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DNA BARCODING OF *Caranx senegallus* FROM THREE WATER BODIES ADJOINING THE GULF OF GUINEA, NIGERIA

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ABSTRACT

Fish species show astonishing phenotypic and genetic diversity in populations. The delimitation and recognition of fish species are not only of interest to taxonomists but also a requirement in studies of ecology. In this study, the genetic structure of *Caranx senegallus* collected from three water bodies adjoining the Gulf of Guinea, Nigeria was characterized using the cytochrome c oxidase I (COI) barcode gene region. Fish samples collected from Qua Iboe estuary, Escravos estuary, and Lagos lagoon were identified morphologically, and muscle tissues were used for DNA barcoding using Fish1 and Fish2 primers. Evolutionary analysis showed the formation of diverging clades in the neighbor-joining tree and indicated the presence of multiple species. The result also revealed that morphological identification was not entirely successful, as the Basic Local Alignment Search Tool (BLAST) prediction confirmed the presence of three specimens initially identified as *C. senegallus* but genetically matching *C. fischeri* and *Trachinotus goreensis.* This observation further emphasized the need for an integrative method for species identifications.

Keywords: Biodiversity, Mitochondrial DNA, Phylogenetic diversity, cytochrome *c* oxidase I, Jackfish

INTRODUCTION

Fish identification is traditionally based on Morphological features. However, due to high diversity and morphological plasticity, in many cases, fish and their diverse developmental stages are difficult to identify by using morphological characteristics alone (Omer, 2017). The characteristics of convergence and divergence in fishes can also lead to controversial classification, distinguishability, and identification of fishes (Keskin and Atar, 2013). Accurate identification of species is a pivotal component in conservation efforts.

The use of traditional methods (morphological characters) in species identification is common in Nigeria. It has been estimated that about 48% of Nigerian freshwater fish species have been characterized using this method (Nwani *et al*., 2011). The utilization of morphological techniques in fish species identification can be incorrect (Ward, 2009) as its accuracy has not yet been tested for many Nigerian fish species. The challenges of the use of morphology lie in the discrimination of closely related organisms. This has paved the way for the development of improved molecular approaches for the identification of fish species (Abdullah and Rehbein, 2017). Unlike the morphological-based method which is faced with inaccuracy due to the existence of synonymous external morphological features, the molecular diagnostic method is free from these barriers and can accurately identify species and also discover cryptic ones (Bingpeng *et al*., 2018). DNA barcoding is one of such molecular technology that has been used for the accurate identification of fish species. This technology relies on the observation that the 'barcode' sequence divergence within species is typically much lower than the divergence exhibited between species, making it an effective marker for species identification and discovery. DNA barcoding has since gained global support as a rapid, more accurate, cost-effective, and broadly applicable tool for species identification, particularly concerning fishes as coordinated by the Fish Barcode of Life (FISH-BOL) campaign. It has been used as an effective tool to perform unambiguous species identification of fishes in countries such as Australia (Ward *et al*., 2005); Mexico and Guatemala (Valdez-Moreno *et al*., 2009) and Indonesia (Nuryanto *et al.*, 2023).

To date, there are scanty details on the DNA barcoding of most fish species in Nigeria. Only the works of Nwani *et al*. (2011), Nwakanma *et al.* (2015), Falade *et al*. (2016), Iyiola *et al*. (2017), Ude *et al*., 2020; Mojekwu *et al.* (2021), Bolaji *et al*. (2023), Uchenna *et al*. (2023) and a few others are available for local fish species. Nigerian fishes need to be studied for adequate knowledge of genetic diversity and possible identification of new species, especially in the estuaries that have not been fully explored. The application of informative molecular markers will provide information on the molecular structure of fish species that will be useful in the identification of unique stocks, stock enhancement, breeding programmes for sustainable yield, and preservation of genetic diversity. Therefore, this study aimed to determine the utility of the COI marker gene in *Caranx senegallus* obtained from three water bodies adjoining the Gulf of Guinea, Nigeria.

MATERIALS AND METHODS

Description of the study area

This study was carried out in three water bodies that have connections to the Gulf of Guinea, Atlantic Ocean. They include the Qua Iboe estuary, Escravos estuary, and Lagos lagoon. The sites were also characterized by landing sites with high species richness and availability of economically important fish species. These three water bodies also provide ample fishing opportunities for the coastal communities, waterways transportation, and offshore oil exploration. The map showing the study area is presented in Figure 1.

