GENETIC DIVERSITY OF TILAPIA IN NIGERIA USING AVAILABLE COI SEQUENCE IN THE GENE BANKS

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ABSTRACT

Tilapia importance in global aquaculture and fisheries has motivated the study and assessment of their genetic diversity in Nigeria. In this article, attempt was made to retrieved all available mitochondrial cytochrome c oxidase subunit I gene (COI) sequences of tilapia species from Nigeria deposited in BOLD and NCBI, to quantify their available DNA barcoding information. It appears output of tilapia species barcoding studies in Nigeria were not much as we got only 112 hits. Maximum likelihood analyses of the sequences with three tilapia reference genomes and three distant outgroups (669 bp fragment; N=112+3+3) showed two groups of Nile tilapia Oreochromis niloticus, identification of some unidentified Tilapia spps and Oreochromis as Coptodon camerunensis, Coptodon dageti and Oreochromis aureus, presesnce of Blue tilapia Oreochromis aureus in both species of O. niloticus and Sarotherodon galilaeus groups independently. These species are readily utilized for aquaculture in Nigeria and could escape from farms into the wild due to poor waste management or flooding. Hence, this may explain why the BLAST search identified an unplaced sequence from Ondo, Nigeria as Coptodon camerunensis (99.2%), a strain endemic to Northern Cameroonian rivers. However, O. niloticus individual from River Benue could be pure based on placement with its genome. Considering the limitations of mtDNA, we recommended an exome gene capture approach in addressing issues of introgressive hybridization, for better-informed conservation of tilapia pure stocks.

KEYWORDS: Cichlids, GenBank, Introgression, Mitochondrial DNA, Phylogenetic, Geographical regions.

INTRODUCTION

Tilapias are freshwater fish of Cichlidae family native to Africa and middle East. They are widely distributed across Africa except Atlas Mountains and Southwest Africa, (McAndrew 2000). There are 76 reported tilapia species in Africa (Philippart and Ruwet 1982; Adesulu 1997), however, 52 tilapia species of the Cichlidae family which includes 32 *Oreochromis*, 13 *Sarotherodon*, and seven *Tilapia* species were suggested by Fishbase (Froese and Pauly 2018). Nigeria is one of the leading producers of farmed tilapias in Africa, with Egypt topping the chain (Adesulu 1997; El-Sayed 2006; Fagbenro *et al*., 2010). There are about six species of tilapia commonly used for aquaculture, namely, *Oreochromis niloticus*, *O. aureus, Sarotherodon galilaeus*, *S. melanotheron, Tilapia zillii and Tilapia. guineensis (*Adesulu and Sydenham 2007; Idodo-Umeh 2003).

Their utilization in aquaculture can also result in the taxonomic classification confusion of tilapia among researchers (Dunz and Schliewen 2013). This is because farmed populations usually colonize non-native water catchments either by intentional introduction or escape from the farm due to poor disposal management (Ford *et al.,* 2015; Syaifudin *et al.,* 2019; Wu and Yang 2012; Shechonge *et al.,* 2019). This can lead to hybridization, habitat alteration, and ecological competition that threatens native species (Bbole *et al.,* 2014; Canonico *et al*., 2005; Deines *et al.*, 2014; Firmat *et al*., 2013; Mwanja *et al*., 2012). Flooding is another major event that can destroy farms, cause the overflowing of rivers, and homogenize different species (Askew 1999; Odufuwa *et al.,* 2012; Umar and Gray 2022). In Nigeria, flooding has been happening and causing overflow of major river systems with devastating impact across Nigeria (Chukwu 2014; Horsfall *et al.,* 2023; Ishaya *et al.,* 2023). This long time level of flooding over several generations of species, questions the purity of any inland aquatic species in Nigeria or Africa. This could lead to introgressive hybridization and taxonomic confusion among researchers, as observed by the recent changes of names of some Tilapia species; *Tilapia dageti* (Thys van den Audenaerde 1971), *Tilapia guineensis* (Günther 1862), *Tilapia mariae* (Boulenger, 1899) and *Tilapia zillii* (Gervais 1848) reclassified as *Coptodon dageti* (Thys van den Audenaerde 1971),

Coptodon guineensis (Günther 1862), *Pelmatolapia mariae* (Boulenger 1899) and *Coptodon zillii* (Gervais 1848), respectively. Hence, there is need for proper molecular taxonomic assessment of tilapia species diversity in Nigeria.

