

DNA Barcoding of Wild Cichlid (*Tilapia guineensis*) from Selected Water Bodies in Southwestern Nigeria

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Abstract

Proper identification of organisms such as fish is essential for breeding programmes and species conservation. One of the most fundamental requirements in taxonomy is the ability to classify scientifically using DNA barcoding because it correctly links biological specimens to actual species. This study aimed to establish a barcoding reference database for *Tilapia guineensis* and assessed the effectiveness of the Cytochrome C Oxidase subunit I (COI) gene for species-level fish identification. DNA was isolated from fresh fish tissue (caudal fin) using the phenol-chloroform isoamyl alcohol method and quantified with a spectrophotometer. The barcode gene region 5' of the mitochondrial Cytochrome Oxidase subunit 1 (COI) was amplified using the Fish F1 primer. Amplified products were visualized using 1% agarose gel electrophoresis, then purified and sequenced unidirectionally. The sequences acquired were edited, examined, aligned, and compared to sequences from the Gene Bank. A phylogenetic tree was created using populations and other sequences from the Gene Bank. Three major clusters were formed, with 99% similarity in identification among populations. Also, 94% - 96% similarity was detected with other species from other countries. This study demonstrated a high level of confidence and accurate taxonomic identification of fish species from the water bodies in Southwestern Nigeria. Hence, pure *Tilapia guineensis* strains could be obtained from the wild as broodstocks.

Keywords: *Tilapia guineensis*, DNA barcoding, Mitochondrial, Cytochrome oxidase, Electrophoresis.

Introduction

The mitochondrial DNA molecular technique (mtDNA) is widely used for species identification. Cytochrome C Oxidase I (COI) gene is one of the most commonly used mitochondrial DNA markers. The COI gene can reflect species identity, phylogenetic pattern, and genetic diversity in aquatic species (Tan *et al.*, 2019). The success of the COI gene in species identification is intrinsically connected to gene amplification using polymerase chain reaction (PCR).

Cichlid, generally known as *Tilapia* is an important seafood commodity. *Tilapia guineensis* is one of the most important estuaries fish widely cultured in many tropical and subtropical countries. It has rapid growth rates, high tolerance to adverse environmental conditions, efficient feed conversion, ease of spawning, resistance to diseases and good consumer acceptance (Shelton, 2002).

Despite these attributes, little is known about DNA sequences, which are a major source of information for advanced understanding of identification, evolutionary and genetic links of the fish species (Hajibabaei, 2007).

This information would enhance fish productivity in aquaculture. The purpose of this study is to use DNA barcoding to assess wild cichlids (*Tilapia guineensis*) from selected water bodies in Southwestern Nigeria.

Materials and Methods

Sampling Location

The sampling locations are Ondo Lagoon, Oyan Dam, and Lagos Lagoon with coordinates N 5°55'05" and E4°59'2"; N7°15'30' and E3°15'20' and N6°29'24" and E3°23'58", respectively.

Collection of Samples

The samples were collected from the sampling locations with cast nets through fishermen. Taxonomic classification was done with the aid of identification texts: Field Guide to Nigerian Freshwater Fish and Freshwater Fishes of Nigeria (Idodo-Umeh, 2003). Their caudal fin clips were clipped and placed in Eppendorf tubes containing 95% ethanol for molecular analysis.

DNA Extraction and Quantification

DNA was extracted from the caudal fin tissues (0.5g - 0.75g) of 6 samples (2 per location) using the phenol-chloroform isoamyl alcohol method (Sambrook and Russell, 2001). The DNA quantification was checked with a nano-spec photometer (Shimadzu Corporation Japan, MODEL UV-1800, 2000 Series) by measuring absorbance at 260 nm and 280 nm. Agarose gel of 0.8% and 1% weight per volume (w/v) were used to run both the DNA and PCR.