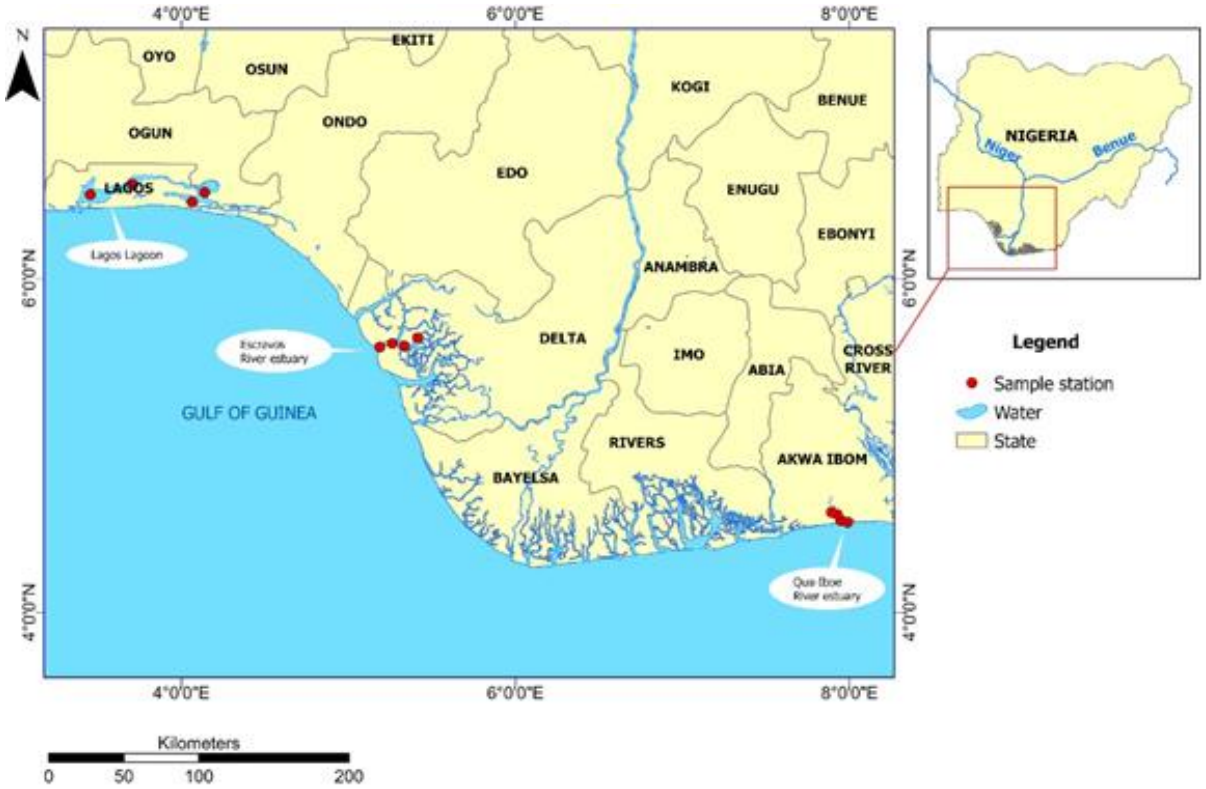

Figure 1: Map showing Qua Iboe Estuary, Escravos Estuary, and Lagos Lagoon adjoining the Gulf of Guinea in Nigeria.

Sampling

Random samples of *C. senegallus* were collected each from Qua Iboe estuary, Escravos estuary, and Lagos lagoon through indigenous fishers operating in the various water bodies, using purse seine and gill nets. The samples were thereafter taken to the laboratory for further identification and analysis. Phenotypically identified samples of *C. senegallus* from the three study sites were dissected and tissues aseptically collected. The muscle tissues were preserved in saline water, kept in an iced pack, and transported to Inqaba Biotechnology, Ibadan for DNA extraction, sequencing, and sequence analysis of COI barcode gene region.

