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DNA Barcoding of Selected Freshwater Fishes in New Calabar River, Rivers State, Nigeria

Anukwu John Uchenna [a*], Nwamba Helen Ogochukwu [a] and Chiaha Ijeoma Emiliana [a]

[a] Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, South-Eastern Nigeria, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The major environmental crisis is the loss of biodiversity and the decline is predominant in the fish population. Although taxonomic history began 250 years ago, there are still undiscovered members of species and new species are waiting to be uncovered. The failure of the traditional taxonomic method to address this issue has resulted to the adoption of a molecular approach-DNA barcoding. It was proposed that DNA barcoding using the mitochondrion cytochrome oxidase subunit I (COI) gene has the capability to serve as a barcode for fish. The aim of this study was to use DNA barcoding in the identification of fish species in the New Calabar River, Rivers State. BLAST result showed the correlation between the sequence queried and the biological sequences with the NCBI database. The names of the samples, percentage ID, predicted organisms, and GenBank Accession numbers were clearly identified. A total of 18 sequences (all > 600bp) belonging to 8 species, 7 genera, 7 families, and 5 orders were validated and submitted to the NCBI database.

**Corresponding author: E-mail: anukwuuchenna@gmail.com;*

Each nucleotide peak was represented by a single colour with various percentage occurrences. Two (22%) out of the 9 original samples analyzed corresponded with the predicted organisms from the BLAST result.) There were a total of 712 positions in the final dataset. Evolutionary analyses were conducted in MEGA11. Pairwise sequence alignment showed different consensus positions and a total of 30 mutations. There was one insertion from *Polynemus dubius* and 29 substitutions (transition-15 and transversion-14) mutations. No deletion and nonsense codons were detected in all the amplified sequences. This work will facilitate more research in other keys areas such as the identification of mislabeled fish products, illegal trading of endangered species, and effective tracking of fish biodiversity.

Keywords: DNA barcoding; New Calabar River; phylogenetic tree; pairwise DNA alignment; mutation.

1. INTRODUCTION

"In every society, the major environmental extremity is the loss of biodiversity and the decline is variable among different taxonomic groups. Still, attempts to conserve biodiversity remained uncertain because there is fragmentary information concerning the exact global birth data and the rate of loss is unknown, particularly in terms of background knowledge" [1]. "Although the history of taxonomy began more than 250 years ago, there are still numerous undescribed members of a species. It has been reported that only 226,000 of the estimated 0.7 – 1 million marine species have been described" [2]. "Formalized techniques need to be developed for data accession of biodiversity in order to manage the current biodiversity crisis. Among the marine organisms, the organism with the highest form of taxonomic extremity is the fish. The term "fish" is generally an accessible description for a group of poikilothermic (cold- thoroughbred) marine invertebrates under the phylum Chordata that breathe with gills" [3]. "Fishes are vertebrates of great diversity in morphological appearances and numerically, accurate scientific descriptions have been noted for more than 35,000 species that contribute significantly to the number of invertebrates" [3,4]. "Globally, there are 86% of unidentified terrestrial species in need of taxonomic identity whereas in marine and brackish territories, this number is extremely high (91% species)" [5]. "Freshwater is any natural liquid or frozen water containing low level of dissolved solids. Although the term specifically excludes seawater and brackish water but does include non-salty mineral-rich waters similar as chalybeate springs" [6]. "Freshwater fishes are vertebrates with streamlined bodies and are rich in different types of nutrients. They live each, or a critical part of their life in either brackish inland or brackish arms" [7]. "Some examples of freshwater fishes are carps, characins, cichlids, salmon, eels, shafts, and sawfish, some estuarine fish, e.g. archer fish and gobies; and soda pop and swab lake fish but exclude coral reef fish and fishes in the ocean and sea. Fish identification is traditionally done with the use of morphological parameters" (Valentina, Andrea and Rita, 2011). "Since humans learned how to hunt fish, species were identified and given names on the ground of their simple anatomical features. The identification of fish on the ground of their morphological features is the most practical, rapid and low-cost system" [7]. "Besides well-experienced fishermen and fishmongers, people who live by the waterside would learn to identify fish at a youthful age. This is due to knowledge and memory acquired from long-term observation or through oral tradition maintained by elders" (Ferreira et al., 2014). Some species like the *Silurichthys indragiriensis and Wallago attu* propel themselves forward or backward by wavelike flexure of long anal fins. Ichthyologists call these fins " strip- fins "[8], and what makes them so easy to identify is that the entire stretch of the anal fin is actuated by muscles along the body length [9-11]. "Patterns and configuration of the cephalic-lateral system on fish bodies are occasionally used for fish identification similar to those from the Kryptoglanis, Pseudorasbora and Caecieleotri genera" [12-14]. A recent study describes an app that could be installed on smartphones in order to take photos of the fish and shoot them to a cloud server for processing and recognition. The answer would also arrive directly at the smart device [15]. This method has been adjudged to be more than 50% accurate and efficient in fish identification and recognition [16]. Even with these records, morphological indices have been adjudged to be inadequate in the unambiguous identification of fish, especially the cryptic species. For this reason, scientists have adopted a DNA-based method that will eclipse the limitations with morphological parameters. "DNA barcoding is a system of species identification using a short section of DNA from a specific