PCR and Sequencing

Amplification was carried out in 20 µl final volume containing 10 µl of 2 x Mastermix (Syd Labs, Inc.,

USA), 8 µl of nuclease-free water, 0.5 µl (about 10 pmol) each of forward and reverse primer (FISH1), and 1 µl (about 50 ng) of the genomic DNA in a thermocycler (Biorad, USA). The cycling conditions for the PCR reactions were: 94°C for 5 minutes of initial denaturation, 30 cycles of 94°C for 60 s, 59.5°C for 60 s, and 72°C for 120 s with final extension at 72°C for 8 minutes. The PCR products of CO1 and IGF-1 genes were sequenced unidirectionally using a Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) at Inqaba West Africa Laboratory in South Africa using the forward primers.

Statistical Analysis

Sequence analysis was carried out using Bio Edit (version 7.0) and Mega 5.0.

Results

DNA Purity and Quantification

The mean concentration ranged from 672.04 ng/l to 3889.40 ng/l and purity ranged from 1.75 to 2.02 (Table 1). There were four major clusters (Figure 1) namely: Cluster 1 (Lagos broodstock TG 1); Cluster 2 (Ondo broodstock TG 11, Lagos TG broodstock); Cluster 3 (Oyan dam broodstock TG 15, Ondo broodstock TG 10) and Cluster 4 (Oyan dam broodstock TG 09), with 99% similarity of identification. Figure 2 shows a dendrogram of the fish population in relation to other countries from Asia (Philippines) and Africa (Mauritania) and three major clusters were generated. It was observed that 94% and 96% of *Tilapia guineensis* (broodstocks) were similar to those from Asia (Philippines) and Africa (Mauritania), respectively.

Table 1: DNA Quantification *Tilapia guineensis* from selected water bodies in Southwestern Nigeria

Locations	Concentration (ng/ul)	Purity
Lagos Lagoon broodstock 1	830.87	1.76
Lagos Lagoon broodstock 2	2036.24	1.93
Ondo Lagoon broodstock 1	1319.40	1.75
Ondo Lagoon broodstock 2	3889.40	1.91
Oyan Dam broodstock 1	672.04	1.89
Oyan Dam broodstock 2	1260.09	2.02