DNA Extraction, PCR Amplification and Sequencing

DNA extraction was carried out using the Quick-DNA™ Miniprep Plus Kit (Zymo Research®) and following the manufacturer's protocol for solid tissues. The fish target region was amplified using the OneTaq Quick Load 2X Master Mix (NEB, Catalogue No: M9486), nuclease-free water, and template DNA with primers for forward and reverse reactions. Primers originally developed for marine and estuarine fish species were used for the amplification. The names of the primers used are presented in Table 1. After thermal cycling, the amplified DNA was stored at -20°C before use as described by Shokrallan *et al.* (2010).

Gel electrophoresis was carried out using the methods described by Lucentini *et al.* (2006). Purified PCR products were directly sequenced in both forward and reverse directions with an automated DNA sequencer (ABI 3730) following the manufacturer's instruction. The nucleotide sequences were viewed and confirmed by eye using SeqManTMII (DNASTAR Lasergene 7). They were aligned in MEGA 7.0 using ClustalW (Kumar *et al*., 2018) with default parameters. The aligned sequences were translated into amino acids to check for premature stop codons and to confirm that the open reading frame was maintained in the protein-coding loci. Basic Local Alignment Search Tool (BLAST) was the program used to confirm the identity of the amplified sequences. This was achieved by comparing nucleotide sequence data that were produced from the genomic DNA to the sequences database at Genbank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Additionally, the FASTA sequences of each of the *COI* sequences were inputted into the BOLD identification request tool (http://www.boldsystems.org/index.php/IDS_OpenIdEngine) . Sequences were submitted for species-level identification under the option "Species Level Barcode Record" following Hebert *et al*. (2003).

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal, 1973). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980). The generated sequence data from the DNA barcoding were aligned and subjected to phylogenetic tree reconstruction using Maximum Likelihood (ML) and Kimura 2-parameter (K2P), with bootstrap 1000 replicates. Genetic diversity distances based on K2P were analyzed using MEGA X.

Table 1: PCR and sequencing primers used in this study.

Primer Name	Forward	Reverse	Reference
Fish1	TCAACCAACCACAAAGACATT GGCAC	TAGACTTCTGGGTGGCCAA AGAATCA	Ward *et al*. (2005)
Fish 2	TCGACTAATCATAA AGATATCGGCAC	ACTTCAGGGTGACCG AAGAATCAGAA	Ward, (2012).

RESULTS AND DISCUSSION

The BLAST prediction for *C. senegallus* from Qua Iboe estuary, Escravos estuary, and Lagos lagoon is presented in Table 2. The BLAST results showed different fish species inherent in the samples sequenced across the three water bodies. The percentage identity of the sequence data ranged from 98.72% to 100% in fish samples across the study sites. The Sequence length (bp) and highest query coverage ranged from 617 to 691 and 94% to 100% respectively. All identifications were confirmed as correct based on BLAST results except for three samples initially identified as *C. senegallus* using morphometric characters but genetically matching *C. fischeri* (2 samples from the Escravos estuary with 98.72% and 99.36% identity) and *Trachinotus goreensis* (from the Lagos lagoon with 100% identity).

Table 2: BLAST prediction for *Caranx spp* from Qua Iboe estuary, Escravos estuary and Lagos lagoon

Water Body	Sample ID	Sequence length (bp)	% Identity	Accession no of BLAST hit	Organism	Alignment score	Highest query coverage (%)
Qua Iboe estuary	QJACK1	646	99.68%	LC646716.1	*Caranx senegallus*	≥200	96%
Qua Iboe estuary	QJACK2	627	100%	LC646716.1	*Caranx senegallus*	≥200	100%
Qua Iboe estuary	QJACK3	618	100%	LC646716.1	*Caranx senegallus*	≥200	100%
Escravos estuary	EJACK1	641	99.36%	LC646709.1	*Caranx fischeri*	≥200	97%
Escravos estuary	EJACK2	638	98.72%	LC646709.1	*Caranx fischeri*	≥200	98%
Escravos estuary	EJACK3	640	98.72%	LC646716.1	*Caranx senegallus*	≥200	96%
Lagos lagoon	LJACK1	691	100.00%	HM883016.1	*Trachinotus goreensis*	≥200	94%
Lagos lagoon	LJACK2	620	100.00%	LC646716.1	*Caranx senegallus*	≥200	100%
Lagos lagoon	LJACK3	617	99.52%	LC646716.1	*Caranx senegallus*	≥200	100%