gene or genes. The premise of DNA barcoding is that by comparison with a reference library of similar DNA sections (also called "sequences"), an individual sequence can be used to uniquely identify an organism to species level, in the same way that a supermarket scanner uses the familiar black stripes of the Universal Product Code (UPC) barcode to identify an item in its stock against its reference database" [17].

These sections of mtDNA called "barcodes" are sometimes used in an effort to identify unknown species or parts of an organism, or simply to catalog as many taxonomic groups as possible, or to compare with traditional taxonomy in an effort to determine species boundaries. Different gene regions are used to identify the different organismal groups using barcoding. Different gene regions are used to identify the different organismal groups using barcoding. The most generally used barcode region for animals and some protists is a section of the cytochrome c oxidase I (COI or COX1) gene, found in mitochondrial DNA. In 2003, Hebert et al. [18] proposed that "the cytochrome oxidase subunit I (COI) gene in the mitochondrial DNA has the capability to serve as a barcode for all animals. DNA barcoding serves as a golden bullet for not only species identification but also delimits species boundaries". "With this revolution in taxonomy, the Barcode of Life (BOL) design was launched to develop a generally accepted barcode system that's hinged on a standard sequence of mitochondrial cytochrome oxidase I (COI) gene to identify eukaryotes and also matriculates global biodiversity at a platform" [19]. In late 2004, the Consortium for the Barcode of Life (CBOL) inaugurated this project. The goal of CBOL became to broaden a popular protocol of DNA extraction, PCR, and sequencing techniques, which is a useful resource for the formation of a worldwide DNA library.

"The CBOL entered a new phase when it launched the International Barcode of Life (IBOL) collaborating with 26 countries that aimed to enlist eukaryotic biodiversity. For the first time, the IBOL concentrated substantially on a collection of maximum barcode sequences in a barcode library from all over the world to achieve the target of five million species barcodes. The IBOL also developed bioinformatics software with a huge data library" [19,20]. Some other important systems include the Fish barcode of life (Fish BOL), Health Barcode of life (HBOL), Lepidoptera Barcode of life (LBOL), Marine Barcode of life (MarBOL), Mosquito Barcode of life (MBI), Mammalia Barcode of life (MBOL), Coral Reef Barcode of life (CRBOL) and Bee barcode of life (BeeBOL) [21]. Many nations of the world have come up with such projects as Norway (NorBOL), Mexico (MexBOL), Japan (JBOL) and Europe (EBOL). To date, no study has been able to provide molecular details of the diversity and distribution of freshwater fish species in New Calabar river, hence the aim of this study was to use DNA barcoding in the identification of fish species in New Calabar river, Rivers State.

2. MATERIALS AND METHODS

2.1 Study Area

Rivers state is in the South South part of Nigeria. The capital of Rivers state is Portharcourt. The state shares borders with Anambra and Imo on the north, Abia and Akwa Ibom on the East and Bayelsa and Delta on the West. The samples were collected from New Calabar River in Rivers West Senatorial district of Rivers state, Nigeria.

