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Mitochondrial Diversity and Time Divergence of Commonly Cultured Cichlids in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OTA, CJE, IOA, AHA and EAA designed and carried out the experiment and wrote the first draft of the manuscript. Authors AOA, MBI and SOD supplied the software and interpreted the data. Author ABA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aims are to study the genetic diversity and population structure of Tilapia fish species in Nigeria using mtDNA D-loop region and time divergence of these various groups of fish to give a baseline information for fish management and conservation programs.
Methodology: Blood samples from four species of tilapia were collected with the use of FTA cards for DNA extraction and PCR amplification. A 941bp long fragment, including the D-loop, was subsequently used for analysis. Molecular diversity indices and Tajima's selective test were determined. A phylogenetic tree was constructed for all the four fish species using UPGMA. The divergence time among the four fish species using MEGA5 software.

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Place and Duration of Study: The study was carried out in the Biotechnology Centre, Federal University of Agriculture, Abeokuta Nigeria between. Genotyping and Optimization of PCR conditions were carried out at STAB VIDA Lda, Madan Parque, Rua dos Inventores, 2825-182 Caparica Portugal. Total duration of study was between February to August 2015.

Results: *Sarotherodon melanotheron* has the highest nucleotide diversity (**π**), mean number of pairwise differences and number of polymorphic site, while the least was observed in *Sarotherodon galilaeus*. The highest number of monomorphic site and the highest sequences conservation percentage was observed in *Sarotherodon galilaeus*.

The Neighbour joining tree revealed six (6) clusters with no clear sub-structuring among the sampled fish populations. The smallest divergence time (about 31 mya) was observed between *Sarotherodon galilaeus* and *Sarotherodon melanotheron*. *Sarotherodon spp* diverged from *Oreochromis niloticus* about 32.5 mya.

Conclusion: The present study revealed that the sampled Tilapia species from Nigeria represent a single stock. The existing low levels of genetic variability observed here may compromise the evolutionary plasticity of the Tilapia species population. These findings indicate an urgent need for the careful monitoring of the harvesting of Tilapia species in Nigeria.

Keywords: Tilapia species; genetic diversity; population structure; polymorphism; haplotype diversity; genotype; clusters.

1. INTRODUCTION

The fishes of the genera *Tilapia*, *Oreochromis* and *Sarotherodon* belong to the family Cichlidae and are collectively and commonly known as tilapia [1]. These fishes are indigenous to tropical and sub-tropical fresh waters of Africa, Mediterranean and Middle East [1]. The three genera are mainly taxonomically distinguished on the basis of their parental care patterns [2].

The cichlid fishes of African lakes are particularly well known for their rapid speciation and extensive adaptive radiation. Reliable molecular clock estimates could help estimate the extent to which speciation and adaptive diversification have been dependent on the presence of long-lasting lacustrine habitats [3].

Aquatic biodiversity provides the genetic resources that are the basis for fisheries and aquaculture. However, despite some exceptions this diversity is still poorly documented, in particular at the intraspecific level and with regard to molecular genetic approaches. It is, however, becoming more widely accepted that such data sets may have essential impacts on the development of management and conservation strategies and the utilization of aquatic genetic resources [4]. The study of genetic diversity is essential for fish conservation, which depends on the knowledge of the amount of variation existing in a local reproductive unit [5]. The importance of this approach lies on its potential for delimiting priority areas for species conservation and sustainable use [6].

Timing divergence events allow us to infer the conditions under which biodiversity has evolved and gain important insights into the mechanisms driving evolution. Their species richness and diversity of morphology, color, and behavior has made them model organisms for the study of speciation and adaptive evolution [7].

In this paper, we studied the genetic diversity and population structure within this fish species using mtDNA D-loop region and time divergence of these various groups of Tilapia fish species to give a baseline information for fish management and conservation programs.

2. MATERIALS AND METHODS

The four species of tilapia (*Sarotherodon melanotheron, Sarotherodon galilaeus, Oreochromis niloticus, Tilapia zilli*) were gotten from three different locations; The Federal University of Agriculture Abeokuta Ogun state, Ministry of Agriculture Odeda farm institute (Eweje Odeda) and IFSERAR (Institute of Food Security, Environmental Resources and Agricultural Research). FUNAAB reservoir. Blood samples from the four species of tilapia were collected with the use of FTA cards for DNA extraction and PCR amplification.

