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Full-length Research Article

An Evaluation of the Genetic Variability of *Tilapia guineensis* Populations Based on Microsatellite Markers

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Summary: Improvement of aquaculture production, conservation and stock management of T. guineensis species are important. The genetic variation of this species, as well as its cross-breeding pattern and growth performance were assessed. One hundred and fifty live samples with average weights of TG (21.90g and 31.60g) for females and males, respectively, were collected from three different water bodies. DNA was extracted using the phenol-chloroform isoamyl alcohol method from randomly selected fish populations. The quality and quantity of DNA were determined using a spectrophotometer. Microsatellites were amplified, and PAGE electrophoresis was analyzed using PopGene version 3.6 software. The DNA concentrations ranged from 100.36 ng/l to 3889.40 ng/l with purity values of 1.69 to 2.00. The partial regions of the gene fish populations amplified were polymorphic, with an average of two allele differences in frequency. The values of gene diversity, polymorphic information content, and inbreeding coefficient were 0.41, 0.30, and -0.41, respectively. Observed heterozygosity was higher than expected heterozygosity in the fish populations. The genetic variations observed among the crossbreeds emphasized the importance of selective breeding for fast growth by increasing productivity, but pure lines should be maintained so that they form part of the baseline population and avoid inbreeding over time.

Keywords*: T. guineensis, genetic variation, cross-breeding, growth performance.*

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INTRODUCTION

Genetic tools are being used to significantly increase production in world aquaculture as well as to conserve and protect. The progress of DNA-based markers has had an innovative impact on gene mapping and, more generally, on all of animal and plant genetics. According to Nazari and Pourkazemi, (2023), different molecular tagging methods have been designed to study fish species characterization and population structure. Microsatellite, or Simple Sequence Repeats (SSR), has been among the tagging methods (Chen *et al*., 2024). The use of molecular genetic techniques such as SSR in fisheries research has increased due to the increasing availability of such techniques in research labs.

The increasing importance of fish farming has necessitated advancements in the technology required to secure the early and fundamental prerequisites for productive aquaculture, namely the production of fish seed for stocking. According to Sanda *et al*. (2024), the status of genetics in aquaculture would positively impact sustainability in the development of fish breeding programs in several developing countries, especially in Africa. Population and quantitative genetics are critical components in assessing genetic diversity or variation, which characterize naturally occurring genetic differences across individuals of the same species (Salgotra and Chauhan, 2023). According to Vieira *et al*. (2025), evaluating genetic diversity and genetic relationship base population is critical for successful control over generations, reducing problems associated with genetic potential loss for selective breeding. According to Mojekwu and Hoareau (2024), detected microsatellite loci might be recommended for characterizing *Tilapia species* in Nigerian water bodies. Such information has implications for future bloodstock selection and breeding management and for further analysis of interactions between different populations of *Tilapia species*. As a result, the goal of this study was to use microsatellite markers to describe *Tilapia guineensis* of both broodstock and crossbreed in order to improve the quantity and quality of fish seeds for hatchery operations for aquaculture purposes.

MATERIALS AND METHODS

Sample Locations: Live fish samples (broodstocks) (50 fish samples from each location) were collected from three locations in Southwestern Nigeria, namely, Ondo (On), Oyan (Oy), and Lagos (La) Lagoons. The broodstocks were collected from Mahin (Ondo) (O) Lagoon, Oyan Lake (Oy), and Lagos Lagoons (L). Ondo Lagoon with coordinates N 5055′05″ and E40590′ 2″; N ′ and E3015′20′ and N6029′ 24″ and E3023′58″, respectively (Fig. 1).

Collection and Paring of Broodstocks: The broodstocks were collected with oxygenated double nylon and transported to Badore NIOMR, Lagos, and acclimated for 2 weeks. The average weight of females and males of 21.9g and 31.6 g, respectively, were paired (each strain of the locations was crossed together) in a ratio of 1:2 in the triplicate: pure strains (La x La; On x On; Oy x Oy) and crossed breeds (La x On; Oy x On; La x Oy), respectively. The spawning and hatching periods occurred. After the fry's absorption of yoke, the fry was transferred into a white tank for culturing; they were fed with 45% COPPENS FEED intensively for 4 weeks.

