that 100% of the total molecular variance was within the population and there was no variation (0%) among the populations.

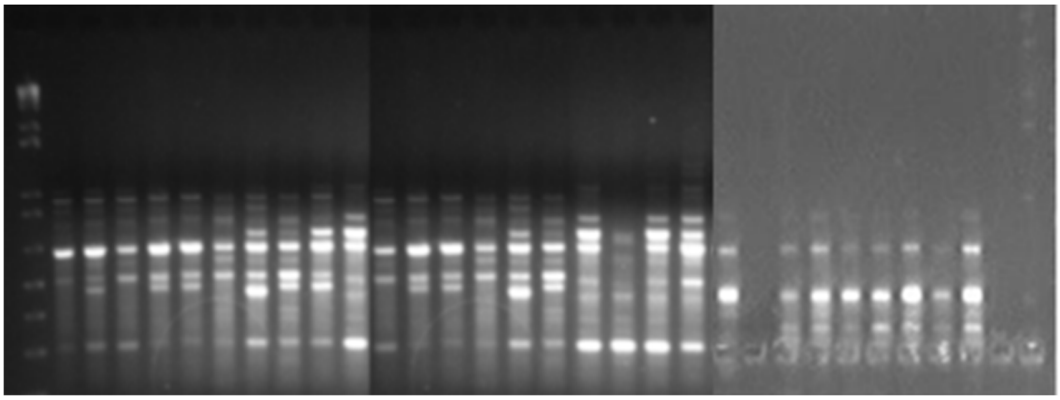

Figure 1: Gel electrophoresis for RAPD marker OPB 08

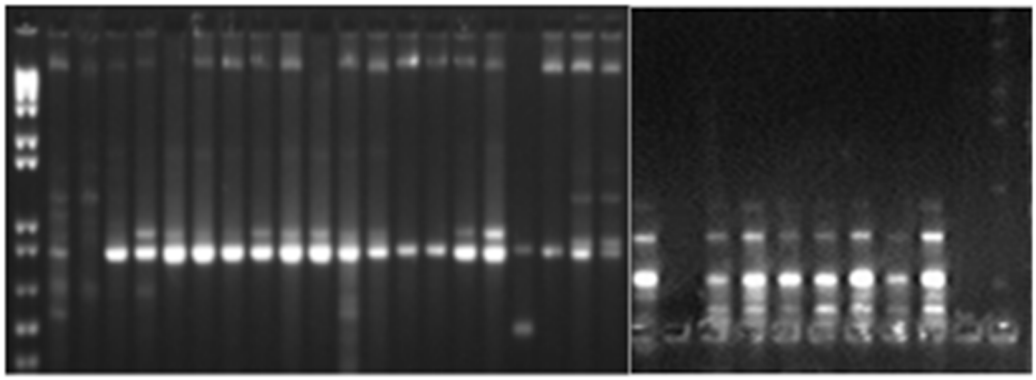

Figure 2: Gel electrophoresis for RAPD marker OPB 14

Table 3: Basic Indicators of Genetic Variation across the Sampled Populations from RAPD Analysis

Population	Polymorphic Loci	Mean no of Alleles (A_N)	Effective no of Alleles (A_E)	Shannon Information Index(I)	Expected Heterozygosity (He)	Unbiased Expected Heterozygosity (UHe)
NF	100	2.00	1.637±0.037	0.561±0.017	0.378±0.015	0.398±0.016
NNK	100	2.00	1.684±0.037	0.582±0.015	0.396±0.014	0.417±0.014
FRF	100	2.00	1.689±0.039	0.582±0.020	0.398±0.017	0.419±0.018
MEAN	100	2.00	1.670±0.033	0.575±0.015	0.391±0.014	0.411±0.014