2.1 DNA Extraction

1 mm disk of the blood sample was punctured from the FTA® classic cards and put in a 1.5 ml eppendorf tube. 50 µL of ddH_20 was added and vortex three times and left to rest for 10 minutes.

The spent water was removed as much as possible. Then, 100 µL ddH_20 was added so as to submerge the disks. The tube with the disks was then transferred to a heating block and heated at 99ºC for 15 mins. The samples were vortex and briefly centrifuged. The extracts were then pipetted and put in a new tube; the preparation has 60-150 µL of DNA.

2.2 PCR Amplification

The PCR amplification reaction consist of 10x PCR Buffer, 50 µM dNTPs, H_2O Nuclease free, 250 µM $MgCl_2$, 10 µM of primer forward, 10 µM of primer forward, and 10 µL Surf Hot Tag. Amplification was performed in a Thermocycler (Agilent Surecycler 8800, Applied Biosystem, Foster City, USA) programmed as follows: an initial denaturation at 96ºC for 15 mins, followed by 40 cycles each consisting of 45 secs denaturing at 56.9ºC for *Sarotherodon melanotheron*, 60ºC for *Tilapia zilli*, 62.7ºC for *Sarotherodon galilaeus* and *Oreochromis niloticus*, 90 secs primer annealing at 72ºC, 7 mins extension at 72ºC and then a final 8 mins extension at 1 2ºC.

PCR was carried out using different primer for the four species:

Tilapia zilli: Czilli forward 5' GGATTTTAACCCTTACCCC 3'
Czilli Reverse 3' AGTAAAGTCAGGACC AAGCC 5'

Oreochromis niloticus: Fish-comum-D-loop Fwd 5' GGATTYTAACCCYTRCCCC 3'
Czilli fwd 3' AGTAAAGTCAGGACCAAGCC 5'

Sarotherodon melanotheron: Fish – D-loop2-fwd 5' RCCCCTAACTCCCAAAGC 3'
Fish-D-loop2 Rev 3'TAAAGTCAGGACCAA GC 5'

Sarotherodon galilaeus: Fish-comum-D-loop Fwd 5' GGATTYTAACCCYTRCCCC 3'
Czilli-Rev 3'AGTAAGTCAGGACCAAGCC 5'

2.3 Sequencing of the mtDNA

The sequencing was carried out in 10µl comprising approximately 250 µM of $MgCl_2$, 50 µM of dNTP, H_2O Nuclease free, 10 µM of primer forward, 10 µM of primer reverse and 10 µM of Surf Hot Tag: initial denaturation at 96ºC for 1mins, followed by 30 cycles of denaturing at 96ºC for 10 seconds, annealing at 50ºC for 6secs and extension at 60ºC for 4 mins and then a final 8 mins extension at 12ºC for 10 mins.

2.4 Sequence Analysis

2.4.1 Data analysis

A 941 bp long fragment, including the D-loop, was subsequently used for analysis. 722 mtDNA polymorphisms were identified in a total of 40 sequences from the 4 species of Cichlidae family. Multiple alignments of the sequences excluding the gap were done using ClustalW in MEGA 6.06 [8]. DnaSP 5.10 [9] was used to determine molecular diversity indices (haplotypic and nucleotide diversity, mean number of pairwise differences, number of polymorphic site, parsimony informative site) and Tajima's selective test.

A phylogenetic tree was constructed for all the four fish species using UPGMA. The divergence time among the four fish species was labelled on the scale bar calculated from the average nonsynonymous nucleotide rate (0.0099 per million year, [10]) using MEGA5 software [11].

3. RESULTS

The result of the molecular diversity indices (Table 1) revealed that *Tilapia zilli* has the highest number of haplotypes (14) and the least number of haplotypes (6) was observed in *Sarotherodon melanotheron*.

Each sampled fish population has a haplotype diversity (*hd*) of 1.00. *Sarotherodon melanotheron* has the highest nucleotide diversity (**π**), mean number of pairwise differences and number of polymorphic site of 0.21, 0.295 and 436 respectively, while the least nucleotide diversity (**π**), mean number of pairwise differences and number of polymorphic site value of 0.01, 0.005 and 16 respectively were observed in *Sarotherodon galilaeus*.

Sarotherodon melanotheron has the highest number of singleton variable site (367) and INDEL site (70) with the least number of monomorphic site (405), while the least number of singleton sites (10) and INDEL site (1) with the highest number of monomorphic site (897) was observed in *Sarotherodon galilaeus.* The highest sequences conservation percentage was observed in *Sarotherodon galilaeus* whle the

lowest sequences conservation was observed in *Sarotherodon melanotheron.*

The Neighbour joining tree (Fig. 1) revealed six (6) clusters with no clear sub-structuring among the sampled fish populations.