DNA extraction: A total of 180 fish samples (DNA) were extracted from the caudal fin tissue of both the broodstock and randomly selected offspring (F1 generation) using a modified chlorophenol / isoamyl / alcohol protocol according to Sambrook and Russell (2001) on the bench at the biotechnology laboratory of NIOMR, Badore Outstation, Nigeria. Electrophoresis on 0.8% ethidium bromide-stained agarose gel (Plates 1a and 1b) was used to assess the integrity of the isolated DNA. A nano-drop spectrophotometer (Shimadzu Corporation Japan, MODEL UV-1800, 2000 series) at an absorbance of 260/280nm was used to check the concentration of the DNA. It was diluted to a final concentration of 50–100 ng/µl in highly purified water for amplification. The isolate was stored at -20°C prior to PCR amplification.

PCR Amplification: Nine microsatellite primers (Ukenye *et al*; 2022), were used to characterize and investigate the genetic variation between and among broodstocks from different locations, as well as the first filial generation (F1) offspring from cross-breeding. The polymerase chain reaction was carried out on a gradient thermal cycler (Biorad, module 10-8731) to determine the annealing temperature of each primer, which ranged from 55oC to 65oC (Table 1).

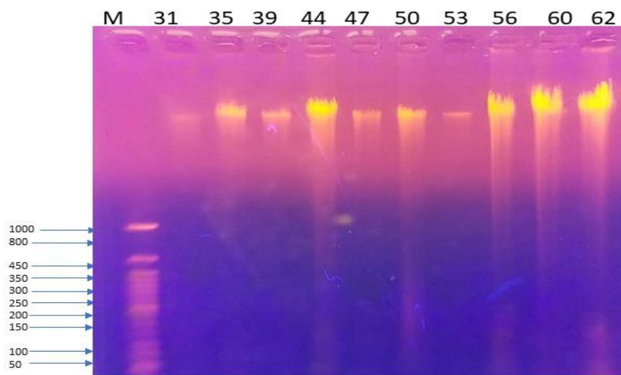

Plate 1a:
Agarose Gel Electrophoresis from randomly selected DNA of *T. guineensis*.

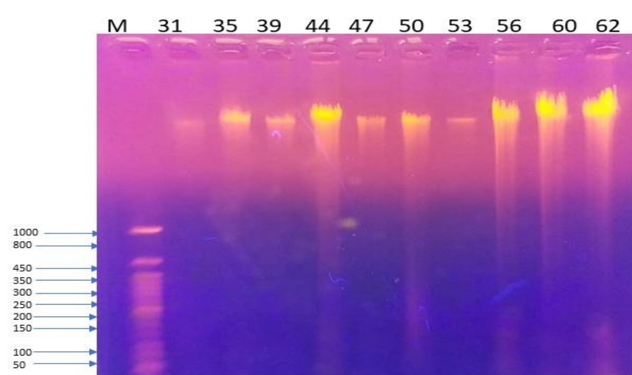

Plate 1b:
Agarose Gel Electrophoresis from randomly selected DNA of *T. guineensis*.

