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Mitochondrial DNA analysis of an unidentified cichlid called 'Wesafu' from Lagos lagoon, Nigeria

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Abstract

The knowledge of molecular biology and biotechnology has contributed significantly to genetics in fisheries and aquaculture across the world, particularly in taxonomy. This study was conducted to explore molecular biology, using DNA technology to characterize the unidentified cichlid 'Wesafu' which will serve as a prelude naming the fish. Total genomic DNA was extracted from 10 specimens for 'Wesafu', *Tilapia guineensis*, *Sarotherodon melanotheron* and *Tilapia mariae* using the standard protocol. Amplification reactions were performed on Gene Amp® PCR System (Applied Biosystems Inc.) in a total volume of 25 µl containing 25 ng of extracted genomic DNA template, 10 mM of Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 200 µM each of dATP, dTTP, dCTP and dGTP, 1 unit of *Taq* DNA polymerase and 12-15 pM each of forward and reverse primers. PCR products were purified using the KT 62 Gene quick PCR purification kit and bidirectional cycle sequencing was performed on ABI 3100 PE automated capillary sequencer. Phylogenetic analysis based on Neighbour Joining consensus produced three distinct clusters, one containing X1, X2, X4 (*Tilapia guineensis*), Y3, Z1 and Z2, the second cluster containing 'Wesafu', W1, W2, W3, W4 and W5, and the third cluster containing *T. mariae*, V1 and V4. As for the data base search of the first cluster, it showed a positive alignment with *T. rendalli* but from unvalidated database, with 91% confidence. The results of molecular analysis using ND2 segment revealed higher degree of similarity between 'Wesafu' and *Sarotherodon galileaus*, *Oreochromis niloticus*, *Oreochromis aureus* *Stomatepia mariae*, *Sarotherodon caroli* and *Stomatepia mongo*. When the nucleotide sequences were translated to protein sequences, it was observed that the sequence of 'Wesafu' was similar to *Sarotherodon galilaeus* than any other cichlids in the data base. This suggest that this cichlid 'Wesafu' is a strain of *Sarotherodon galilaeus*.

Keywords: Mitochondrial, DNA, Lagos lagoon, 'Wesafu'

Introduction

The application of genomic studies has made a significant contribution to the field of genetics and reproductive biology in the fields of fisheries and aquaculture [1]. The recognition of the level of variation that is necessary to the placement of a particular population to a new species, subspecies or strain has generated a lot of controversy amongst experts in the field of taxonomy. It has been observed for some fish species, amphibians, birds and monkeys that when previously reproductively isolated allopatric species were brought together by geological events, they sometimes start inter-breeding [13]. The situation has brought confusion and has led to the concept of a 'supra-species' [14]. The conventional system of nomenclature using genus, species and subspecies is good in describing a natural population, but may not be adequate for aquaculture populations where inter-breeding takes place.

The presence of "Wesafu", an unidentified cichlid of Epe Lagoon, Lagos, which grows to 1500g in the wild [9, 10, 4-6] was originally thought to be *Tilapia guineensis*. However several studies have differentiated the cichlid from *T. guineensis*, the authors reported. This cichlid has a deep body and grows to a mass of 1,500g in the wild [6-8]. It appears to be an excellent candidate for genetic improvement of commercial strains for the growing aquaculture industry in Nigeria.

'Wesafu' has been a subject of scientific investigation in Nigeria by a number of workers. A number of studies have been conducted on the meristic and morphometric, nutrient utilization, food and feeding habit as well as protein and amino acid profiling. The results of meristic and morphometric showed that 'Wesafu' is different from other known cichlids. Studies on the biology, growth performance as well as nutrient compositions of this tilapia have been

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reported [2-4]. This present study set to explore DNA technology to characterize the cichlid which will serve as a prelude naming the fish.

