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Original Research Article

Evaluation of Genetic Variation in *Oreochromis* Tilapia Species from South-South Nigeria using Mitochondrial DNA Hypervariable Region

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ABSTRACT

Understanding genetic variation among species is essential for effective selection and breeding enhancements. This study was conducted to assess the genetic diversity among three tilapia species (*Oreochromis niloticus*, *Oreochromis aureus*, and *Oreochromis mossambicus*) from several rivers located in South-South Nigeria. A total of 300 samples representing the three species were used for this research. Blood samples were collected from all individuals for DNA extraction, amplification, and sequencing of the mitochondrial (mt) control region. Analysis of mitochondrial DNA revealed that *Oreochromis aureus* exhibited the greatest number of polymorphic sites, with a total of 225, compared to *Oreochromis niloticus* and *Oreochromis mossambicus*, which had 129 and 84 polymorphic sites, respectively. The number of haplotypes was highest in *O. niloticus* with five, while *O. aureus* and *O. mossambicus* each had three haplotypes. *O. niloticus* also demonstrated the highest haplotype diversity (0.796), whereas *O. aureus* showed the highest nucleotide diversity (0.139). The largest genetic distance was found between *O. aureus* and *O. mossambicus* (0.388), whereas the smallest genetic distance was noted between *O. niloticus* and *O. mossambicus* (0.217). Enhancing tilapia production in Nigeria can be achieved by selectively breeding tilapia from the Itu, Ethiope, and New Calabar Rivers, which exhibited high genetic variation.

***Keywords*:** Genetic distance, River, Selection, Tilapia, Variation.

Introduction

In Sub-Saharan Africa, changes in diet, economic instability, insecurity, and population growth have led to a greater need for fish, surpassing the available supply. Over 200 million Africans rely on fish as a crucial source of protein, mineral, and micronutrient source.[1] In approximately 20 African nations, fish contributes more than 20% of animal protein intake.[2] Globally, there has been a recent trend towards animal protein, including meat and fish.[3, 4] From 2007 to 2015, fish consumption in Sub-Saharan Africa ranged from 25% to 50%.[5] Factors like population growth, accessibility, affordability, and the health benefits associated with fish consumption are driving this demand.[5, 6] Despite increasing demand, fish production in Africa has remained slow.[7, 8, 9] Factors such as indiscriminate fishing, ineffective management, and environmental abuse have led to a decline in African fish stocks, risking the genetic erosion of crucial species.[1]

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Scholars emphasize the importance of employing data-driven approaches to characterize and measure aquatic food systems for both short and long-term sustainability.[10, 11, 12, 13] Access to robust data is vital for decision-making [14] and supporting investments in aquaculture,[15, 16] leading to a more sustainable, equitable, inclusive, and resilient food system.[17] In light of these concerns, urgent action is needed from the government, policymakers, and research institutions to explore, exploit, and conserve fish resources in Nigeria. Tilapia stands out as a highly sought-after fish due to favourable aquaculture characteristics, with its farming increasingly popular to meet rising demand.[18, 19, 20] Globally, tilapia is the third most farmed fish after grass carp and silver carp [21], contributing significantly to food security in countries like China, Egypt, the Philippines, Brazil, Thailand, and Bangladesh.[21] Worldwide aquaculture production of tilapia is estimated at 6.1 million tonnes [21] and Nigeria contributed only 69,579 tonnes. [22] Tilapia's ability to thrive on omnivorous diets and its straightforward reproductive processes make it one of the easiest and most profitable fish to farm. In 2018, global tilapia sales reached an estimated $12 billion.[20] China leads in tilapia production with 1,241,410 tonnes, followed by Indonesia with 1,172,633 tonnes, and Egypt with 954,154 tonnes.[19] Other significant contributors to tilapia production include Thailand, Bangladesh, Brazil, Vietnam, Myanmar, Mexico, Ecuador, Costa Rica, Honduras, Uganda, and Kenya.[20] In Africa, Egypt dominates tilapia production, accounting for 80% of the continent's total, while the rest of Africa contributes the remaining 20%.[20-21] In Nigeria, aquaculture plays a crucial role in providing an affordable and nutritious source of protein, with tilapia species being among the most commonly cultivated.[23] Nigeria's tilapia production lags behind other nations, leading to a heavy reliance on imports costing an estimated 125 billion naira annually.[23] This situation highlights the urgent need to adopt effective strategies to boost domestic fish production and improve

food security. A critical first step in this effort is the comprehensive assessment of genetic diversity among tilapia stocks and species in key water bodies. Without valid genetic information on diversity, it is challenging to make informed decisions for stock improvement and selection, which are essential for advancing production and enhancing sustainability.[20, 24]