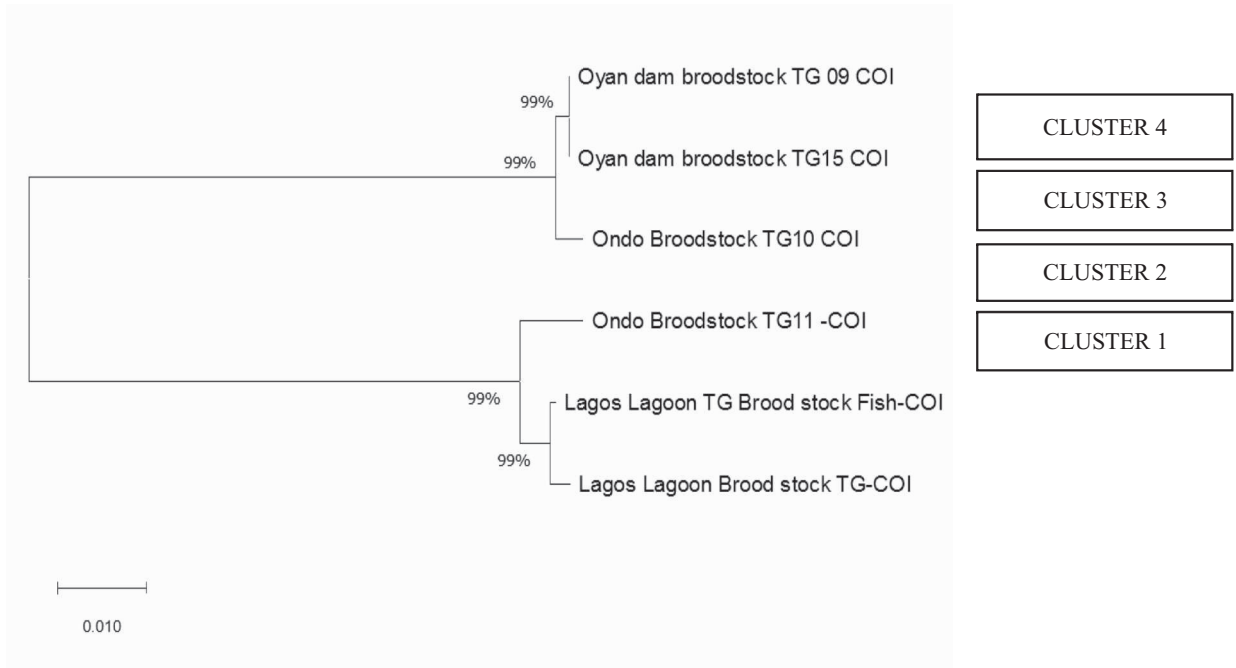


Figure 1: Dendrogram showing the relationship among *Tilapia guineensis* broodstocks populations in Southwestern Nigeria

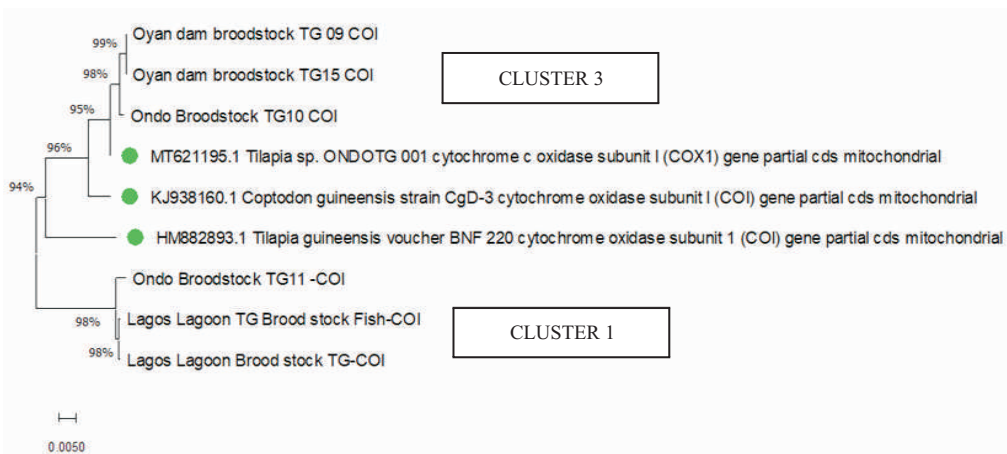


Figure 2: Dendrogram showing the relationship between *Tilapia guineensis* broodstock populations from Southwestern Nigeria, Asia (Philippines), and Africa (Mauritania)

Discussion

The DNA concentration showed good quantity and quality with high purity. This is in agreement with Usman *et al.* (2021) who observed high quality and quantity of DNA produced using the modified phenol-chloroform isoamyl alcohol protocol for extraction. The COI sequence revealed the identity of broodstocks from different locations with low intraspecies variation and high divergence from closely allied taxa. This

suggests high genetic similarity among the species from different locations. Popoola *et al.* (2022) observed distinct clusters on a phylogenetic tree developed for *Tilapia* species from North-eastern, Nigeria, using DNA barcoding. The high similarity of Nigerian species with those of other countries corroborates the findings of Hubert *et al.* (2008) and Mohanty *et al.* (2015). These authors used the COI gene to distinguish between North American freshwater fishes and those that were cultivable.

Conclusion

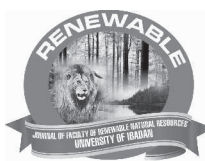
This study provided preliminary information on the potential use of DNA barcoding for taxonomic identification of *Tilapia guineensis* in Nigeria. The study revealed that pure strains of *Tilapia guineensis* broodstocks could be obtained from the wild. Nevertheless, there would be a need for proper conservation and management of the species through breeding programs.

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