Materials and Methods

Study Area

The fish specimens (40 specimens per species) used in this study were obtained from the commercial artisanal catches at Ebute Chief on Epe Lagoon (Lat. 06°35' 00.2¹¹, Long. 04° 00' 01.4¹¹). The location falls within the rain forest zone of Nigeria. Fish identification was carried out using identification guide from Nigerian Institute for Oceanography and Marine Research, Lagos. The caudal fin was collected and preserved in 70% ethanol. The species were 'Wesafu', *Sarotherodon melanotheron*, *Tilapia mariae* and *Tilapia guineensis* (650±45g).

DNA Extraction

Total genomic DNA was extracted from 10 specimens for 'Wesafu', *Tilapia guineensis*, *Sarotherodon melanotheron* and *Tilapia mariae* using the standard protocol [15]. Amplification reactions were performed on Gene Amp® PCR System (Applied Biosystems Inc.) in a total volume of 25 µl containing 25 ng of extracted genomic DNA template, 10 mM of Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 200 µM each of dATP, dTTP, dCTP and dGTP, 1 unit of *Taq* DNA polymerase and 12-15 pM each of forward and reverse primers. The temperature profile for the amplifications was for initial denaturation (95 °C) for 2 mins followed by 35 cycles of 94°C for 30s, annealing at 54°C for 30s and 72°C for 1 min and a final extension of 72°C for 10 minutes. Primers for the mitochondrial Cytochrome oxidase subunit I (COI) used were:

FishF1-5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR2-5'-ACTTCAGGGTGACCGAAGAATCAGAA-3' [18].

PCR products were purified using KT 62 Gene quick PCR purification kit and bidirectional cycle sequencing was performed on ABI 3100 PE automated capillary sequencer.

The sequences were first edited using Bio Edit version 7.0.5.3, aligned using the computer software Clustal W multiple alignments and corrected manually. Cytochrome Oxidase subunit I nucleotide sequences were translated to amino acid sequences using software Primer Premier version 5.00.

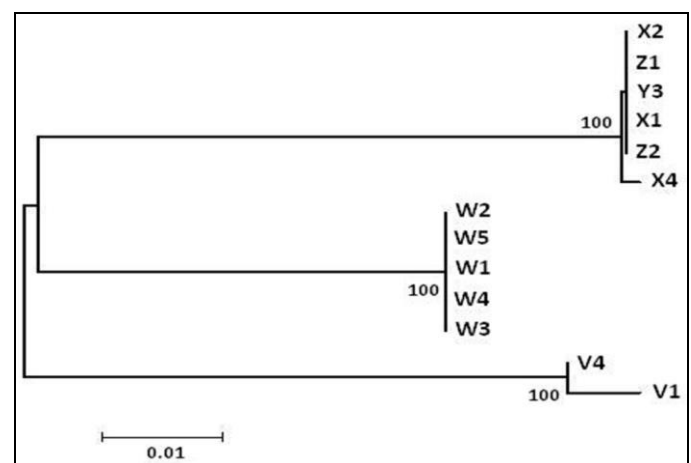
Phylogenetic analyses were performed using software program MEGA version 4.0 [16]. Neighbor Joining consensus trees were generated using Maximum Composite Likelihood model with 500 replicates for bootstrap analysis.

For ND2 Sequence of 'Wesafu', the sequence file was converted to MEGA format and then copied to gene bank interphase and blasted. Then all similar blast sequences that are 99% similar were downloaded and taken to MEGA version 16 [17]. 'Wesafu' ND2 sequence (969 base pair sequence) was inserted and aligned using cluster W. The genetic distance was then computed, followed by drawing phylogenetic tree using maximum likelihood model.