DNA barcoding is a short fragment that is easily used to identify species by comparing it to a reference library of DNA sequences for a given taxonomic group. The standard tool for DNA barcoding mostly used to differentiate species and detect diversity in animals is the mitochondrial genes such as cytochrome c oxidase subunit I gene (COI) (Kadarusman *et al.,* 2012; Kakioka *et al.,* 2018). In Nigeria DNA barcode library has been employed to study the taxonomy of Nigerian fishes (Nwani *et al.,* 2011; Nwakanma *et al*., 2015; Falade *et al*., 2016; Sogbesan *et al*., 2017; Iyiola *et al.,* 2018; Nneji *et al*., 2020; Mojekwu *et al.,* 2020; Popoola *et al.,* 2022). However, information on the utilization of DNA barcodes to identify and distinguish tilapia species is dearth. Most of the samples from NW and SS (Nwani et al., 2011; Falade et al., 2016; Sogbesan et al., 2017; Mojekwu et al., 2020; Popoola et al., 2022). The barcode CO1 sequence has been shown to be highly variable and specific due to sequence divergence and conservation between and within species. This makes DNA barcoding an effective technology used in verifying several animal groups (Ward *et al.,* 2005; Hajibabaei *et al.,* 2007).

Nevertheless, mtDNA is maternally inherited and cannot account for events such as hybridization and incomplete lineage sorting prevalent among tilapia species. Hence, a significant limitation in utilizing mitochondrial markers in assessments of that phenomenon. However, some authors have used both mtDNA and nuclear markers in addressing introgressive hybridization (Anane-Taabeah *et al*., 2019; Ford *et al*., 2019). Assessment of tilapia diversity in Nigeria will require detailed sampling from various geographical river systems with proper morphological and genomic approaches to avoid taxonomic inaccuracies.

The present study aimed to assess relationships between tilapias from Nigeria stored in GenBank(Gene database). Confirm if there are taxonomic discrepancies between species based on the COI sequences. The author analyzed DNA barcodes of wild individuals found on the Barcode of Life Data system (BOLD) database(https://www.boldsystems.org/) and NCBI using a systematic method.

MATERIAL AND METHODS

Mining COI 5' (barcoding fragment) from BOLD and NCBI

BOLD system version 4 (www.boldsystems.org) and NCBI (https://www.ncbi.nlm.nih.gov) were used for the search on the 15th of July 2023. The search term used was "COI tilapia in Nigeria" (BOLD database → data portal → search query in public data →Input "tilapia COI in Nigeria" → search →download sequences as FASTA while in NCBI→ nucleotide → input "tilapia COI in Nigeria"→ click search → send to file → download sequences as FASTA). We discovered that *O. aureus* spp were not present using the above search terms. Hence to capture any tilapia like *O. aureus* not shown in previous search result, further search using the term ("Oreochromis COI in Nigeria") was done. A Complete mitochondrion reference genome sequences of *O. nil x O. aureus* Hybrid NC_025669.1, *Coptodon zillii* NC_026110.1, and *Oreochromis niloticus* NC_013663.1 were added for easy taxonomic placement. Furthermore, three species of non haplotilapiines COI sequences from different genera; *Heterochromis multidens* MK074351.1, *Tylochromis lateralis* MK074681.1 and *Pelmatochromis nigrofasciatus*, KT193197.1 were included as distant outgroups based on Dunz and Schliewen 2013. The combined sequences were trimmed in MEGA X (Kumar *et al*., 2018) and ready for phylogenetic analysis.

Phylogenetic Analyses and BLASTN

Sequences retrieved from databases were edited, assembled and aligned using MEGA X (Kumar *et al.,* 2018). To compare and ascertain the best standard nucleotide substitution models, feature in MEGA X was applied for the model test, which ranked K2+G as the top models. Phylogenetic analysis derived from a maximum likelihood phylogeny was computed based on the best K2P+G substitution model (Tamura *et al.,* 2004), branch support of 500 bootstrap replicates and pair-wise deletion option to remove ambiguous positions for each sequence pair. BLAST search were further used to identify some unidentified Tilapia species based on the sequence similarity and coverage.