Table 1:
SSR Primer Code, Sequence, Annealing Temperature and Band Size

Primer code	Sequence	Annealing Temperature (°C)	Molecular size (bp)
GM 211	Forward 5' GCAAGTTGAGAGGCTACTGT 3'	60	178–398
	Reverse 3' AAACAACCCACAACCTTAGTT 3'		
UNH 995	Forward 5' CATCATCACAGAGACAGATTAGA 3'	55	180-350
	Reverse 3 ' GCAGCACAACCACAGTGCTA 3'		
UNH 123	Forward 5' CATCATCACAGACAGATTAGA 3'	55	145-208
	Reverse 3' GATTGAGATTTCATTCAAG 3'		
UNH 207	Forward 5' ACACAACAAGCAGATGGAGAC 3'	55	140-220
	Reverse 3' CAGGTGTGCAAAGCAGAAGC 3'		
UNH 146	Forward 5' CCACTCTGCCTGCCCTCTAT 3'	55	130-300
	Reverse 3' AGCTGCGTCAAACTCTCAAAAG 3'		
GM 538	Forward 5' CAGCATGTTGTCTGGATCTTG 3'	60	140-300
	Reverse 3' TTTGTTGCTGTGGTCTGTTCTT 3'		
GM 531	Forward 5' AAAGCCAACGGTCTGAATTG 3'	55	140-190
	Reverse 3'AGCAGAGGACACCCCTCAT 3'		
UNH 104	Forward 5' GCAGTTATTTGTGGTCACTA 3'	55	170-250
	Reverse 3' GGTATATGTCTAACTGAAATCC 3'		
UNH 185	Forward 5' CAGACACACTAGACACATTCTA 3'	55	120-150
	Reverse 3' GTGTTTCCATGTGTCTGTAC 3'		

Lee *et al.* (2005) and Saad *et al.* (2013)

Each PCR tube received a total volume of 25µl of PCR components, which included 2.5µl of 10 x PCR buffer, 1µl of 25mM MgCl2, 1µl of each primer (forward and reverse), 1µl of DMSO, 2µl of 2.5mM DMSO, 0.1µl of 5u/ul Taq DNA polymerase, 3µl of 10ng/l of DNA, and 13.4µl of nuclease-free water. The ingredients were run on a thermocycler (Biorad, module 170-8731) for amplification. The PCR condition is: 96oC for 2 minutes (initial denaturation); 30 cycles of 94oC for 30 seconds (denaturation); 55oC (optimal temperature of each primer, which varies for each of the primers) at 30 seconds; 72oC for 30 seconds, followed by a final extension of 72oC for 6 minutes.

Polyacrylamide gel electrophoresis: 6% of polyacrylamide gel was prepared by mixing 7.5ml of acrylamide (instant page buffer), 2.5ml of TBE buffer (Tris-borate/EDTA electrophoresis buffer), 50µl of TEMED, and 500µl of ammonium per sulphate (APS) in 40ml of distilled water, giving a total volume of 50ml. A total 8µl volume of solutions, which consist 6µl of PCR product and 2µl of 6x loading dye, were loaded into the wells created by the combs. A 1 kb base pair (bp) ladder was used as the size standard, which was loaded alongside the PCR products on the gel. The gels were then viewed with a UV transilluminator (SpectrolineR TC 312 E), and the pictures were taken using a camera (Alpha Imager) (Plate).

Statistical Analysis: Population genetics data generated was analyzed using PopGene version 3.6 software to obtain the number of alleles per Simple Sequence Repeat (SSR) locus (Na), effective number of alleles (Ne), Shannon information index (I), observed heterozygosity (Ho), expected heterozygosity (He), and Nei's Pairwise genetic distance.

RESULTS

DNA purity quantification: Concentration and purity values are shown in Table 2, with mean concentration values ranging from 100.36 ng/l to 3889.40 ng/l and purity values ranging from 1.69 to 2.00 using a nano-spectrophotometer.

Genetic Variability of *Tilapia guineensis* among Microsatellite Loci: A total of 18 alleles were found in the study of 9 populations of both *Tilapia guineensis* (broodstocks, pure lines, and crosses). The mean average number of alleles per locus generated by each marker was 2, with variations in the frequency of alleles. Table 3 shows the allelic diversity of the coding region of *Tilapia guineensis* with the same alleles as two (2). Locus UNH 146 had the highest number of allele frequencies (0.95), while Locus UNH 185 had the lowest allele frequency (0.45). The level of diversity revealed by the studied loci ranged from 0.04 to 0.50, with an average of 0.39. The PIC value of each primer is evaluated on the basis of its alleles. The value for *Tilapia guineensis* varied greatly for all the tested Simple Sequence Repeat (SSR) loci, from 0.04 to 0.38 with an average of 0.30 (Table 4). The highest PIC value of 0.38 was obtained for UNH 123 and UNH 104, followed by UNH 185 and UNH 211 (0.37), UNH 995 (0.36), GM538 (0.31), UNH 146 (0.30), UNH 207 (0.20), and GM 531 (0.04), respectively, with an average value of 0.30 of major allele frequency.