Understanding the genetic diversity of tilapia and the various populations they inhabit offers significant advantages for selection, breeding enhancement, and conservation efforts. Genetic diversity studies are crucial for evaluating the genetic richness within populations and serve as a guide for selecting individuals with favourable genetic traits for breeding programmes.[24, 25] While traditional methods like meristic counts and morphometric measurements are commonly used to assess genetic diversity,[26, 27] the introduction of molecular markers has revolutionised these studies, yielding promising results that contribute to animal improvement and increased agricultural productivity.[6, 28] Sequence variation in mitochondrial DNA has proven useful in discriminating between tilapia species.[29, 30, 31, 32] The present study addresses the critical need to understand genetic diversity among tilapia species to improve selection, breeding, and conservation efforts. While most previous research has focused on single species, particularly the Nile tilapia, at the population level, our study pioneers a comparative analysis of genetic diversity across three tilapia species sourced from five populations in South-South Nigeria. By utilizing mitochondrial hypervariable regions, we aim to uncover stock variations, providing a foundation for enhanced breeding programmes and conservation strategies essential for sustainable aquaculture in the region.

Materials and Methods

Location and sample collection

From 5th May to 9th August 2022, a total of 300 mature tilapia were sampled from five sites across the Niger Delta region of Nigeria. The sample comprised 100 individuals each of *Oreochromis niloticus*, *Oreochromis aureus*, and *Oreochromis mossambicus*. The locations of the samples were recorded using the global positioning system (GPS) as follows: Itu River in Akwa Ibom State (5°12′5″N, 7°58′39″E), Anangtigha River in Cross River State (4°54′56″N, 8°20′39″E), New Calabar River in Ikwerre, Rivers State (4°59′55″N, 6°53′45″E), Kpansia River in Bayelsa State (4°56′55″N, 6°19′52″E), and Ethiope River in Delta State (5°54′25″N, 5°40′58″E). In each location, 20 samples per species (*O. niloticus, O. aureus* and *O. mossambicus*) were collected, resulting in a total of 60 tilapia per state. The fish included both males and females with an average weight of 0.734 kg, but sex was not considered a factor in the research.

Ethical statement

This research was conducted under the ethical approval of the Faculty of Biological Sciences Ethical Committee, University of Calabar, Nigeria (BIOSC22-08). The ARRIVE guidelines (https://arriveguidelines.org) for experimenting with animals were fully adopted.

DNA extraction from blood samples

Mitochondrial DNA was extracted at the Biotechnology Laboratory Unit in the Animal Science Department of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The Quick-DNA MiniPrep kit from Zymo Research, USA, was used for the extraction from whole blood samples. Beta-mercaptoethanol was added to the lysis buffer to a final concentration of 500 µl per 100 ml, following the manufacturer's instructions. The lysis buffer was mixed with 200 µl of blood in an Eppendorf tube at a 4:1 ratio (800:200 µl), vortexed briefly, and incubated at room temperature for ten minutes. The mixture was then transferred to a Zymo-Spin column in a collection tube and centrifuged at 10,000 rpm for one minute using a Centurion Scientific microcentrifuge (Model: C2015, USA). The flow-through was discarded, and the column was transferred to a new collection tube. Next, 200 µl of DNA pre-wash buffer was added, followed by centrifugation at 10,000 rpm for one minute. Then, 500 µl of g-DNA wash buffer was added and centrifuged at the same speed. The column was transferred to a clean microcentrifuge tube, 50 µl of DNA elution buffer was added, and the mixture was incubated at room temperature for five minutes before a final centrifugation at 15,000 rpm for 30 seconds. The DNA was then stored at -20 ℃ for further analysis.

Polymerase chain reaction (PCR) amplification

PCR amplification was carried out at STABVIDA Laboratory, Quinta de Torre, Portugal. The D-loop region was targeted using the primers Marinefish_Dloop_Thr_F (5'-AGCACCGGTCTTGTAAACCG-3') and Marinefish_Dloop_Phe_R (3'-GGGCTCATCTTAACATCTTCA-5'). Each PCR reaction mixture had a total volume of 15 µl, consisting of 2 µl of genomic DNA, 8.6 µl of double-distilled water (ddH_2O), 0.5 µl of $MgCl_2$, 1.5 µl of dNTPs, 1.5 µl of 10x PCR buffer, 0.37 µl of each forward and reverse primer, and 0.15 µl of STABVIDA proprietary Taq polymerase. The amplification was performed using a GeneAmp® PCR System (9700 thermal cycler, USA) with the following cycling protocol: initial denaturation at 95 ℃ for five minutes, followed by 25 cycles of denaturation at 94 ℃ for 40 seconds, annealing at 54 ℃ for 45 seconds, extension at 72 ℃ for one minute, and a final extension at 72 ℃ for seven minutes. The PCR products were then purified according to the Exofast protocol provided by the manufacturer.