RESULTS

BOLD and NCBI search of tilapia COI indicated that all the 102 sequences downloaded in NCBI were present in Bold except one tilapia spp MT621195 (Table 1). The sequences were combined after filtering out duplicates to obtain 103

DNA barcodes. The search results for other tilapia spp using the term "Oreochromis COI in Nigeria" gave a total of 39 *Oreochromis spp*, which includes 30-number *O. niloticus* previously captured, 6-number *O. aureus* and 3-number *Oreochromis* unidentified. This showed that 9 sequences (6-number *O. aureus* and 3-number *Oreochromis* unidentified) were added to 103 sequences to give 112 (103+9). The three reference genomes from *Onil x Oureus* Hybrid, *Coptodon zillii*, and *Oreochromis niloticus* were also added while *Heterochromis multidens*, *Tylochromis lateralis* and *Pelmatochromis nigrofasciatus*, were included as outgroups. This brings it to a total of 118 (102+1+ 6+3+3 genomes+ 3 outgroups) sequences of COI from the database (Table 1). The sequence alignment was inspected and confirmed to be of good quality with no gaps or frameshift mutations. The final data set was a matrix of 118 COI sequences of wild tilapias trimmed to a length of 669bp, ready for phylogenetic analysis (Supplementary table: https://www.researchgate.net/publication/376189282).

Table 1: Number of Tilapia COI barcodes sequences in Nigeria retrieved from databases

S/N	Species	Accession numbers	Region	Number BOLD	Number NCBI	Others searches	Reference genomes	**Total**	BLASTn
1	*Coptodon zillii*	HM882904/ ON072283/, MG824685,4,2 /KY784688)	SE(Otuocha), NC(Jos /Kwara 3) NE (Yola)		36		1		
2	*Coptodon dageti*	HM882891, HM882900/ JF510523	SE(Afikpo 2/ Abakaliki		3				
3	*Coptodon guineensis*	HM882893,5, HM882908,07/ HM882916, HM882923,22, HM882911)	SE(Abakaliki 4/ Afikpo 4)		8				
4	*Oreochromis niloticus*	ON072283/ HM882892/ Mk497146-8/ KY784674/ MK130701-4/ NC 013663.1	NC (Jos)/ SE (Afikpo /osha), NE (Yola), NC (Makurdi 4)/ (Genome)		30		1		
5	*Pelmatolapia mariae*	HM882912, HM882905, HM882901/02/03	SE (Otuocha Anambra state)		5				
6	*Sarotherodon galilaeus*	KY784677, KY784681 / HM882890.	NE(Yola), SE(Abakaliki)		19				
7	*Unidentified Tilapia sp*	HM882892 /	SE(Afikpo)	1					*C. dageti*
		MT621195	SW (Ondo)		1				*C. camerunensis*
8	*Oreochromis aureus*	MG824627/8/9. MK130701/3/4	NC (Kwara 3), (Markurdi 3)			6			
9	*Unidentified Oreochromis*	MG824630/ MG824631/ MG824632	NC (Kwara),			3			*O. aureus*
10	*Onil x Oureus* Hybrid	NC025669.1 OnilXQaur	Hybrid Genome				1		
11	*H. multidens* *T. lateralis* *P.nigrofasciatus*	MK074351.1, MK074681.1, KT193197.1	DR Congo					3	
	Total			1	102	9	3	**118**	

Phylogenetic analysis showed two groups (Group 1 & 2) of Nile tilapia *O. niloticus* (Fig.1). Group 1 with 98% support value comprises sequences of *O. niloticus* and *O. aureus* from South East, North East and North Central, the three unidentified Oreochromis (MG824630 – 32) and the complete genome hybrid of *O. niloticus x O. aureus* species. Group 2 comprises of complete *O. niloticus* genome (NC 013663.1) and two *O. niloticus* species (MKY130700.1 & 2) from North Central.

Some Blue tilapia *O. aureus* from North Central were associted with *S. galilaeus* from South East and North East at 97% support value (Fig. 1). However, Group 1, *O. niloticus* and *S. galilaeus* are monophyletic at 100% support value and sister to group 2 at the same support value.