Table 2:
DNA concentration (ng/µl) and ratio of absorbance (260/280nm) of DNA extracted from fish population broodstocks, pure lines and crossbreed lines of *T. guineensis*

Sample source	Species	Concentration (ng/l)	Purity	Mean Purity(X^{-1})	Mean ±SD
Lagos Broodstock	*T. guineensis*	830.87 - 2036.24	1.76 - 1.93	1.88	1.88±0.06
Lagos (Pure line)	*T. guineensis*	100.36 - 2459.85	1.57 - 1.93	1.85	1.85±0.10
Lagos X Ondo (Cross breed)	*T. guineensis*	573.79 - 1666.40	1.70 - 1.96	1.69	1.69±0.27
Ondo Broodstock	*T. guineensis*	1319.40-3889.40	1.75 - 1.91	1.86	1.86±0.05
Ondo (Pure line)	*T. guineensis*	873.02-1031	1.50 - 1.95	1.88	1.88±0.98
Lagos X Oyan (Cross breed)	*T. guineensis*	704.67-835.93	1.68-1.98	2.00	2.00±0.11
Oyan Broodstock	*T. guineensis*	672.04-935.30	1.89 – 2.02	1.94	1.94±0.58
Oyan (Pure line)	*T. guineensis*	830.52-949.30	1.65 – 2.03	1.87	1,87±0.54
Oyan X Ondo (Cross breed)	*T.guineensis*	546.20-635.29	1.78 – 1.95	1.83	1.83±0.36

Table 3:
Characteristics of SSR loci analyzed for *Tilapia guineensis*

Maker	Freq	Sample size	NA	Gene Diversity	PIC
GM 538	0.88	180	2	0.38	0.31
GM 531	0.85	180	2	0.40	0.04
UNH 104	0.69	180	2	0.50	0.38
UNH 123	0.86	180	2	0.49	0.38
UNH 185	0.45	180	2	0.23	0.37
UNH 207	0.91	180	2	0.49	0.20
UNH 211	0.68	180	2	0.38	0.37
UNH 146	0.95	180	2	0.47	0.30
UNH 995	0.88	180	2	0.39	0.36
Means	**0.79**	**180**	**2**	**0.41**	**0.30**

Legend: **Freq** -Major allele frequency, **NA** – Number of alleles, **PIC** – Polymorphic Information content

Table 4:
Locus Specific Indices of Genetic Diversity in the *Tilapia guineensis* Population

Locus	Na	Fis	Heterozygosity	Fit	Inbreeding coefficient	Fst	D
GM 538	2	0.061	0.311	0.181	0.185	0.127	-0.59
GM 531	2	-0.049	0.040	-0.020	-0.017	0.027	-0.59
UNH 104	2	-1.000	1.000	-1.000	-1.000	0.000	-0.48
UNH 123	2	-0.988	0.994	-0.987	-0.988	0.000	-0.60
UNH 185	2	-0.336	0.506	-0.030	-0.019	0.244	-0.59
UNH 207	2	-0.378	0.264	-0.150	-0.150	0.165	-0.62
UNH 211	2	-0.868	0.916	-0.850	-0.844	0.025	-0.40
UNH 146	2	-0.393	0.466	-0.249	-0.241	0.104	-0.48
UNH 995	2	-0.278	0.426	-0.075	-0.086	0.276	-0.63
Mean	**2**	**1.63**	**0.387**	**0.65**	**-0.412**	**0.086**	**-0.55**