Sequencing of D-loop

The D-loop region of mitochondrial DNA was sequenced for all tissue-DNA samples using the primers Marinefish_Dloop_Thr_F (5'-AGCACCGGTCTTGTAAACCG-3') and Marinefish_Dloop_Phe_R (3'-GGGCTCATCTTAACATCTTCA-5'). Sequencing was performed at STABVIDA Laboratory, Quinta de Torre, Portugal, with an AB13730×L sequencer. The sequencing reaction involved a 20 µl mixture containing approximately 20 ng of purified PCR product as the DNA template, 8 µl of Big Dye™ Terminator Reaction Mix (which included dNTPs, ddNTPs, buffer, enzyme, and $MgCl_2$), 8 µl of deionised water, and 2 µl of the primer. The process was set for 25 cycles with conditions of 96 ℃ for 10 seconds, 60 ℃ for 5 seconds, and 60 ℃ for 4 minutes.

Statistical analysis

Sequences were viewed and edited using ChromasPro version 2.6.6. Multiple sequence alignment for all samples was performed with MEGA 6.06.[33] Polymorphism metrics, including nucleotide diversity (π) and haplotype diversity (Hd), were calculated using DnaSP 5.1 software.[34] Genetic distances within and between species were assessed using MEGA 6.06. Natural selection on codons across different tilapia species was evaluated using the HyPhy method available in MEGA 6.06.[33] Mutation analysis of SNPs in the aligned sequences was conducted with CodonCode Aligner version 6.06.[33]

Results and Discussion

Mitochondrial DNA (mtDNA) stands out as one of the most commonly used markers for distinguishing and characterising organisms at both species and population levels. Its notable advantage in genetic diversity studies over nuclear DNA lies in its high mutation rate. [35] Among the 37 genes of mtDNA, the D-loop, also known as the hypervariable region, exhibits the highest mutation rate, consequently yielding the highest level of variation.[6, 36, 37] This distinctive feature of the mtDNA D-loop has sparked considerable interest among researchers in evolutionary biology, population biology, genetics, and related fields, as it offers a valuable tool for addressing critical research questions about species and population discrimination. In the field of fish genetics, numerous previous studies have highlighted the mtDNA D-loop as a highly reliable genetic marker for distinguishing both populations and species. [38, 39, 40, 41, 42]

Genetic variation among the tilapia species

Table 1 presents the mitochondrial DNA polymorphism data for the three tilapia species studied. *Oreochromis aureus* displayed the greatest polymorphism with 225 polymorphic sites, compared to *Oreochromis niloticus* and *Oreochromis mossambicus*, which had 129 and 84

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polymorphic sites, respectively. Among the species, *Oreochromis niloticus* had the highest number of haplotypes, totalling five, while both *Oreochromis aureus* and *Oreochromis mossambicus* each had three haplotypes. *Oreochromis niloticus* also showed the highest haplotype diversity (0.796 ± 0.001), followed by *Oreochromis mossambicus* (0.703 ± 0.004) and *Oreochromis aureus* (0.692 ± 0.002). In terms of nucleotide diversity, *Oreochromis aureus* had the highest value (0.139 ± 0.001), with *Oreochromis niloticus* (0.058 ± 0.00012) and *Oreochromis mossambicus* (0.052 ± 0.0002) following. Sequence conservation was highest among *O. mossambicus* at 87% and lowest among *O. aureus* at 72.6%. Table 2 has the result of the mt polymorphism between the three tilapia species within each location. The number of polymorphic sites was highest in *O. aureus* across the five locations followed by *O. niloticus* and *O. mossambicus* except in Kpansia River where mtDNA polymorphism was in the order *O. aureus* > *O. mossambicus* > *O. niloticus*. The presence of polymorphic sites was notably higher in *O. aureus*, indicating the potential for greater genetic variation within this species compared to *O. niloticus* and *O. mossambicus*. One method of quantifying genetic variation within and between species is by evaluating gene diversity, also known as haplotype diversity. Haplotypes represent genes preserved as sequences that persist through multiple generations of reproduction. *Oreochromis niloticus* exhibited the highest number of haplotypes, whereas *O. aureus* and *O. mossambicus* displayed similar haplotype counts. This indicates that *Oreochromis niloticus* has more conserved genes, showing a higher degree of similarity among individuals than the other two species. Previous studies identified 5 haplotypes in both *O. niloticus* and *O. aureus*.[29] Similarly, another investigation found six haplotypes in *O. niloticus* populations in South West Nigeria, which closely aligns with the haplotype count observed in the current study.[30] Authors [43] also identified 13, 11, 11, and 7 haplotypes in four populations of *O. niloticus*, while *O. esculentus* was identified with five and 11 haplotypes in two populations. These findings suggest that haplotype numbers may be species-specific and influenced by the environment. Thus, the differences in haplotype numbers observed among the three tilapia species in this study may stem from variations in their mitochondrial genome, with a particular emphasis on the mtDNA D-loop.