Results

This study compared 'Wesafu' with other cichlids found in the lagoon using cytochrome oxidase subunit 1. Phylogenetic

analysis based on Neighbour Joining consensus produced three distinct clusters (Figure 1), one containing X1, X2, X4 (*Tilapia guineensis*), Y3, Z1 and Z2, the second cluster containing 'Wesafu', W1, W2, W3, W4 and W5, and the third cluster containing *T. mariae*, V1 and V4. As for the data base search of the first cluster, it showed a positive alignment with *T. rendalli* but from unvalidated database, with 91% confidence. The third cluster also suggested *Serranochromis sp.* with 89.88% confidence from database. These two groups of sequences did not yield any matches from the validated database, hence not reliable. The 'Wesafu', W series (second cluster) could be at least one of three species. The validated database says it could be *Sarotherodon boulengeri* (99.84%), *Oreochromis niloticus* (99.36%) or *O. aureus* (99.33%). The encouraging aspect of the last cluster is that these sequences could find match in the validated database. This implied that 'Wesafu' formed a separate cluster, making it different from the rest of the fish species found in the lagoon.



Legend: V1-V5, (*T. mariae*); W1-W5, (Wesafu); X1-X5, (*T. guineensis*); Z1-Z5 (*S. melanotheron*)

Fig 1: Phylogenetic analysis of tilapia fishes of Epe lagoon, Lagos, Nigeria.

The results of molecular analysis using ND2 segment revealed higher degree of similarity between 'wesafu' and *Sarotherodon galileaus*, *Oreochromis niloticus*, *Oreochromis aureus* *Stomatepia mariae*, *Sarotherodon caroli* and *Stomatopia mongo*. When the nucleotide sequences were translated to protein sequences, it was observed that the sequence of 'wesafu' was similar to *Sarotherodon galileaus* than any other cichlids in the data base (Figure 2). However, the similarities between 'wesafu' and *Sarotherodon galilaeus*, *O. niloticus*, *O. aureus* were found much higher than between 'wesafu' and *Stomatepia mariae*, *Sarotherodon caroli* and *Stomatopia mongo* (Figure 2). Considering the phylogenetic relationship of these fish species, the clustering of 'wesafu' is more around *Sarotherodon galilaeus* than any other species mentioned. When the DNA sequences were translated into protein sequences, more similarities were equally found between 'wesafu' and *Sarotherodon galilaeus* than *O. niloticus*, *O. aureus*. With *Stomatepia mariae*, *Sarotherodon caroli* and *Stomatopia mongo*, the similarities were greatly reduced.