2.2 Sample Collection

A total of nine (9) fish samples were collected and used for this study. Samples were collected by the help of fishermen using a standard fishing gear. Random sampling method was used for the collection of the fish samples as described by Eyo and Akpati [22].

2.3 Transportation of Fish to Laboratory

The fishes were placed in a glass aquarium where they were transported to ETF lab of Applied Biology and Biotechnology, Enugu State University of Science and Technology for sample collection.

2.4 Identification of Fish Samples by a Taxonomist

Before sample collection, a taxonomist was called to identify the fish samples. The identification was done as described by Fischer [23].

2.5 Collection and Preservation of Sample

A sharp blade was used to cut the fish muscle as described by Dowgiallo [24]. Prior to DNA extraction, the muscle samples were collected

and preserved in 50 ml of DNA/RNA and were transported to Inqaba Biotech, Ibadan for further analysis.

2.6 DNA Extraction

Quick DNA Miniprep Plus kit (D4068, Zymo Research) was used for the DNA extraction as described by Zymo Research Corp. It has the advantage of working reproducibly with almost any kind of plant or animal specimen. A 15mg Fish muscle was excised from each sample and used for DNA extraction. The fish muscle sample was grinded and homogenized using mortar and pestle for 2 minutes. The samples were then placed in clean micro centrifuge tubes of 1.5mL and were labelled with an identification number (1 to 9) according to the number of samples. Ninety five (95) µL of water, 95ul of solid tissue buffer and ten (10) µL of proteinase k were added to the samples in the microfuge tubes. The tubes were vortexed 10-15 seconds and then incubated at 55^{o}C for 1-3 hours until the tissue solubilized. The tubes were centrifuged at 12,000 x g for one minute to remove the insoluble debris. The aqueous supernatants were transferred to a new microfuge tube of 1.5mL. This was followed by the addition of 400 µL of Genomic Binding Buffer and was votexed for 10-15 seconds. The mixture was transferred to a Zymo-Spin^{TM} IIC-XLR column in a collection tube and centrifuged at 12,000 x g for 1 minute. The collection tube was then discarded with the flow through. Four hundred (400) µL of DNA Pre-wash Buffer was added to the spin column in a new collection tube and centrifuged at 12,000 x g for 1 minute. The collection tube was emptied and was immediately followed by the addition of 700ul g-DNA Wash Buffer to the spin column. It was centrifuged at 12,000 x g for 1 minute. The collection tube was then discarded with the flow through. The spin column was transferred to a new clean microfuge tube. Fifty (50) µL of DNA Elution Buffer was added to the matrix, incubated for 5 minutes at room temperature, and then centrifuged at maximum speed for 1 minute to elute the DNA. The eluted DNA was stored at 20^{o}C in preparation for amplification. The Nanodrop Spectrophotometer was used to determine the purity and concentration of DNA samples.

2.6.1 Amplification of DNA by PCR

The fish target region was amplified using the OneTaq Quick Load 2X Master Mix (NEB, Catalogue No: M9486), nuclease free water, template DNA with two primers for forward and reverse reactions (FISH F1-TCAACCAACCACAAAGACATTGGCAC and FISH F2-TAGACTTCTGGGTGGCCAAAGAATCA). After thermal cycling, the amplified DNA was stored at -20°C as described by Shokrallan et al., [25].

2.6.2 Analyzing PCR product by gel electrophoresis

The gel-casting tray was used for the gel electrophoresis. A total of 1g of powdered agarose gel was dissolved in 100ml of 1X Tris Acetate EDTA (TAE). It was heated until agarose was completely dissolved in the buffer and it was allowed to cool after which 4ul of SafeView Classic (gel stain) was added. It was allowed to cool for about 60^{o}C and was poured into the tray with casting dams fit on both ends of the tray and combs in correct position and allowed to set. After the gel is set, the combs and casting dams were removed while the tray was placed in the electrophoresis tank containing the buffer of choice (TAE). The ladder and samples were carefully loaded into the wells and tank covered with its lid and connected to the negative (–ve) and positive (+ve) electrodes and power supply. The gel was kept for approximately 30 minutes 130v and was viewed using UV transilluminator as described by Lucentini et al. [26].