Table 1: Variation in mitochondrial DNA among three species of tilapia

Polymorphism indices	*O. niloticus*	*O. aureus*	*O. mossambicus*
Number of sequences (NSQ)	100	100	100
Number of sites (NS)	813	807	776
Monomorphic sites (MNS)	684	582	692
Polymorphic sites (PS)	129	225	84
Singleton variable sites (SVS)	08	14	10
Parsimony information sites (PIS)	121	211	74
Number of haplotype (NH)	5	3	3
Haplotype (gene) diversity (Hd)	0.796 ± 0.001	0.692 ± 0.002	0.703 ± 0.004
Nucleotide diversity (Nu)	0.058 ± 0.00012	0.139 ± 0.001	0.052 ± 0.0002
Average number of nucleotide difference (ANND)	42.35	112.830	40.527
Sequence conservation (SC)	0.839 (83.9%)	0.726 (72.6%)	0.870 (87.0%)
Minimum number of recombination (MNR)	6	0	0

Table 2: Variation in mitochondrial DNA between species of tilapia from five rivers of South South, Nigeria

		Polymorphism Indices											
Locations	**Species**	**NSQ**	**NS**	**MNS**	**PS**	**SVS**	**PIS**	**NH**	**Hd**	**Nu**	**ANND**	**SC**	**MNR**
ITU-RV	O.n	20	825	759	66	66	0	2	0.333 ± 0.046	0.027 ± 0.0003	22	0.920 (92.0%)	0
	O.a	20	807	582	225	215	10	3	0.700 ± 0.048	0.119 ± 0.004	96.200	0.727 (72.7%)	0
	O.m	20	823	776	47	47	0	2	0.500 ± 0.270	0.029 ± 0.0003	23.500	0.943 (94.3%)	0
ANT-RV	O.n	20	818	766	52	52	0	2	0.167 ± 0.0018	0.0011 ± 0.00008	8.667	0.937 (93.7%)	0
	O.a	20	807	582	225	200	25	3	0.833 ± 0.049	0.150 ± 0.007	121.333	0.726 (72.6%)	0
	O.m	20	816	811	5	5	0	2	1.00 ± 0.250	0.006 ± 0.00002	5.00	0.994 (99.4%)	0
IKW-RV	O.n	20	814	723	91	91	0	2	0.222 ± 0.028	0.0248 ± 0.00031	20.222	0.889 (88.9%)	0
	O.a	20	815	715	100	100	0	2	0.400 ± 0.056	0.049 ± 0.0001	40.00	0.878 (87.8%)	0

	O.m	20	778	749	29	09	0	2	0.500 ± 0.070	0.019 ± 0.0001	14.50	0.998 (99.8%)	0
KPN-RV	O.n	20	882	805	17	17	0	2	0.167 ± 0.018	0.0035 ± 0.00001	2.833	0.979 (97.9%)	0
	O.a	20	808	605	200	200	0	2	0.50 ± 0.070	0.124 ± 0.005	100	0.757 (75.7%)	0
	O.m	20	776	692	84	84	0	3	1.00 ± 0.074	0.076 ± 0.002	59.333	0.870 (87.0%)	0
ETH-RV	O.n	20	820	769	51	51	0	2	0.400 ± 0.056	0.025 ± 0.0002	20.40	0.938 (93.8%)	0
	O.a	20	808	592	216	216	0	2	0.400 ±0.056	0.107 ± 0.004	86.400	0.738 (73.8%)	0
	O.m	20	778	745	33	33	0	2	0.500 ± 0.070	0.021 ± 0.0002	16.500	0.958 (95.8%)	0

