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Molecular Characterization of Two Catfish Species (*Chrysichthys nigrodigitatus* and *Chrysichthys auratus*) from the Cross River, Nigeria, Using Ribosomal RNA and Internal Transcribed Spacers

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EAU and DAA designed the experiments. Authors DAA, EAU, MU and CMA carried out field sampling and data collection. Author VON designed the primers. Authors VON, EAU, GMU, ORI, CMA and CO carried out the molecular analysis while authors DAA, EAU and VON wrote and proofread the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Studies were conducted on *Chrysichthys nigrodigitatus and C. auratus* from the Cross River, Nigeria. The objective was to document intra- and inter-specific variations in molecular structure among four populations of the genus in the river. DNA was extracted from caudal fin and primers

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designed for the ribosomal RNA and Internal Transcribed Spacer Region were used for amplification. Molecular characterization yielded a fragment containing partial sequences of 18S rRNA, ITS-1, ITS-2 and 28S rRNA genes and a complete sequence of 5.8S rRNA. The 5.8S rRNA was the only gene amplified consistently in all samples indicating that it is more conserved among the samples studied. The percentage of GC contents fall within the range observed in other catfish species. They were generally higher in *C. auratus* than in *C. nigrodigitatus* and also higher than the AT contents in both species. The high GC content could be an adaptation to warmer climates. Alignment of the nucleotide sequences of the two species from the two regions showed that the rRNA and ITS genes are highly conserved with >99% nucleotide identity. However, a total of 284 single nucleotide polymorphisms (SNPs) were observed in these genes in the two species, made up of deletions (0.35%), single base insertions (11.27%), multiple base insertions (14.39%), single base transition substitutions (29.93%), single base transversion substitutions (33.8%) and multiple base substitutions (11.27%). The overall ratio of transition to transversion polymorphisms was approximately 1: 1 indicating a low level of genetic instability in *Chrysichthys* spp. The indels were responsible for length variations in the sequences. Sequence information for the ITS region of these fishes is reported for the first time. This study has provided an insight into polymorphism patterns of these genes in *Chrysichthys* spp across the four populations studied which could form the basis for further genomic characterization and mapping of these species.

Keywords: Internal transcribed spacer; ribosomal RNA; DNA sequencing; silver and golden catfishes; SNPs.

1. INTRODUCTION

Chrysichthys nigrodigitatus is a prized fish in Niger delta region of Nigeria. Commercially, it forms the most important freshwater single-species fishery in the region, providing up to 90% of the total catch each year. Thus, *Chrysichthys* fishery provides numerous job opportunities for the artisanal fishery sector. The species occurs alongside *Chrysichthys auratus* and both species are widely distributed in freshwaters of West Africa where they are highly valued in human nutrition, [1]. In addition, both species are among the wild commercial catches with all-year- round fishery in the Cross River system. *Chrysichthys nigrodigitatus* alone contributes significantly to the economy of the people of the Cross River basin, coming third behind *Pseudotolithus elongatus* and *Ethmalosa fimbriata* [2].

The genus *Chrysichthys* contains more than 35 species that are not easily distinguished morphologically because of lack of distinctive characters among these species [3]. According to [4], *Chrysichthys* is found in Nile, West Africa and western coast of Central Africa. Both *C. nigrodigitatus and C. auratus* are found in commercial landings in the Cross River, Nigeria, all year round. In their study of morphological identification and the taxonomic relationship of farmed species of *Chrysichthys* in Ivory Coast, [5] concluded that *C. nigrodigitatus* was morphologically different from *C. maurus* and *C. auratus.* They, however, suggested further studies on genetic differentiation of individuals from different species. Ama-Abasi et al. [6] also stressed the need for stock identity of the species of *Chrysichthys* in the Cross River.

The use of molecular markers such as mitochondria DNA, random amplification of polymorphic DNA and microsatellite DNA has greatly facilitated studies on the genetic structure, diversity and evolutionary divergence of different fish populations including *Chrysichthys spp.* [7-13]. The development of high-throughput methods for the detection of SNPs and small indels has revolutionized their usage as markers in many organisms [14]. The Internal Transcribed Spacer (ITS) regions of ribosomal DNA (rDNA) are a component of an rDNA cistron, which consists of a transcriptional unit which codes for 18S, 5.8S and 28S, separated by two internal transcribed spacers (ITS-1 and-2) and surrounded by intergenic spacers (IGS). In most eukaryotes, ITSs exist in several hundred copies and are located in one or several loci and distributed in one or several chromosomes. Within a genome, the genes coding for 18S, 5.8S and 28S rRNA are highly conserved because of concerted evolution of intra- and inter-chromosomal loci [15].

In Nigeria, the Niger Delta has suffered extensive environmental pollution from oil and gas related activities [16]. As a result, natural water bodies near such activities are subject to contamination [17]. An increase in contamination levels could increase mutation levels in the fishes found in this area. Genetic information of the fishes

occurring in these waters are scanty and up until the commencement of this study, no sequence information on the ribosomal RNA as well as the Internal Transcribed Spacer (ITS) genes of *Chrysichthys. nigrodigitatus* and *Chrysichthys auratus* was available in the GenBank. The present study was, therefore partly designed to address this issue, thereby providing baseline information on the DNA sequence of these two species. Additionally, the study was to provide insight into the polymorphism patterns of these genes and document genetic differences between individuals from the two geographical locations in the river system. Such knowledge will form the basis for further genomic characterization and mapping of these species.