Na - Number of alleles, **Ne** – effective number of alleles, **Fit** – Maker Fitness, **Fst**- Maker Fixation Index, **Fis**- Inbreeding co-efficient of individual and **D**- Heterozygote deficiency calculated as D = (Ho-He)/He. **Ho** – Observed heterozygosity and **He**- Expected heterozygosity

DISCUSSION

The used of the phenol-chloroform isoamyl alcohol protocol for the extraction of the DNA, which proved to be good for obtaining pure DNA from the fish caudal fin. This finding is in agreement with the results obtained in a comparative study of an improved method of DNA extraction from fish fins and fish scales (Tayyab, 2021), in which repeated DNA phenol chloroform isoamyl alcohol showed no sign of degradation and spectrophotometer absorbance at 260/280 indicating a good DNA template for PCR analysis.

In the present study, nine (9) microsatellite markers were utilized to characterize and investigate the genetic variation in *Tilapia guineensis* populations for both broodstocks and their crosses, with the aim of having information in the base population for crossbreeding, minimizing inbreeding, and improving the seed quality among the sample's populations for breeding and conservation programs (Kumari *et al*; 2025)..

This study revealed an average Polymorphism information Content (PIC) which suggested that the makers are moderately informative, indicating that they have good merit to distinguish different *Tilapia species*. This is in line with the report of Marques, *et al*. (2023), which stated that the PIC values ranged from 0.25 to 0.50, which is somewhat informative.

The number of alleles in these fish populations studied shows the variation in the number of alleles across multiple loci within the populations. This is similar to the study conducted by Amoussou *et al*. (2025), in which the number of alleles ranges from 2 to 4 in the same locus positions on the chromosomes of Nile Tilapia (Oreochromis niloticus) in Volta Lake, Ghana, using microsatellite makers.

A total of 18 alleles were also observed in this study on fish populations, which is incomparable with the report of Sadler *et al*. (2023), who reported 75 allele frequencies in Oreochromis niloticus populations. The low gene variation encountered in this study may suggest there is a need for an increase in the diversity of these species through selective breeding and conservation programs.

In qualitative terms, a marker is considered polymorphic if it has at least two alleles, and the most frequent allele has a frequency range of 50% to 99% (Rezk *et al*., 2024). This is in accordance with this study, in which the average means allele frequencies of SSR markers used for *Tilapia guineensis* (81%). This finding suggests that there is a relatively high percentage of allele frequency among the fish populations, which can lead to genetic drift or new mutations within the populations.

The average means of gene diversity and inbreeding coefficient of the populations using microsatellite markers is relatively informative when compared with other markers such as ISSR (Hesamzadeh Hejazi., 2024). The gene diversity based on locus specifies that there is genetic differentiation between populations; this implies that there are a range of different inherited traits within a species. This is in agreement with the results obtained by Ekerette *et al*. (2024) on genetic variations of Oreochromis niloticus.

In this present study, the fish populations showed negative values of inbreeding co-efficient, which implies excess heterozygosity among the populations in disparity to what is expected in the intensive production of selective growth traits, which can lead to homozygosity among species. This is in accordance with the research findings of Böhne *et al*. (2023), who observed excess heterozygosity in a natural population of the West African cichlid fish Pelvicachromis taeniatus, which showed clear kin mating in experiments but no inbreeding depression. It is nevertheless not in agreement with Ukenye *et al*. (2022), in which some of the loci showed positive inbreeding co-efficient among *Tilapia guineensis* populations in some coastal water in Nigeria.

This present study deviated from the Hardy-Weinberg equilibrium principle, in which there are differences in genotype and allele frequency among the fish populations (Singh *et al*; 2024). These differences could be the result of forces such as mutations, natural selection, non-random mating, genetic drift, and gene flows. Therefore, microsatellite variation at most estimated loci was more informative in characterizing *Tilapia species* differences than the ISSR markers (Chang *et al*; 2024).

In conclusion, these results will provide useful information for genetic variations of T. guineensis and therefore provide the needed genetic information for effective decision-making toward the management of these fishes for improvement on aquacultural purposes. This information may be needed for effective management strategies by the government to plan toward managing the fisheries resources.

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