ITU-RV = Itu River, ANT-RV = Anangtigha River, IKW-RV = New Calabar River, KPN-RV = Kpansia River, ETH-RV = Ethiope River, O.n = *Oreochromis niloticus*, O.a = *Oreochromis aureus*, O.m = *Oreochromis mossambicus*, NSQ = Number of sequences, NS = Number of sites, MNS = Monomorphic sites, PS = Polymorphic sites, SVS = Singleton variable sites, PIS = Parsimony information sites, NH = Number of haplotypes, Hd = Haplotype (gene) diversity, Nu = Nucleotide diversity, ANND = Average number of nucleotide differences, SC = Sequence conservation, MNR = Minimum number of recombination

Haplotype and nucleotide diversity are crucial metrics for assessing variation in DNA sequences among organisms. Haplotype diversity quantifies the probability that two or more randomly selected sequences are distinct,[44] while nucleotide diversity measures genetic variation influenced by mutation rate and effective population size.[45] These metrics are typically expressed as coefficients, where values approaching one indicate higher diversity estimates. Among the three tilapia species studied, *O. aureus* exhibited the highest nucleotide diversity, whereas *O. niloticus* and *O. mossambicus* displayed similar nucleotide diversity. Similar trends were observed within each species across locations. As a result, *Oreochromis aureus* exhibited higher within-species genetic variation and was more genetically distinct from *Oreochromis niloticus* and *Oreochromis mossambicus*. This pattern aligned with the findings of genetic distance analysis, which indicated a greater genetic separation between *O. aureus* and the other two species. There was an earlier report of nucleotide diversity of 0.237 in 20 samples of *O. niloticus* and 0.276 in 26 samples of *O. aureus*[29]. Similarly, a range of 0.001 to 0.006 nucleotide diversity was reported in a fragmented population of tilapia fish [46]. Discrepancies between the nucleotide diversity reported in previous studies and the current investigation likely stem from environmental variations. Evaluation of sequence conservation among the three tilapia species revealed the lowest conservation in *O. aureus* sequences, underscoring the primary reason for the species' higher within-species diversity and greater genetic distance from *O. niloticus* and *O. mossambicus*.

Genetic distance within and among the tilapia species

The genetic distances among the three tilapia species are detailed in Table 3. The greatest distance was found between *O. aureus* and *O. mossambicus* (0.388), whereas the smallest distance was observed between *O. niloticus* and *O. mossambicus* (0.217). Table 4 presents the genetic distance among the three tilapia species within and between the different study locations. The greatest distance was found between *O. niloticus* from Kpansia River and *O. aureus* from New Calabar River (0.509). Generally, higher genetic distance values were observed between *O. aureus* and all species in the different locations. The lowest genetic distance values were recorded within species between locations. For instance, *O. aureus* between Itu and Ethiope River was 0.000. Similar results were obtained between *O. aureus* from Anangtigha, Itu, and Ethiope rivers. The within-species genetic distance between *O. mossambicus* from Ikwerre and Ethiope Rivers; Anangtigha and Kpansia rivers was also 0.000. Thus, the genetic distance was higher between species than within species, irrespective of location. Genetic distance is a useful indicator for evaluating the extent of genetic divergence both within and between populations, especially among closely related species.[44] A lower genetic distance indicates a closer genetic relationship due to shared alleles, while a higher distance reflects greater genetic differences. The analysis of genetic distances among the three tilapia species revealed that *Oreochromis aureus* was notably distinct from *Oreochromis niloticus* and *Oreochromis mossambicus*, which had a closer genetic relationship to each other. This finding suggests that *O. aureus* exhibited greater genetic variation compared to *O. niloticus* and *O. mossambicus*. This increased genetic diversity in *O. aureus* is supported by a higher number of variable sites, greater nucleotide changes, and less conservation in its mitochondrial D-loop region. The observed allelic differences among species from different locations, particularly in *O. aureus*, underscores the importance of genetic distance estimation for understanding population genetics. This variability could serve as an informative indicator for fish breeders in selection and breeding improvement efforts. Consequently, selecting *O. niloticus* for crossbreeding with *O. aureus*, aiming to harness their genetic heterogeneity, may prove advantageous, especially considering the substantial variation observed in their mtDNA D-loop sequences. Furthermore, considering nucleotide diversity within each location, *O. niloticus* from the Itu River and Ethiope River exhibited the highest diversity compared to other locations. This suggests that selecting *O. niloticus* individuals from these rivers for breeding improvement could yield more favourable outcomes, aligning with the principles of genetic improvement in farm animals.[47] However, for interspecies breeding and genetic enhancement, *O. niloticus* from the Itu and Ethiope rivers, alongside *O. aureus* from the New Calabar River, may offer a preferable choice. This is because *O. aureus* individuals from the New Calabar River demonstrated a higher genetic distance from *O. niloticus* individuals from the Itu and Ethiope rivers, presenting an opportunity for effective crossbreeding and genetic improvement initiatives.