NF =Normal Feathered, FRF = Frizzled Feathered, NNK = Naked Neck

Microsatellite Result

A total of 25 alleles were scored in the microsatellite analysis with the sizes ranging from 210 base pairs to 1500 base pairs while 95% success was recorded upon amplification. Electrophoresis gel plate for two of the microsatellite primers are represented on Figures 3 and 4. Allelic diversity ranges from two to eight alleles per microsatellite locus. The overall heterozygosity for all samples at all the five microsatellite loci was 30% and no locus was monomorphic. The mean number of alleles (Na) per population, the effective number of alleles (NE) contributing to the population, Shannon information index (I), observed and expected heterozygosities as well as percentage of polymorphic loci are shown on table 4. Analysis of molecular variance (AMOVA) showed that 83% and 17% of the total molecular variance was within and among the populations respectively. The highest genetic identity (1.1036) was recorded between frizzle feathered and naked neck population, while the lowest genetic identity value (0.7586) was recorded between frizzle feathered and normal feathered population (table 5). The phylogenetic consensus tree constructed using Nei's unbiased genetic distance (Figure 5) grouped all the three strains into two clusters. The dendrogram showed that frizzle feathered and naked necked populations clustered together and was separated from normal feathered. Thus, indicating that the frizzle feathered and naked necked populations are more similar genetically than the normal feathered population based on the microsatellite DNA analysis.

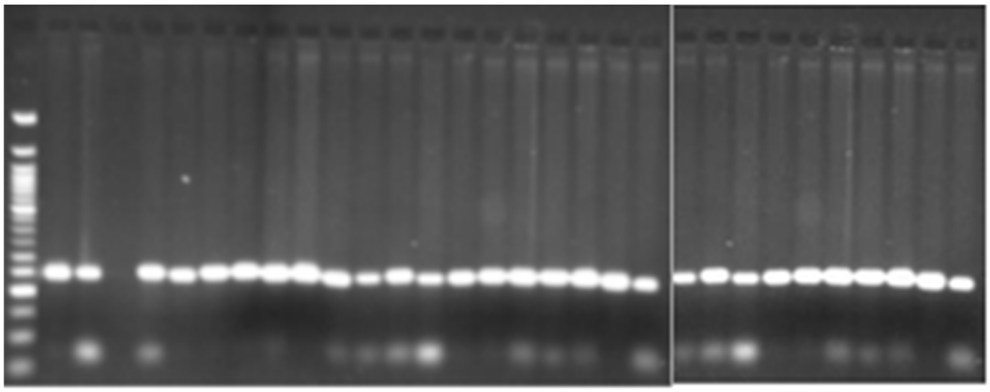

Figure 3: Gel electrophoresis for SSR marker MW0029

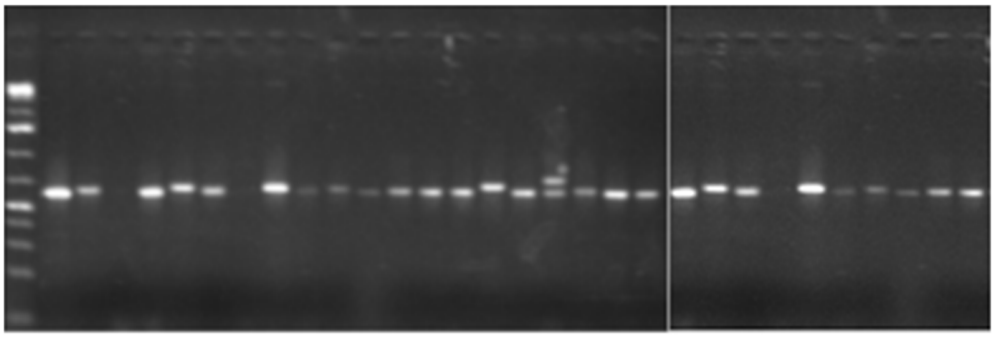

Figure 4: Gel electrophoresis for SSR marker MW0040

Table 4: Genetic Diversity Information for the three populations of *Gallus domesticus* estimated from five microsatellite loci

Population	Mean number of alleles (A_N)	Effective number of alleles (A_E)	Shannon's Information index (I)	Observed Heterozygosity (Ho)	Expected Heterozygosity (He)
NF	3.600±0.510	2.616±0.396	1.031±0.159	0.301±0.055	0.634±0.104
NNK	4.400±0.678	2.877±0.442	1.138±0.211	0.317±0.062	0.590±0.077
FRF	4.000±0.632	3.143±0.509	1.172±0.181	0.523±0.152	0.574±0.073

