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 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 (CO1) 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. 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 and Cytochrome C Oxidase I (COI) gene is one of the most commonly used mitochondrial DNA markers. The CO1 gene can reflect species identity, phylogenetic pattern, and genetic diversity in aquatic species (Tan *et al*., 2019). The success of the CO1 gene in species identification is intrinsically connected to gene amplification using polymerase chain reaction (PCR).

Cichlid, generally known as Tilapia is an important sea food 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 was 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 were Ondo Lagoon, Oyan dam and Lagos Lagoon with coordinates N $5°55'05''$ and E$4°59'$ $2''$; N$7°15'30'$ and E$3°15'20'$ and N$6°29'$ $24''$ and E$3°23'58''$, respectively.

Collection of Samples
The samples were collected from the sampling locations with cast net 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 phenol-chloroform isoamyl alcohol method (Sambrook and Russell, 2001). The DNA quantification was checked with 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 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 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 showed 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

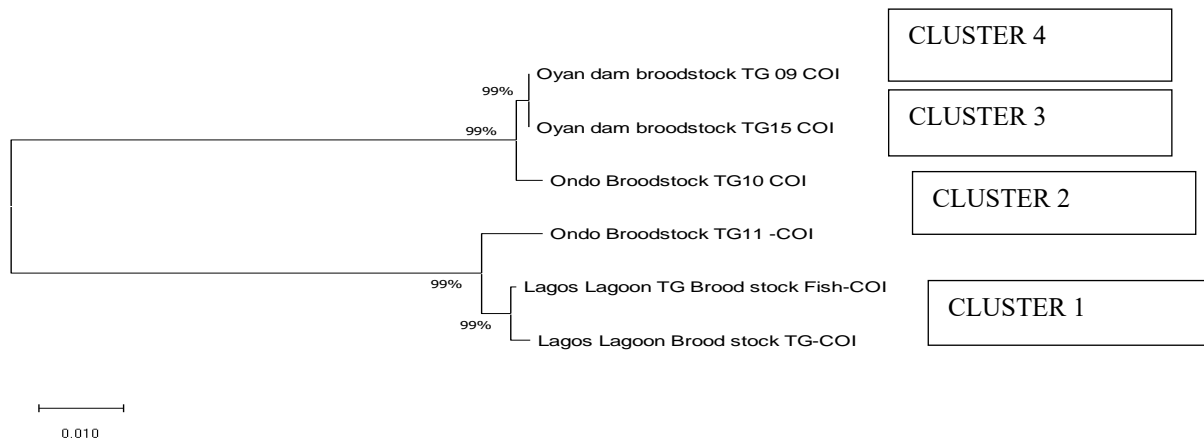| Locations	Concentration (ng/ul)	Purity
Lagos Lagoon broodstock 1	830.87	1.76
Lagos Lagoon broodstock 2	2036.24	1.93
Ondo Lagoon broodstock 1	1319.40	1.75
Ondo Lagoon broodstock 2	3889.40	1.91
Oyan dam broodstock 1	672.04	1.89
Oyan dam broodstock 2	1260.09	2.02

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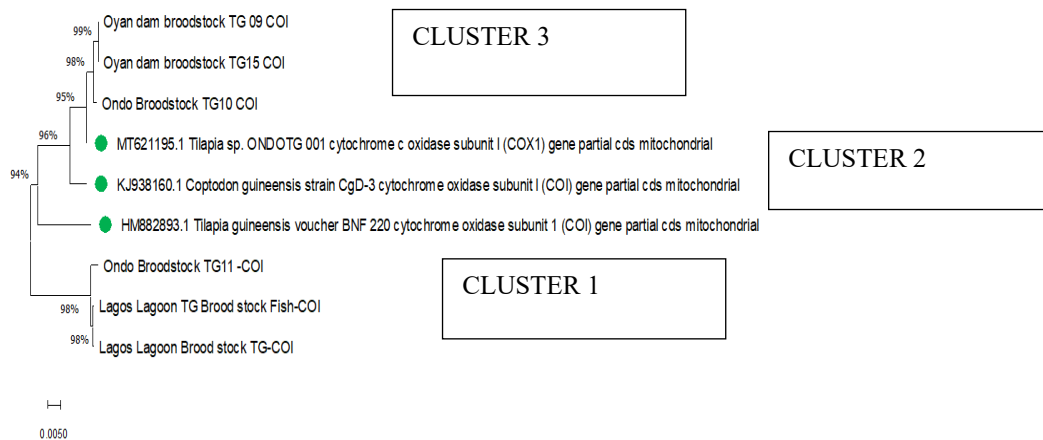

Figure 2: Dendrogram showing the relationship between *Tilapia guineensis* broodstocks populations from Southwestern Nigeria, Asia (Philippines) and Africa (Mauritania)

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 for DNA produced using the modified phenol-chloroform isoamlyl alcohol protocol for extraction. The CO1 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 phylogenetic tree developed for Tilapia species from North-eastern, Nigeria, using DNA barcoding. The high similarity of Nigerian species with those of other countries corroborate the findings of Hubert *et al.* (2008) and Mohanty *et al.* (2015). These authors used CO1 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 need for proper conservation and management of the species through breeding programs.

ACKNOWLEDGMENTS

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