Positive selection, non-synonymous amino acid substitution, and transversion mutations are the predominant forces of variation in mtDNA D-loop of the tilapia species.

Positive selection, non-synonymous amino acid substitution, and transversion mutations are the predominant forces of variation in mt-DNA D-loop of the tilapia species

The result of the selection pressure among the three tilapia species measured as positive, negative and neutral is presented in Table 5. It was revealed that the majority of the sites in mtDNA sequence of the three species were under neutral selection pressure. *O. niloticus*, *O.*

aureus, and *O. mossambicus* experienced more positive selection pressure with 48, 83, and 45 site indexes than negative selection pressure with 33, 56, and 22 site indexes. Table 6 shows selection pressure on the species based on location. It was revealed that positive selection pressure predominated in each location, except in the Ethiope River, where a higher negative site index of 92 was observed compared to a positive site index of 86. Table 7 illustrates the single nucleotide polymorphisms (SNPs) identified in the mitochondrial DNA sequences of the three tilapia species. *Oreochromis niloticus* had 129 SNPs, *Oreochromis aureus* had 225 SNPs, and *Oreochromis mossambicus* had 84 SNPs. Out of the 129 SNPs in *O. niloticus*, 98 (76%) were non-synonymous mutations, while 31 (24%) were synonymous mutations. The 129 SNPs also resulted in 72 (55.8%) and 57 (44.2%) transversion and transition mutations, respectively. In *O. aureus*, there were 201 (89.3%) non-synonymous and 24 (10.7%) synonymous mutations from the 225 SNPs detected, which also resulted in 137 (60.9%) transversion and 88 (39.1%) transition mutations. *Oreochromis mossambicus* also had higher non-synonymous mutation than synonymous mutation [70 (83.3% and 14 (16.7%), respectively]. Transversion mutations (52= 61.9%) were also higher than transition mutations (32= 38.1%) in *O. mossambicus.* In the three species of tilapia, it was generally observed that SNPs resulted in more non-synonymous mutations than synonymous mutations. There were also more transversion mutations than transition mutations. There is growing interest in quantifying selection pressure and its impact on genetic variation within populations. Several approaches have been developed to detect emerging mutations that might provide a selective advantage in a population.[43, 47] Positive or directional selection occurs when an extreme character (phenotype) is preferentially selected over other variants within the population,[48, 49] whereas negative selection, or purifying selection, works to gradually eliminate deleterious genes[49, 50] resulting in a stabilizing effect on population phenotypes. Conversely, neutral selection (balancing selection) does not affect an organism's ability to survive and reproduce.[44, 48,51] In this study, most site indices of mitochondrial sequences were found to be under balancing or neutral selection, suggesting no discernible influence on the fitness of tilapia species. However, positive selection pressures were more prevalent than negative site indices. This aligns with the findings of [52], who also reported a higher number of positive selection sites in frizzled feather chicken genotypes. Positive selection is often observed when populations face new environmental pressures due to migration between different environments, contributing to swift alterations in allele frequencies and facilitating speciation.[49, 53] The higher positive selection pressure observed among tilapia species may be linked to the elevated dN/dS substitution rate for positive site indices, suggesting that many alleles of mtDNA in these species are under the advantage of positive selection, potentially leading to population structuring and speciation over time. Negative selection pressure was also observed among the three tilapia species, characterized by a lower dN/dS substitution rate and negative site index. This suggests that negative or purifying selection is relatively weak in the D-loop sequences of the tilapia species, implying a lower rate of elimination of deleterious genes.[54] This may enhance the survivability of these species by mitigating the effects of harmful mutations resulting from genetic drift and inbreeding.[54,55]

Table 3: Genetic distance between three species of tilapia

Species	*O. niloticus*	*O. aureus*	*O. mossambicus*
O. niloticus	0	0.294	0.217
O. aureus	0.294	0	0.388
O. mossambicus	0.217	0.388	0

It has been suggested that the selection pressure on mitochondrial genes may be influenced by environmental factors affecting metabolic processes, which may vary among taxa or populations.[54] Combining sequences from the three species at each study location revealed a higher positive selection index compared to negative selection. This suggests that environmental factors play a crucial role in maintaining alleles through positive selection and removing alleles through negative selection. The analysis of single nucleotide polymorphisms among the three tilapia species indicated a higher prevalence of non-synonymous mutations than synonymous mutations, with nucleotide changes resulting in more transversion mutations than transition mutations. A similar submission had been made earlier, where non-synonymous and transversion substitutions were said to have contributed significantly to the variations among West African Dwarf sheep. [56] Non-synonymous mutations occur when nucleotide substitutions lead to the production of entirely new amino acids. In contrast, transversion mutations involve the substitution of a purine with a pyrimidine, or vice versa. These mutations contribute to genetic variation within populations, indicating that the observed variation among tilapia species primarily stems from non-synonymous and transversion mutations.