The smallest divergence time (about 31 mya) was observed between Sarotherodon galilaeus and Sarotherodon melanotheron. Sarotherodon spp diverged from Oreochromis niloticus about 32.5 mya.

Table 1. Molecular diversity indices of the four fish species in Nigeria

Parameters	***Tilapia zilli***	***Oreochromis niloticus***	***Sarotherodon melanotheron***	***Sarotherodon galilaeus***
Sample size	14	11	6	9
Haplotype (h)	14	11	6	9
Haplotype diversity (hd)	1.00	1.00	1.00	1.00
Nucleotide diversity (π)	0.05	0.04	0.21	0.01
Mean no of pairwise differences	0.054	0.048	0.295	0.005
No of polymorphic site	160	182	436	16
Singleton variable site	52	172	367	10
Monomorphic site	669	675	405	897
Parsimony informative site	108	10	69	6
INDEL site	65	44	70	1
Sequence conservation (%)	79.4%	72.9%	47.7%	98.2%
Tajima's D for INDEL	-1.033 (p>0.1)	-2.04 (p<0.01)	-1.13 (p>0.1)	0.16 (p>0.1)

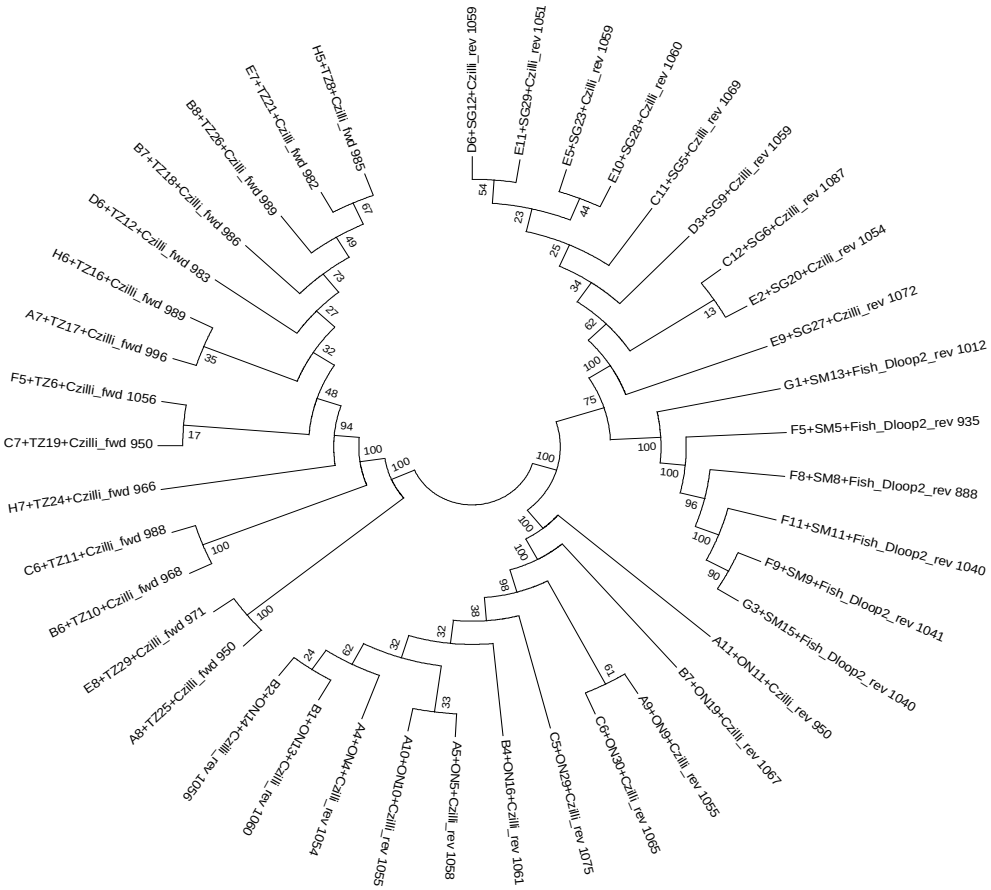

Fig. 1. Phylogenetic relationships between the four species of fish in Nigeria

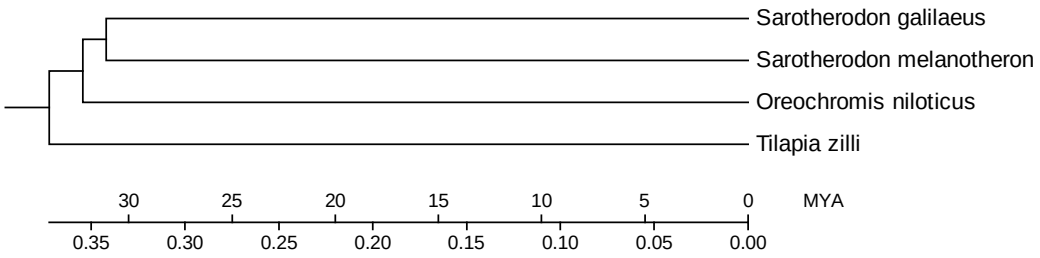

Fig. 2. Phylogenetic tree showing the time of divergence of the fish species

4. DISCUSSION

Sequences of the same segment of the mitochondrial D-loop have been employed in a number of studies to investigate genetic structuring and demographic history in populations of overexploited fishes especially Tilapia in Nigeria. The sampled fish populations showed high haplotype diversity and low nucleotide diversity which indicated a strong species structure pattern in the distribution of mtDNA haplotype and relatively low level of genetic diversity, which may be as a result of genetic drift believed to cause reduction in the genetic variation. A higher haplotype and nucleotide diversity value was reported for *Scianenops ocellatus* (*hd* = 0.98 and π = 0.030; [12]), *Lutjanus campchanus* (*hd* = 0.97 and π = 0.018; [13]), *Lutjanus purpureus* (*hd* = 0.99 and π = 0.027; [14], *Lutjanus erythropterus* (*hd* = 0.99 and π = 0.030; [15]) and *Cynoscion acoupa* (*hd* = 0.892 and π = 0.003; [16]). Over-exploitation and environmental degradation could be some of the factors responsible for the observed low of genetic diversity because despite improvement in technology, catches have declined drastically over the past few years [17].

Indices of neutral evolution (Tajima's *D*) applied to identify evidence of strong selective sweeps or balancing selection, is negative and significant in ON and as well negative in others except SG. The observed negative Tajima's *D* test result was taken not to be statistically significant as well as consistent with population at drift-mutation equilibrium which indicated that the sampled fish populations except *Sarotherodon galilaeus* showed a departure from equilibrium, and these may be due to past or recent population expansion, bottleneck effect or heterogeneity of mutation rates [18].