Evolutionary analysis by Maximum likelihood (ML) method using base substitution model HKY+I at Bootstrap values of 1000 showed the various lineages (Figure 2). The formation of diverging clades in the neighbor-joining tree indicated the presence of multiple species. The lineage comprised of three species from different genera and locations at 84% support value. Two species *Caranx senegallus* and *Caranx fischeri* are of the same genus. However, the third species *Trachinotus goreensis* is a sister to the former species. The overall mean genetic distance between the 9 fish samples computed based on the Kimura 2-parameter (K2P) was identified to be 1.05 with a standard error of 0.28. The interspecific distance within the species ranged from 0.00 - 0.187.

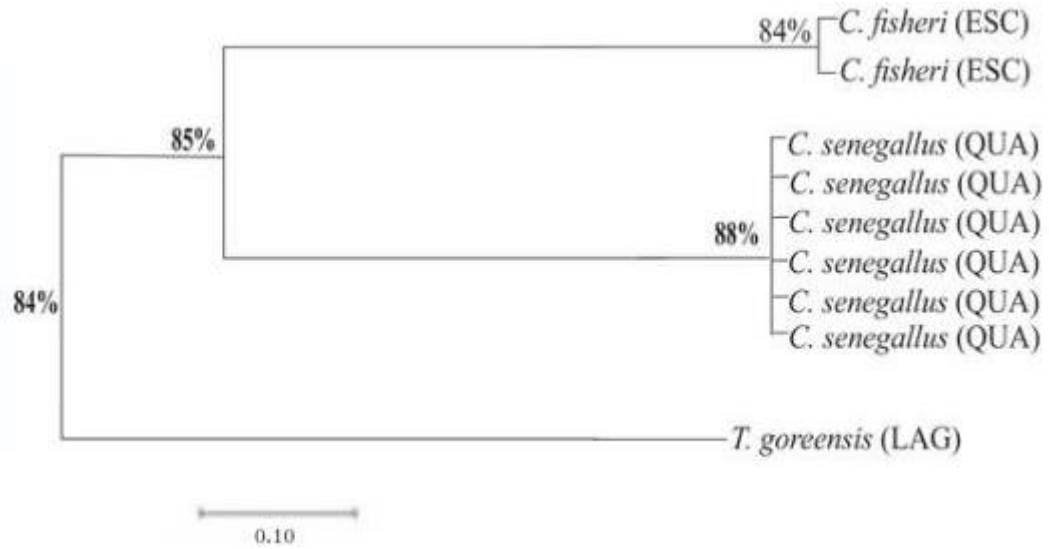

Figure 2: Phylogenetic relationship among the species sampled from various locations
Note: QUA (Qua Iboe estuary), ESC (Escravos estuary), LAG (Lagos lagoon)

Discussion

The study revealed that the identification of *Caranx* species was not entirely successful based on morphological characters alone. The BLAST prediction confirmed the targeted fish species except for three specimens initially identified as *C. senegallus* using morphometric characters but genetically matching *C. fischeri* (2 samples) and *Trachinotus goreensis*. Similar observations have been reported in many studies for different species of Caranx. Bolaji *et al*. (2023) have reported that samples of *C. hippos* that were previously identified using morphometric features were genetically identified *as Caranx fischeri* (with 100% identity on BOLD). Wilette and Padin (2014) have also stated that samples initially identified as *C. sexfasciatus* were actually *C. papuensis*, which was determined through diagnostic coloration patterns and cytochrome *b* phylogenetic reconstruction. The proportion of fish species identified and confirmed using the morphological and DNA barcoding approach that was observed in this study (66.67%) was higher than the 39.60% confirmation rate reported by Iyiola *et al.* (2017).

The observed discrepancies between the morphometric and genetic characteristics could be attributed to species misidentification. *Caranx senegallus* and *C. fischeri* belong to the Caranx genus, sharing many anatomical features and behaviors common to jackfish. *Trachinotus goreensis* on the other hand belongs to the Trachinotus genus, which is distinct from Caranx but still within the Carangidae family. The jackfish generally have elongated, compressed bodies, forked caudal fins, and two separate dorsal fins (one spiny, one soft). Although, *C. senegallus* is larger and slenderer than *C. fischeri*. Trachinotus on the other hand are oval-shaped bodies with a deeper profile. They are distinguishable for the possession of only one continuous dorsal fin, unlike the two separate fins of the Caranx species (Smith-Vaniz and Carpenter, 2007; Carpenter and De Angelis, 2016).