2.7 DNA Sequencing

The PCR products were further purified using EXOSAP method. The purified fragments were analyzed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, Therm Fisher Scientific) for each reaction and every sample. The extracted fragments were sequenced in the forward and reverse directions as described by Sanger et al. [27].

2.8 Data Analysis

A total of 18 sequences were generated from the ABI 3500xl Genetic Analyzer (Applied Biosystems). Each sample was identified to the specie level using BLASTn program on the GenBank (National Center for Biotechnology Information) [28]. The sequencing results were carefully trimmed and edited using the DNA subway as described by Merchant et al. [29]. Pairwise alignment of sequences was done using MEGA 11 software [30]. The evolutionary history was inferred using the Neighbor-Joining method as described by Saitaou and Nei [31]. DNASTAR

was used to analyze the ab1 files generated by the ABI 3500XL Genetic Analyzer.

3. RESULTS

3.1 Estimation of the Concentration and Purity of DNA Samples

The data obtained from the Nanodrop Spectrophotometer is shown in Table 1. The Nanodrop was used to measure the concentration and purity of the DNA samples. The column measured light passing through the DNA at a wavelength of A280/260nm. The ideal purity value for DNA samples at A260/280 is 1.8 and between 2.0 and 2.2 for A260/230. Scores lower than these usually indicate that there are contaminants present in the sample. The highest DNA concentration of 271 was observed in sample 13 (*Lutjnus agennes*) with ratios of 1.89 and 1.92 for A280/260 and A260/230 respectively. The lowest concentration of 43.9 was observed in sample 7 (*Pseudotolithus elongatus*) with ratios of 1.81 and 0.47 respectively. Samples 10 (*Polynemius dubius*) and 8(*Ethmolosa fambriata*) had the highest and lowest purity ratios of 1.97 and 1.76 respectively.

3.2 BLAST Result from NCBI Database

Table 2 shows the BLAST result which corresponds to the similarity between the sequences queried and the biological sequences with the NCBI database. The names of the original samples, percentage identity (ID), predicted organisms from blast result and GenBank Accession numbers were clearly identified. A total of 18 sequences (all > 600bp) belonging to 8 species, 7 genera, 7 families and 5 orders were validated and used for the analysis. The highest percentage ID of 100% for predicted organisms was found in *Neochelon falcipinnis, Lutjanus agennes* and *Pseudotolithus elongatus* while *Polynemus dubius,* hadthe lowest percentage ID of 86.2%. Only two (22%) out of the 9 original samples analyzed corresponded with the predicted organisms from BLAST result. The organisms are *Ethmolosa fambriata* and *Chloroscombrus chrysurus.* The remaining 7 samples (77%) differed from the predicted organisms in BLAST analysis. A total of one class (Actinopterigii), 5 orders (Perciformes, Siluriformes, Clupeiformes, Mugiliformes, Carangiformes) and 7 families (Claroteidae, Sciaenidae, Clupeidae Polynemidae, Mugilidae, Lutjanidae, Corangidae) were detected among the analyzed samples. The highest number of orders and families were found in Perciformes (n=5) and Sciaenidae (n= 3) respectively.

3.3 Evolutionary Relationships of Taxa

The phylogenetic tree is shown in Fig. 1. The evolutionary history was inferred using the Neighbor-Joining method [31]. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei and Kumar, 2004) and are in the units of the number of base substitutions per site. This analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 712 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [32]. Table 3 shows the intra and intergenetic distances among the analyzed fish species.