NF= Normal feathered, FRF= Frizzle feathered, NNK= Naked neck

Table 5: Pairwise Population of Nei's unbiased genetic distance (above diagonal) and genetic identity (below diagonal)

Population	NF	FRF	NNK
NF	***	0.4683	0.3912
FRF	0.7586	***	0.3317
NNK	0.9384	1.1036	***

NF= Normal feathered, FRF= Frizzle feathered, NNK= Naked neck

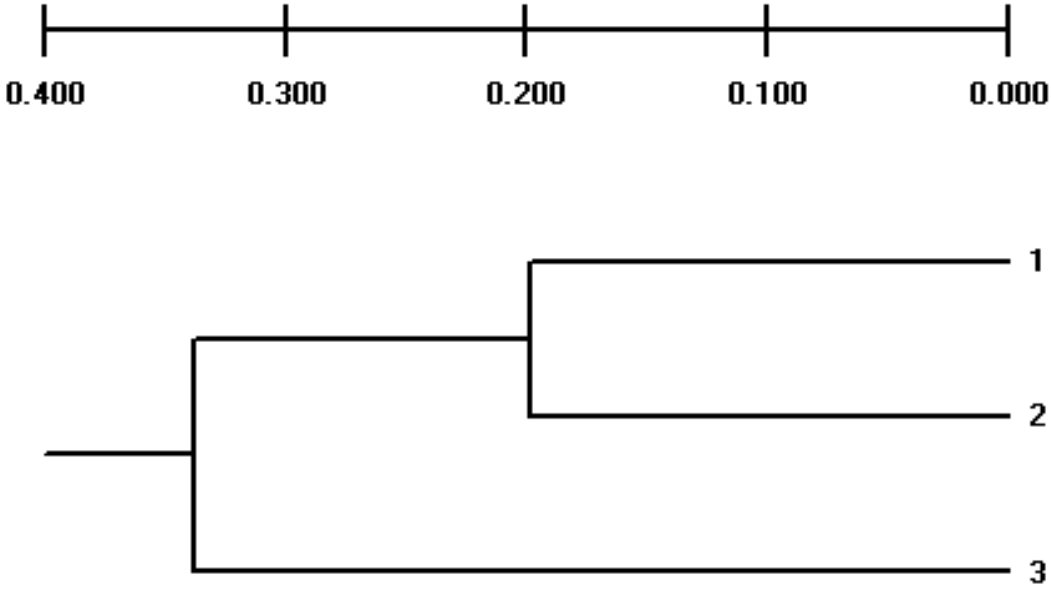

Figure 5: Dendrogram based on Nei's (1972) showing genetic distances between the three populations of *Gallus domesticus*.
1 represents Frizzled feathered, 2 represents Naked Neck while 3 represents Normal feathered.

Genetic structure

Microsatellite analysis revealed a high level of genetic differentiation within populations/strains. The coefficient of hierarchical Fst estimated among the three strains had an average value of 0.364 (Table 6). The overall gene flow (Nm) among the populations was 0.442, which gave an estimate of the average number of migrants between all studied populations per generation. The observed value shows that gene exchange between the populations is low. Test for departure from Hardy- Weinberg (H-W) equilibrium showed significant deviation ($P<0.001$) for at least one locus per population (Table 7). The deviations were attributed to deficit of heterozygotes in the populations. Fit which is the overall inbreeding coefficient of an individual relative to the whole set of populations had a mean value of 0.473 (Table 6). This shows that the three populations have deficiency of heterozygotes. Also, Fis which is the inbreeding coefficient of an individual relative to its own population had an average value of 0.562. This also shows that there is inbreeding within populations and a regular heterozygote deficiency.