One unidentified Tilapia spp from South East (HM882892) were placed as sister to *C. dageti* while the other unidentified Tilapia spp. MT621195, collected from ondo in South West were identified as *C. camerunensis* (Fig. 1; Table 1) sharing a common clade with *C. guineensis* and *C. Zilli.*

All outgroups are from DR Congo as we could not find any COI sequences of those species from Nigeria on the database. Some other tilapia species found in Nigeria includes; *Chromidotilapia guntheri*,*Hemichromis camerounensis, Hemichromis fasciatus, Pelvicachromis taeniatus, Rubricatochromis bimaculatus, Rubricatochromis cristatus, Rubricatochromis cf. Guttatus, Thysochromis ansorgii, Tylochromis sudanensis* and many others.

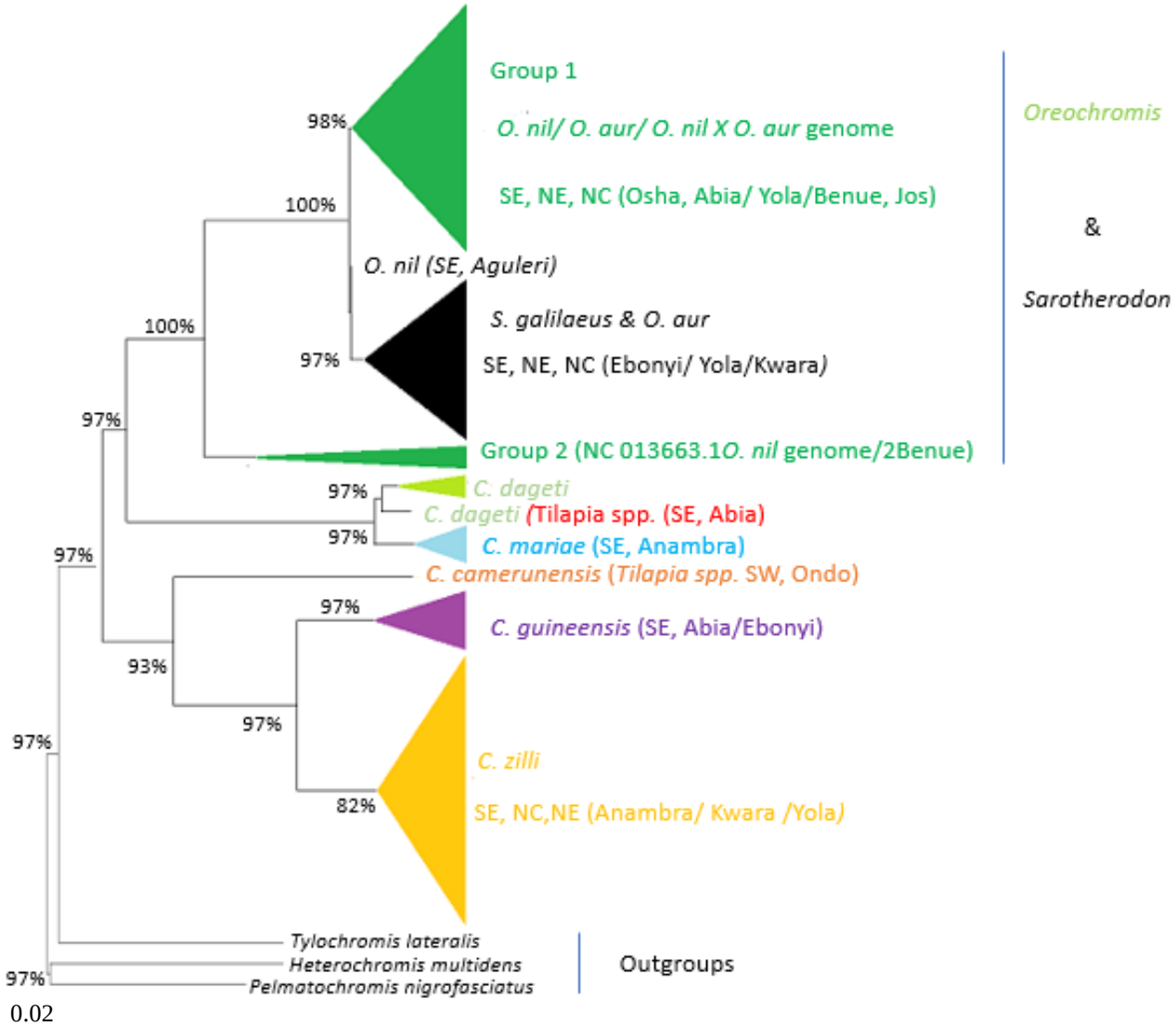

Fig 1: Maximum Likelihood tree of COI tilapia species barcodes in BOLD & NCBI from Nigeria

DISCUSSION

The presence of two groups of *O. niloticus* (Fig. 1) can be attributed to evolutionary complex history resulting from successive dispersal, divergence and secondary contact, reflecting history of river connections that resulted in the extensive native range of *O. niloticus* (Agnese *et al.*,1997; Bezault *et al.,* 2011; Decru *et al.,* 2016; Syaifudin *et al.,* 2019; Mojekwu *et al*., 2020). Group 1 comprises sequence of *O. niloticus* and *O. aureus* from South East (Niger River), North East (Yola) and North Central (N`gell/Gyel river Jos). The three unidentified Oreochromis (MG824630 – 32) from the second search term were placed in this group as *O. aureus*. Group 2 comprises of complete *O. niloticus* genome (NC 013663.1) and two *O. niloticus* species (MKY130700.1 &2) from Benue state (North Central), hence pure stocks of *O. niloticus* could exist in this region based on their association with the genome. Further assessment using nuclear markers will be required to validate these observations. Some authors have also reported several lineages of *O. niloticus* using mtDNA, attributing it to geographic barriers due to its large distribution, and introgression between aquaculture escapees and wild individuals (Bezault *et al*., 2007, 2011; Ford *et al.,* 2019; Mojekwu *et al.,* 2020).