Table 1. Estimation of the concentration and purity of DNA samples

Name	Concentration	A260/A280	A260/230	A260	A280
Sample 5	170.3	1.95	1.63	3.41	1.75
Sample 6	125.6	1.86	1.85	2.51	1.35
Sample 7	43.9	1.81	0.47	0.88	0.48
Sample 8	61	1.76	1.23	1.22	0.69
Sample 10	53.2	1.97	1.85	1.06	0.54
Sample 11	194.8	1.84	1.52	3.90	2.11
Sample 12	130	1.86	1.38	2.60	1.40
Sample 13	271.9	1.89	1.92	5.44	2.88
Sample 14	98.9	1.81	1.65	1.98	1.09

3.4 Electropherogram Trace Files

The electropherogram trace files obtained from the ABI 3500XL Genetic Analyzer (Applied Biosystem) were trimmed, filtered and edited using Finch TV and BioEdit (Hall 1999; Bousalem *et al.,* 2000) [30]. Short sequences with stop codons known as pseudogenes or junk DNA were not detected when sequences were submitted to the NCBI database. Tables 4 and 5 show the percentage occurrence of the four different nucleotides and the total number of base pairs for each predicted organism. In the reverse strand, the table shows that *Pseudotolithus brachygnathus* had the highest number of base pairs (673bp) while the least number of base pairs was found in *Ethmolosa fambriata* (600bp). The intraspecific species (*Pseudotolithus elongatus and Pseudotolithus elongatus*) showed little similarity in their sequence and occurrence of bases both in the reverse and forward strands. For instance, in reverse strand, the occurrence of bases in A was 21.75% and 23.88%, T (28.99% and 28.51%), C (28.70% and 28.8%) and G (20.56% and 18.81) respectively. The observed baseline noise was minimal and did not affect calling of the peaks. Each peak with a particular colour represents a nucleotide. Adenine (A) is represented with green colour, Cytosine (C) with blue, Guanine (G) with black and Thymine (T) with red colour.

Table 2. Result obtained from BLAST analysis using COI gene marker

Name of sample	Percentage ID	Predicted organism	GenBank accession
Pimelodus pictus	97.25%	*Chrysichthys nigrodigitatus*	MG824571.1
Menticirrhus americanus	100%	*Pseudotolithus elongatus*	KY442727.1
Pseudotolithus senegalensis	100%	*Pseudotolithus elongatus*	KY442723.1
Ethmolosa fambriata	98.99%	*Ethmolosa fambriata*	AM911179.1
Polydactylus quadrifili	86.2%	*Polynemus dubius*	KU199001.1
Mugil cephalus	100%	*Neochelon falcipinnis*	HM208829.1
Micropoganias undulatus	99.84%	*Pseudotolithus branchygnathus*	KP722767.1
Lutjanus argentimaculatus	100%	*Lutjanus agennes*	KY442726.1
Choloroscombrus chrysurus	99.84%	*Chloroscombrus chrysurus*	KY442711.1

Table 3. Inter and intragenetic distances among fish samples from New Calabar river

	1	2	3	4	5	6	7	8
Pseudotolithus brachgnathus								
Lutjanus agennes	0.204							
Chloroscrombrus chrysurus	0.212	0.183						
Chrysichthys nigrodigitatus	0.290	0.266	0.266					
Pseudotolithus elongatus	0.105	0.208	0.211	0.307				
Pseudotolithus elongatus	0.141	0.224	0.266	0.271	0.164			
Ethmolosa fambriata	0.273	0.210	0.264	0.283	0.253	0.243		
Polynemus dubius	0.281	0.238	0.239	0.285	0.271	0.248	0.251	
Neochelon falcipinnis	0.212	0.184	0.188	0.266	0.205	0.212	0.201	0.234

Table 4. Summary of DNA bases and their percentage occurrence (reverse)

S/N	Name of organism	A	%	T	%	C	%	G	%	Total
1	Chrysichthys nigrodigitatus	170	27.78%	166	27.12%	116	18.95%	160	26.14%	612
2	*Pseudotolithus elongatus*	185	27.78%	150	22.52%	135	20.27%	196	29.43%	666
3	*Pseudotolithus elongatus*	183	27.52%	161	24.21%	124	18.65%	197	29.62%	665
4	*Ethmalosa fimbriata*	178	29.67%	145	24.17%	125	20.83%	152	25.33%	600
5	*Polynemusdubius*	201	29.91%	160	23.81%	124	18.45%	187	27.83%	672
6	*Neochelonfalcipinnis*	188	28.75%	158	24.16%	117	17.89%	191	29.20%	654
7	*Pseudotolithus brachygnathus*	182	27.04%	155	23.03%	131	19.47%	205	30.46%	673
8	*Lutjanus agennes*	173	25.90%	172	25.75%	125	18.71%	198	29.64%	668
9	*Chloroscombrus chrysurus*	196	29.39%	163	24.44%	123	18.44%	185	27.74%	667