The inconsistency between the morphometric and genetic characteristics observed in the *Caranx spp* could also be due to sequencing error, contamination during sample processing in the laboratory and mismatch as a result of the occasional disparity between BOLD and GenBank platforms (Radulovici *et al*., 2010; Ward, 2012). Iyiola *et al.* (2017) have reported 7.5% cases of mismatches in which query sequences for GenBank and BOLD showed a sequence match with different species names within a 97% similarity cutoff. While BOLD employs distance-based algorithms that compare query sequences to the closest matching reference sequences in the database, GenBank on the other hand utilizes various algorithms, including BLAST and phylogenetic analyses, which can provide more nuanced identifications (Kelly *et al*., 2007; Sanderson *et al*., 2008). Inaccuracies in the query sequence itself can lead to mismatches with reference sequences in either database (Spouge and Mariño-Ramírez, 2012). Also, if reference sequences lack key diagnostic regions, accurate identification may be challenging (Kekkonen and Hebert, 2014; DeSalle and Goldstein, 2019). Errors in the reference database itself can propagate through to identifications of query sequences (Meyer and Paulay, 2005; Shen *et al*., 2013). This observation further emphasized the need for an integrative method for species identification, using both morphological and molecular techniques to ensure the correct identity of fish samples.

The overall mean genetic distance between the fish samples computed based on Kimura 2-parameter (1.05%) was lower than reports of 15.38% from Canadian freshwater (Hubert *et al*., 2008) and 15.46% from Australian freshwater (Ward *et al*., 2005). Variations could be attributed to the heterogeneous nature of the fish species analyzed in the two separate researches. The interspecific distance within species was lower than the range of genetic distances within the genera (6.90 - 28.1%) and families (16.00 - 25.70%) reported in Nigerian waters by Ude *et al*. (2020). Variations could be due to the heterogeneous nature of the studied population. The intraspecific K2P genetic distance obtained in this study was within the range of 0.091 and 1.31 reported in economically important freshwater fish species from north-central Nigeria (Iyiola *et al*., 2017). The observed genetic distance was also consistent with the range of 0 - 0.17 reported by Nwanni *et al*. (2011) in freshwater fishes collected in southeastern Nigeria. The intraspecific K2P distance in this study was in agreement with the findings of Hubert *et al*. (2008) in which the calculated intraspecific distance for 190 Canadian freshwater fish species was 0.3%. This range was, however, drastically smaller than the range of 0.00 - 4.12 reported in *Sparus aurata* obtained from Teluk Penyu Beach Cilacap of Indonesia (Nuryanto *et al*. 2023) and the value of 8.19% reported among morphologically similar *Caranx* species from Batangas, Philippines (Torres and Santos, 2019). The reduced intraspecific variation in the studied population could be a result of infrequent mating of members and genome-based alteration arising from exposure to environmental toxicants.

Nwani *et al*. (2011) have also reported that such differences could arise from genetic recombination and mutation. Intraspecific genetic variation could also occur as a result of hybridization and introgression. The Sequence length (bp) observed across the three water bodies (617 – 691) was within the acceptable DNA barcoding region (Mccusker *et al*. (2013).

CONCLUSION

Misidentification of fish species is a common challenge in taxonomy, especially for morphologically similar species, and accurate identification of the species also relies on the level of expertise of the taxonomist. The accurate identification of fish species is crucial for studying habitat use, diet, life cycles, interactions between species, and evolutionary relationships and adaptations. This study validated the reliability of DNA barcoding against the traditional method of morphological evaluation as genetic analysis revealed samples of *C. fischeri* and *Trachinotus goreensis* that were initially identified as *C. senegallus*. This highlights the limitations of relying solely on morphological evaluation for species identification, as it may lead to misclassification and misinterpretation of species diversity. These observations therefore have important implications for conservation efforts, as accurately identifying and understanding the genetic diversity of fish species is crucial for implementing effective conservation strategies and ensuring the preservation of biodiversity in Nigerian estuaries. The results obtained in this present study can facilitate decision-making and selections for biodiversity, breeding, and conservation in fisheries management.

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