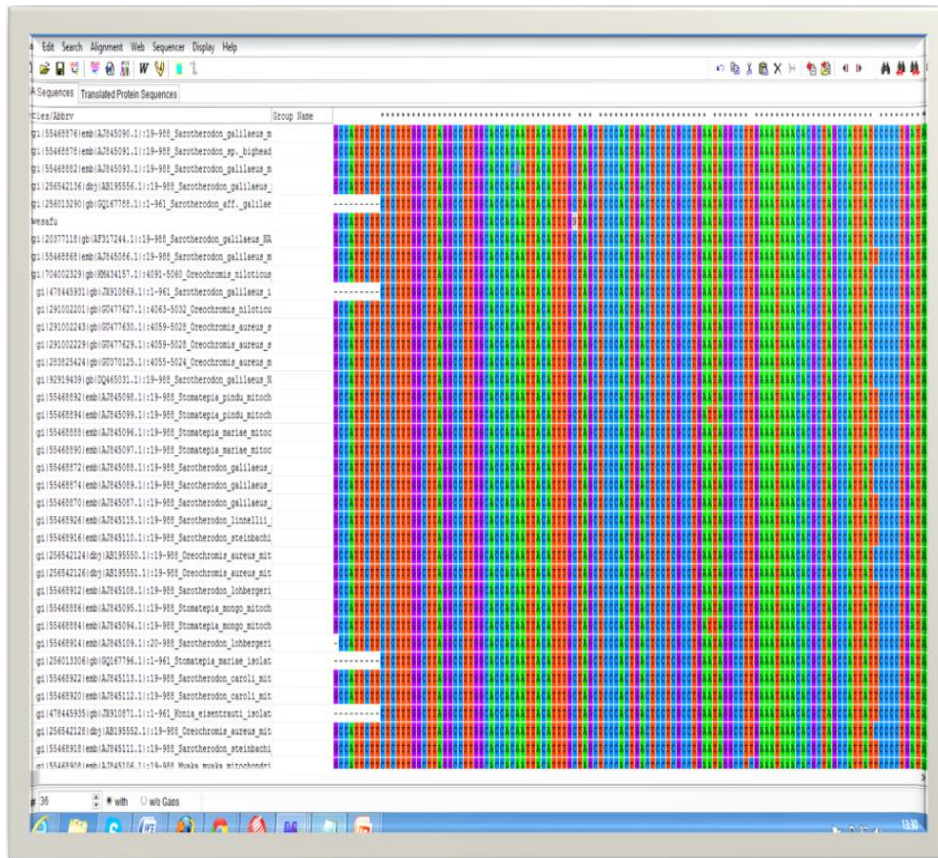


Fig 2: DNA Sequences translated protein sequences



Fig 3: Phylogenetic relationship of similar genome of cichlid with over 99 % similarities.

Discussion

Cichlid fishes have played an important role in evolutionary biology and aquaculture industry [12]. Mitochondrial DNA sequence alignment analyses has been described as one of the more effective approaches to making prediction and recognize different species or populations, particularly in Fisheries. Usually, mtDNA is inherited from the female and does not undergo male and female DNA recombination.

In the present study, phylogenetic analysis based on Neighbour Joining consensus produced three distinct clusters 1, 2 and 3, with cluster 2 representing 'wesafu'. The study, however, is not conclusive; yet suggesting any of three known species of *O. niloticus*, *O. aureus* or *Sarotherodon galileus boulengeri* for 'wesafu' (second cluster). The encouraging aspect of the cluster W ('wesafu') is that these sequences could find match in the validated database.

With ND2 segment of the DNA, the result showed a high level of similarity between wesafu and *S. galileus*, *Oreochromis niloticus* and *O. aureus* than with other cichlids such as *Stomatepia mariae*, *Sarotherodon caroli* and *Stomatopia mongo* found in the data base, although the clustering is most with *S. galileus*. This suggest that this cichlid 'wesafu' is a strain of *Sarotherodon galileus* The question as to whether 'wesafu' is a *Tilapia guineensis* or not should be laid to rest from the above findings.

Pullin [14] reported that a species is never stable in nature. The author opined that the species may die out or spread to form other species. In taxonomy, species with a very restricted distribution are referred as 'young' species and those with a broader distribution are tagged 'old' species. When a particular species has a very wide distribution, it may 'fall apart'. This often happens with fish species when the body of water becomes mutually isolated and the independent populations change through new mutations (change in DNA sequences) and genetic flux the author reported. Such information is very important to the overall study of tilapia. This will equally assist in the management programs for its genetic resources that will facilitate its farming, breeding and development of superior strains and breeds through marker-assisted selection [12]. For this reason wesafu could be product of speciation.

Despite the fact that over 70 species of tilapia have been described, there is considerable argument as to whether these species are truly separate species [2]. The taxonomic classification of tilapia is still not clear and has been subject to continuous changes. This is because of the similarities and overlaps of their morphological features and also partially due to hybridization the frequently observed in nature.

Conclusion

A mitochondrial DNA study has contributed to the cichlid fishes of the lagoon. It has been reported that mtDNA has been explored in the identification of stocks and analysis of mixed fishery and provide information on hybridization between fishes. Earlier breeding studies had suggested 'wesafu' as a maternal mouthbrooder. Considering the results of the breeding characteristics, suggesting 'wesafu' as a maternal mouthbrooder, the present study conclude that wesafu is suspected to be hybrid between *Oreochromis species* and *Sarotherodon galileus* producing a new species altogether.

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