Table 5. Summary of DNA bases and their percentage occurrence (forward)

S/N	Name of organism	A	%	T	%	C	%	G	%	Total
1	*Chrysichthys nigrodigitatus*	170	26.90%	172	27.22%	178	28.16%	112	17.72%	632
2.	*Pseudotolithus elongatus*	147	21.75%	196	28.99%	194	28.70%	139	20.56%	676
3	*Pseudotolithus elongatus*	160	23.88%	191	28.51%	193	28.81%	126	18.81%	670
4	*Ethmolosa fambriata*	152	23.94%	189	29.76%	173	27.24%	121	19.06%	635
5	*Polynemus dubius*	155	23.07%	205	30.51%	186	27.68%	126	18.75%	672
6	*Neochelon falcipinnis*	154	23.73%	189	29.12%	187	28.81%	119	18.34%	649
7	*Pseudotolithus brachygnathus*	149	22.51%	182	27.49%	200	30.21%	131	19.79%	662
8	*Lutjanus agennes*	169	25.26%	174	26.01%	197	29.45%	129	19.28%	669
9	*Chloroscombrus chrysurus*	161	24.03%	197	29.40%	185	27.61%	127	18.96%	670

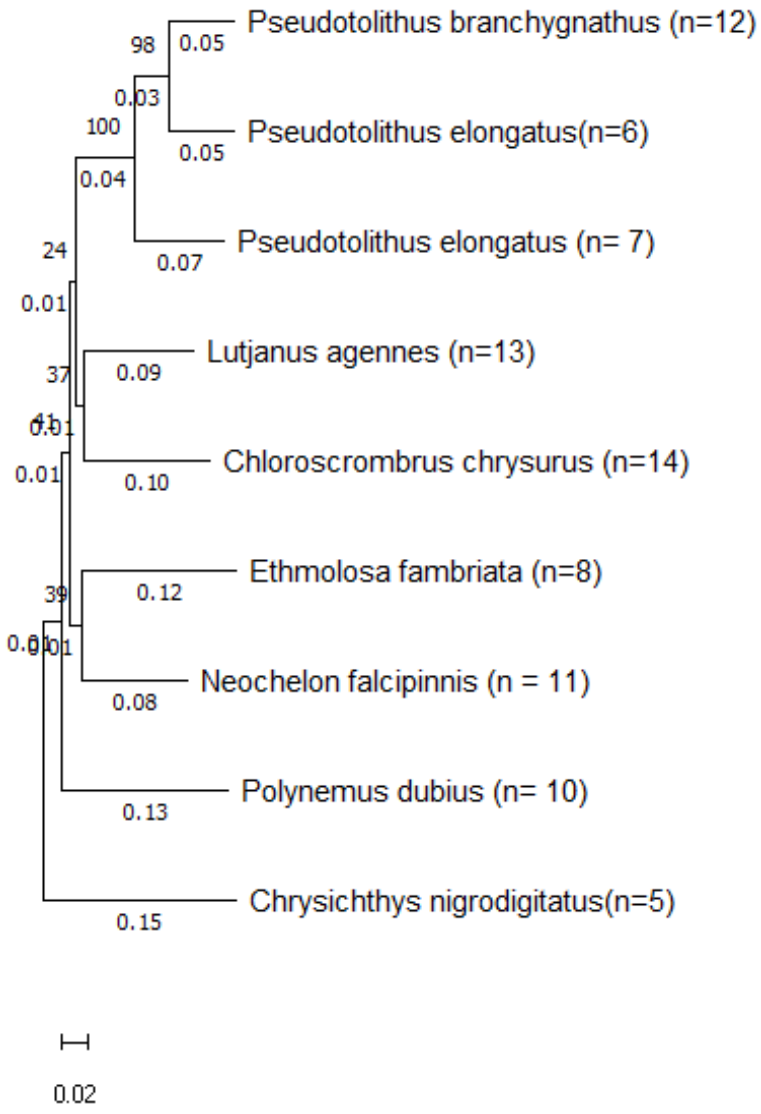

Fig. 1. Phylogenetic tree of fish species from New Calabar River

4. DISCUSSION

The use of DNA barcoding for species identification has been authenticated by several studies [33,34]. The detected 8 species, 7 genera, 7 families and 5 orders are lower than the studies involving 44 fish species [35] but higher than the study involving 3 species [36]. Some of the identified fish species corresponded with the ones earlier discovered by researchers