Previous studies based on nuclear markers suggested that the clustering of *Sarotherodon galilaeus* within *Oreochromis* could be due to past hybridization events (Dunz and Schliewen 2013; Ford *et al.*, 2019). This is not surprising as both genera are common aquaculture species in Nigeria, known to hybridize even in captivity (Ayinla 2007; Otubusin 1988).

Phylogenetic and BLASTn search showed that Unidentified Tilapia spp (MT621195) and HM882892 collected from ondo in SW and Afikpo in SE were identified as *C. camerunensis* and *C. dageti* respectively (Table 1). Tilapia spp MT621195 shared a common clade with *C. guineensis* and *C. zilli*, its BLASTN search showed a 99.2% match (100% coverage) to *C. camerunensis* strain KJ938224.1 (BOLD ID: AEV0357) deposited by Kide *et al.,* 2016. This species is endemic to the Meme, Mungo, and Wouri Rivers of Cameroon (Lamboj 2004; Stiassny et al 2008), and needs to be conserved as its status on the IUCN Redlist is vulnerable (Moelants 2010). *C. guineensis* and *C. zilli* linked to this species are from river systems of NC (Jos, Kwara), SE (Afikpo, Abia Anambra, Enugu, Ebonyi, Abakaliki), and NE (Yola) that border Northern Cameroon. The presence of this (*Coptodon camerunensis*) species that is native to Cameroon could be a result of flooding that harmonizes local river systems within and between the two countries. Flooding is linked to heavy rainfall due to the impact of climate changes, worsened by the releases from the Ladgdo dam in Northern Cameroon causing overflowing of the Niger and Benue rivers with an impact on their tributaries. The BLASTN search of the second unidentified Tilapia sp with accession number HM882892 (Bold ID: BOLD: AAL6362) from Afikpo in Abia state of SE region showed it is a *C. dageti* with 98.92% closeness (100% coverage).

Hence, DNA barcode data is an important molecular resource for improving the knowledge of genetic variation within tilapia species. However, introgressive hybridization of tilapias in Nigeria can only be addressed properly using genomic assessment. Otherwise, how can you prove the purity of the tilapia species you are working with? We, therefore, recommend a multigene approach based on exome gene capture in addressing this problem.

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