The Neighbour joining network phylogenetic analysis clearly showed that there is a single population of Tilapia species in the Nigeria, with no clear evidence of genetic sub-structuring while the three populations (ON, SM and SG) aggregate into a larger panmictic population and might have descended from a single ancestry lineage. Considering the large number of singletons observed in each population, the star-like phylogenetic tree and the evidence of population expansion observed in TZ, ON and SM might have experienced bottleneck events, which tends to have erased much of their original variability, followed by a recent process of expansion.

The time of divergence observed between Oreochromis and Sarotherodon observed in this study is slightly similar to about 30 mya reported by [19] in Africa using Gondwanan fragmentation calibration mean earliest divergence estimate.

5. CONCLUSION

The present study revealed that the sampled Tilapia species from Nigeria represent a single stock. They exist a low levels of genetic variability observed here may compromise the evolutionary plasticity of the Tilapia species population. These findings indicate an urgent need for the careful monitoring of the harvesting of Tilapia species in Nigeria.

6. RECOMMENDATIONS

In view of the study outcome, the four species of Tilapia has low nucleotide diversity, the highest was found in *Sarotherodon melanotheron* 21%. The only conserved population with positive expansion could be found in *Sarotherodon galilaeus*, others have experienced recent and sudden population expansion which has led to departure from equilibrium.

The resultant effect of the mitochondrial diversity of these four Tilapia species are tending towards

low growth, inbreeding, genetic drift where nucleotide coding for a particular variant may be lost or deleted and may never be recovered. If conservation programme is not urgently exercised on these species, they can eventually go into extinction.

We therefore recommend the following measures for immediate solutions to the low levels of genetic variability observed in these species. The creation of monosex population, Interbred with high genetic variant species, Production of genetically modified species (GMO), Introduce chromosome set manipulation techniques to produce sterile triploids to combat the prolific breeding habit of Tilapia and finally the crossbreed of only F_i and F_2 generation should be permitted in their breeding exercise.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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