within and outside the country [35-38]. The COI sequences effectively clustered intraspecific and congeneric species at a bootstrap value of 100%. Similar result was obtained in the study involving 363 freshwater fishes from Southeastern Nigeria [38] and 194 Canadian freshwater fishes [37]. This study recorded more than 95% success rate and it corresponded with other studies on DNA barcoding of freshwater fishes [37-39]. The genetic distances were similar between conspecific and congeneric individuals but different at confamilial taxonomic level. This agrees with the study done by George et al. [35] where they reported computations of genetic distance between (interspecific) and within (intraspecific) species.

The genetic distances between *Chrysichthys nigrodigitatus* and *Pseudotolithus elongatus, Ethmolosa* and *Polynemus dubius, Neochelon falcipinnis,* and *Micropogonias undulatus, Lujanus argentimaculatus* and *Chloroscrombrus chrysurus* are 0.31, 0.25, 0.21 and 0.183% respectively. These values fall within the range (0-7.42%) reported in the DNA barcoding of 72 commercial fish species in USA [40] and 194 Canadian fish species [37]. The genetic distance between the two species of croaker fish-*Pseudotolithus elongatus* was 0.16% and it was also reflected in the occurrence of their bases and their position in the phylogenetic tree. The genetic distance is a little higher than the threshold of 0.02%. On a global scale, the mean intraspecific genetic distance does not exceed 0.01% while interspecific (within genus) distances are usually higher than this value to favour the argument of separation of individual species based on their genetic distances (Tsoupas*et al.,* 2022). This intraspecific variation in species of cassava croaker fish could be as a result of infrequent mating of members and genome based alteration arising from exposure to environmental toxicants. This agrees with Nwani et al. [38] who said that such differences could arise from mutation and genetic recombination. Furthermore, the genetic variation between the two species of croaker fish (*Pseudotolithus*) could also occur as a result of introgression and hybridization. The infrequent intraspecific and frequent interspecific mating can result to the production of hybrid offsprings and may ultimately result to phylogenetic paraphyly (Barraclough and Nee, 2001).

Another explanation to the intraspecific variation is the concept of allopatric speciation. This occurs when a population is separated by a barrier and such isolation prevents the two or more sub-populations from mating. Given time, the lack of gene flow among the sub-populations will cause biological incompatibility and genetic divergence would be triggered (Singh, 2012). Generally, the intergeneric divergences observed in the phylogenetic tree are lower than the one reported in the study in the study of 35 fishes (Lara et al., 2010) and 44 fishes [35]. The Nanodrop Spectrophotometer showed that all the samples (88%) except sample 10 (*Polynemus dubius*) had a standard purity value of 1.8 at a wavelength of A260/280nm. This value suggests that the DNA samples were relatively free of contaminants. Pairwise sequence alignment showed different consensus positions and a total of 30 mutations found in *Polynemus dubius* (n=30). There were 1 insertion, 29 substitution mutations (15 transition and 14 transversion). No deletion mutation was reported. There were no and nonsense codons in all the amplified sequences. This aligns with earlier studies reported by Ali et al. [41] and George et al. [35], [42-49].

5. CONCLUSION

This work has proven that the mitochondrial cytochrome oxidase gene 1 (COI) is unambiguous and effective in discriminating species boundaries. It has generated a barcode library for fish population in New Calabar river, and will serve as a molecular framework for identification of fish species in other freshwater sources in the Rivers state. The use of DNA barcoding in the identification of fish species appears to hold great potential for discrimination of fish species and authentication monitoring system by governments, agencies, regulatory bodies and industries to improve transparency and fair trade on domestic fish management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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