Table 4: Genetic distance among three species of tilapia within and between five rivers of South-South, Nigeria

Species and location	O. n ETH-RV	O. n KPN-RV	O. n ITU-RV	O. n IKW-RV	O. n ANT-RV	O. a ETH-RV	O. a KPN-RV	O. a ITU-RV	O. a IKW-RV	O. a ANT-RV	O. m ETH-RV	O. m KPN-RV	O. m ITU-RV	O. m IKW-RV	O. m ANT-RV
O. n ETH-RV	0														
O. n KPN-RV	0.105	0													
O. n ITU-RV	0.079	0.153	0												
O. n IKW-RV	0.023	0.107	0.070	0											
O. n ANT-RV	0.018	0.101	0.068	0.021	0										
O. a ETH-RV	0.176	0.242	0.204	0.177	0.169	0									
O. a KPN-RV	0.133	0.204	0.182	0.124	0.131	0.064	0								
O. a ITU-RV	0.176	0.242	0.204	0.177	0.169	0.000	0.064	0							
O. a IKW-RV	0.452	0.509	0.464	0.453	0.453	0.356	0.328	0.356	0						

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O. a ANT-RV	0.176	0.242	0.204	0.177	0.169	0.000	0.064	0.000	0.356	0					
O. m ETH-RV	0.156	0.240	0.212	0.157	0.151	0.208	0.171	0.208	0.482	0.208	0				
O. m KPN-RV	0.177	0.248	0.238	0.176	0.166	0.223	0.180	0.223	0.491	0.223	0.043	0			
O. m ITU-RV	0.226	0.299	0.279	0.224	0.219	0.282	0.231	0.282	0.553	0.282	0.098	0.096	0		
O. m IKW-RV	0.156	0.240	0.212	0.157	0.151	0.208	0.171	0.208	0.482	0.208	0.000	0.043	0.098	0	
O. m ANT-RV	0.177	0.248	0.238	0.176	0.166	0.223	0.180	0.223	0.491	0.223	0.043	0.000	0.096	0.043	0

ITU-RV = Itu River, ANT-RV = Anangtigha River, IKW-RV = New Calabar River, KPN-RV = Kpansia River, ETH-RV = Ethiope River, O.n = *Oreochromis* niloticus, O.a = *Oreochromis* aureus, O.m = *Oreochromis* mossambicus

Table 5: Selection pressure in three species of tilapia

Species	Selective types	dN	dS	dN/dS	Site index	p-value
O. niloticus	Positive	25.985	0.00	25.985	48	0.04
	Negative	6.829	33.871	-27.042	33	0.02
	Neutral	0.000	0.00	0.000	149	N.A
O. aureus	Positive	46.166	4.666	41.50	83	0.05
	Negative	14.626	58.054	-43.428	56	0.05
	Neutral	0.00	0.00	0.00	108	N.A
O. mossambicus	Positive	13.598	0.00	13.598	45	0.02
	Negative	4.129	44.028	-39.899	22	0.05
	Neutral	0.00	0.00	0.00	174	N.A

NA = Not available, dN = Non-synonymous, dS = Synonymous

Table 6: Selection pressure of tilapia from five rivers of South-South, Nigeria

Locations	Selection types	dN	dS	dN/dS	Site index	P-value
ITU –RV	Positive	73.122	6.383	66.739	100	0.0101
	Negative	29.852	96.214	-66.362	81	0.0101
	Neutral	0.00	0.00	0.00	50	N.A
ANT-RV	Positive	61.119	7.181	53.938	95	0.010
	Negative	26.357	107.179	80.822	84	0.002
	Neutral	0.00	0.00	0.00	57	N/A
IKW-RV	Positive	57.999	7.993	50.006	98	0.606
	Negative	17.791	77.722	59.931	70	0.007
	Neutral	0.00	0.00	0.00	56	N.A
KPN-RV	Positive	68.768	12.859	55.909	90	0.002
	Negative	32.202	114.598	82.396	88	0.002
	Neutral	0.00	0.00	0.00	43	N.A
ETH-RV	Positive	63.139	3.617	59.522	86	0.005
	Negative	33.609	112.603	78.994	92	0.005
	Neutral	0.00	0.00	0.00	43	N.A