Table 6: Analysis of Genetic differentiation and estimates of gene flow over all Populations for Each Locus

Locus	Fis	Fit	Fst	Nm
MCW0018	0.7638	0.8506	0.3676	0.4300
MCW0029	-0.0169	0.3258	0.3371	0.4917
MCW0032	0.3502	-0.6247	0.4225	0.3417
MCW0036	0.8507	0.9042	0.3578	0.4487
MCW0040	0.8611	0.9077	0.3354	0.4954
Mean	**0.5618**	**0.4727**	**0.3641**	**0.4415**

Fis = inbreeding coefficient at population level
Fit = Inbreeding coefficient at total sample level
Fst = Proportion of differentiation
Nm = Gene flow

Table 7: Test of Conformity to Hardy – Weinberg Equilibrium

Populations	Locus	Df	ChiSq	Probability	Significane
NF	MCW0018	1	10.000	0.002	**
NF	MCW0029	6	10.044	0.123	ns
NF	MCW0032	15	13.333	0.577	ns
NF	MCW0036	10	21.667	0.017	*
NF	MCW0040	10	30.000	0.001	***
NF	MCW0018	1	10.000	0.002	**
FRF	MCW0029	3	9.184	0.027	*
FRF	MCW0032	6	10.111	0.120	ns
FRF	MCW0036	10	17.357	0.067	ns
FRF	MCW0040	6	10.111	0.120	ns
NNK	MCW0018	1	9.000	0.003	**
NNK	MCW0029	6	21.000	0.002	**
NNK	MCW0032	15	12.833	0.615	ns
NNK	MCW0036	6	8.750	0.188	ns
NNK	MCW0040	6	7.389	0.286	ns

ns = not significant, *P<0.05, **P<0.01, ***P<0.001

DISCUSSION

The results of this study showed that all the RAPD and microsatellite markers used were highly polymorphic, thus establishing the usefulness of this markers in chicken's genome analysis (21). Genetic characterization using microsatellites, yield reliable estimates of variability among chicken populations, as demonstrated in several studies (22, 23, 24). In this study, microsatellite marker was able to characterize the populations more efficiently than the RAPD marker due to its high rate of mutation which brings about high standing allelic diversity. Microsatellite variability enables a clearer differentiation, even between closely related breeds, and provide more evidence of the predicted divergence (25, 26). The low heterozygosity values recorded in this study from both RAPD and microsatellite analysis is an indication that inbreeding is evident at the population level in the three strains of *G. domesticus* examined. Studies have reported negative correlation between mean heterozygosity from multiple loci in non-coding DNA regions and inbreeding depression in wild populations; as individuals that are inbred are relatively homozygous through out the genome unless there is a recent mutation (27, 28). Inbreeding usually leads to reduced fitness, a phenomenon referred to as inbreeding depression. Inbreeding increases the chance that an individual will be homozygous for segregating harmful recessive alleles which is the major factor responsible for inbreeding depression in populations (29). The heterozygosity values obtained in this study are comparable to those recorded in breeds of *G. domesticus* from Iran, India, Ethiopia, and Brazil in earlier reports (23, 24, 30). Significant average Fst value (0.36) recorded among the three strains indicates that the strains are genetically differentiated; thus, suggesting that each of the strains be treated separately for conservation and management. This will help to preserve the uniqueness of their genetic identity and reduce potential threat to their long-term health and persistence. The test of departure from Hardy-Weinberg equilibrium showed that none of the studied populations of *G. domesticus* is at random mating as they all deviated significantly from H-W equilibrium ($P< 0.001$) at not less than two, out of the five microsatellite DNA loci examined. This also accounts for the positive inbreeding values observed in the chicken populations. The significant deviation of the populations from H-W equilibrium at several loci is also an indication that the populations are under the influence of evolutionary forces such as mutation, migration, genetic drift, and selection; therefore, the populations can be said to be evolving.

CONCLUSION

The study concluded that the three *G. domesticus* strains/populations need conservation intervention in order to ensure their increased productivity and sustainable yield. Also, each of the strains should be treated with separate management and conservation approach in order to preserve the uniqueness of their genetic identity and forestall probable extinction in the nearest future.

Competing Interest

The authors declare that they have no conflicts of interests.

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