NA = Not available, dN = Non-synonymous, dS = Synonymous

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Table 7: Single nucleotide polymorphisms (SNPs) variation in three species of tilapia fish from five rivers of South-South, Nigeria

	O. niloticus				***O. aureus***				***O. mossambicus***			
	SNP	**Amino Acid change**	**dN/dS**	**Transversion/ Transition**	**SNP**	**Amino acid change**	**dN/dS**	**Transversion/ Transition**	**SNP**	**Amino acid change**	**dN/dS**	**Transversion/ Transition**
	1T>C	STP1Gln	d_N	Transition	1T>C	STP1Gln	d_N	Transition	1T>A	STP1Lys	d_N	Transversion
	7A>T	Lys3STP	d_N	Transversion	3A>G	STP1del	d_N	Transition	7A>C	Arg3Arg	d_S	Transversion
	13T>C	Phe5Leu	d_N	Transition	4T>A	STP2Lys	d_N	Transversion	18A>T	STP6Tyr	d_N	Transversion
	21C>G	Pro7Pro	d_S	Transversion	7T>G	Leu3Glu	d_N	Transversion	131A>T	Lys44Ile	d_N	Transversion
	23T>G	Leu8Arg	d_N	Transversion	8T>A	Leu3Glu	d_N	Transversion	153A>C	Thr51Thr	d_S	Transversion
	24A>G	Leu8Leu	d_S	Transition	11G>A	Gly4Glu	d_N	Transition	216G>A	Lys72Lys	d_S	Transition
	26C>T	Pro9Leu	d_N	Transition	14G>C	Ser5Thr	d_N	Transversion	266C>G	Thr89Arg	d_N	Transversion
	37A>T	Lys13Trp	d_N	Transversion	17T>G	Leu6STP	d_N	Transversion	283G>A	Ala95Thr	d_N	Transition
	38A>G	Lys13Trp	d_N	Transition	19A>C	Asn7Leu	d_N	Transversion	289T>C	Leu97Leu	d_S	Transition
	39A>G	Lys13Trp	d_N	Transition	20A>C	Asn7Thr	d_N	Transversion	315T>C	Ser105Ser	d_S	Transition
	40G>A	Ala14Thr	d_N	Transition	21T>C	Asn7Thr	d_N	Transition	352G>A	Val118Ile	d_N	Transition
	44G>C	Arg15Thr	d_N	Transversion	23C>T	Pro8Leu	d_N	Transition	369G>C	Gln123His	d_N	Transversion
	49C>G	Leu17Val	d_N	Transversion	24T>G	Pro8Leu	d_N	Transversion	465G>A	Gln155Gln	d_S	Transition
	54T>G	Thr18Thr	d_S	Transversion	25G>A	Gly9Ser	d_N	Transition	472T>A	STP158Lys	d_N	Transversion
	83C>G	Ser28STP	d_N	Transversion	26G>C	Gly9Ala	d_N	Transversion	485T>C	Phe162Ser	d_N	Transition
	97T>G	Tyr33Asp	d_N	Transversion	27C>T	Gly9Gly	d_S	Transition	556A>G	Ser186Gly	d_N	Transition
	103T>A	Tyr35Ser	d_N	Transversion	28T>C	Cys10Pro	d_N	Transition	597T>G	Phe199Leu	d_N	Transversion
	104A>G	Tyr35Ser	d_N	Transition	29G>A	Cys10Asp	d_N	Transition	602T>C	Phe201Ser	d_N	Transition
	114A>G	Ser38Ser	d_S	Transition	30T>A	Cys10STP	d_N	Transversion	603C>A	Phe201Leu	d_N	Transversion
	118T>C	Leu40Leu	d_S	Transition	32A>C	Asn11Thr	d_N	Transversion	604A>C	Ile202Leu	d_N	Transversion
Total	129		31/98	72/57	225		201/24	137/88	84		70/14	52/32

SNP = Single nucleotide polymorphism, dN = Non-synonymous, dS = Synonymous

Conclusion

The genetic analyses conducted on the three tilapia species revealed distinct genetic variations among them, as evidenced by mtDNA D-loop sequences. These findings support the significance of genetic diversity in informing breeding strategies aimed at enhancing tilapia populations for sustainable aquaculture and food security initiatives. It is recommended to crossbreed *O. niloticus* from the Itu and Ethiope Rivers with heterogeneous *O. aureus* from the New Calabar River in tilapia breeding progammes for possible genetic gains in hybrids. This can potentially contribute significantly to the genetic improvement of tilapia stocks, thereby advancing the prospects of robust and resilient aquaculture systems capable of meeting the escalating demands of burgeoning human populations and optimizing tilapia production for the betterment of global food security agendas.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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