2. MATERIALS AND METHODS

2.1 Isolation of Genomic Dna And Quantification

Total DNA was extracted from fins of the collected fish samples using cetyltrimethylammonium bromide (CTAB) method of [18]. The DNA concentration was measured using a JenWay Genova nano system. The concentrations were adjusted by diluting the DNA with TE buffer to bring to 250ng/ul concentration.

2.2 Polymerase Chain Reaction (PCR) Amplification

The gene sequences of several Catfish species with gene IDs GQ465242.1, GQ465241.1, GQ465240.1, GQ376028, GQ465248, GQ465244, GQ465239, KF650737, KU707949, AJ412874 and AJ412864 were downloaded from National Centre for Biotechnology Information (NCBI). Several primers were designed from these species to amplify the gene sequences based on the NCBI catfish, in order to also amplify catfish collected from the Cross River. All primers were submitted to Inqaba Biotech West Africa for synthesis.

PCR amplifications for rRNA and ITS were done in a 40 µl reaction volume containing 2 µl genomic DNA (100 ng/µl), 4 µl of 10X PCR buffer, 0.8 µl of dNTP,1 µl of 10 µM of each primer, 0.2 µl Taq polymerase and 31 µl nuclease free water using the Arktik Thermal cycler (84195000, Finland). PCR conditions were as follows: initial denaturation step at 95°C for 5 minutes followed by 34 cycles of denaturation at 94°C for 30 seconds, annealing at 55-58°C (depending on the primer pair) for 30s, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes. After amplification, 10 µl of PCR product was loaded on 1% agarose gel, electrophoresed at 100 V for 45 min and detected by Biotium GelRed. The ones that gave single sharp bands of expected sizes, ignoring primer dimers were selected for sequencing.

2.3 Sequencing of the Target Fragment

A total of 16 *C. nigrodigitatus*, samples (8 from each site), and 12 C. *auratus* individuals (6 from each site) were prepared for sequencing using the BigDye Terminator v3.1 sequencing system (Thermo Fisher). PCR products were purified with QIA quick PCR purification Kit (Qiagen) according to the instruction manual and used for Sanger sequencing. PCR sequencing reaction was performed in thermal cycler using 20 µl reaction containing 5 µl of genomic DNA, 4 µl of 5X sequencing buffer, 2 µl of Big Dye Terminator, 1 µl of 100 ng/µl of either forward or reverse oligo of primers and 8 µl of nuclease free water. PCR amplification conditions were: initial denaturation step at 96°C for 2 min, followed by 40 cycles of denaturation at 96°C for 10 s, annealing at 50°C for 10 s, extension at 60°C for 4 min, and final extension at 72°C for 4 min. The PCR sequencing reaction product was then purified by adding 50 µl of 100% ethanol, 2 µl of 3 M sodium acetate and 2 µl of 125 5 mM EDTA. The reaction was mixed by inverting and incubated at room temperature for 15 min. The mixture was centrifuged at 13000 rpm for 25 min. The pellets were washed with 70% ethanol, air dried for 15 min in the hood. The pellets were then resuspended in 10 µl HiDi formamide and incubated at 65°C for 5 min, 95°C for 2 min and cooled in ice for 2 min. Thereafter the samples were sequenced using ABI 3130 DNA sequence (Applied Biosystems, California and USA).

2.4 Sequence Editing and Alignments

Bioedit software version 7.2.5 was used to view and edit the sequences. The sequencing data were analyzed using SnapGene software (WWW.snapgene .com). MEGA 6.0 was used for multiple sequence alignment of all the samples. To obtain the consensus nucleotide sequence of each species from each location, 8 and 6 individual nucleotide sequences for *C. nigrodigitatus* and *C. auratus* respectively were aligned in each region. The consensus sequence was adopted if at least 70% of the individual sequences had same nucleotide identity.

2.5 Analysis of Single Nucleotide Polymorphisms (Snps)

To illustrate the SNPs in the nucleotide sequences between the two species or between same species from different locations, pairwise alignment of the consensus sequences was done. The absolute frequencies of the SNPs recorded for each pair were presented as bar charts.

2.6 Analysis of Synonymous (Ds) and Non-Synonymous (Dn) Substitutions

Synonymous and non-synonymous polymorphisms were analyzed from pairwise alignment of the consensus nucleotide sequences using DnaSP version 5.10.1 [19].

3. RESULTS

3.1 PCR Amplification Results

Of the various primer combinations tested, only one combination, Cat_5b F:GGATCATTAACGGGTAGCGC and Cat_7R: GGGATTCGGCGCTGGGCTCT amplified about 850 bp fragment in the tested samples. Therefore, this primer combination was selected for PCR for the remaining fish samples. The result showed amplification of about 850bp fragment in most of the samples (Plate 1).

3.2 Variation in ITS Genes between the Two Species

The length of ITS-1 sequence ranged from 251 bp in *C. auratus* to 363 bp in *C. nigrodigitatus* with GC contents of 68.5% and 66.4%, respectively (Table 1). The length of 5.8s rRNA is quite close in both species with GC content ranging from 54.2% in *C. nigrodigitatus* to 56.4% in *C. auratus*. The length of ITS-2 sequence ranged from 203 bp in *C. auratus* to 217 bp in *C. nigrodigitatus* with GC contents of 71.4% and 61.9%, respectively. The GC contents in ITS-1, 5.8s rRNA and ITS-2 were generally higher in *C. auratus* than in *C. nigrodigitatus* and also higher than the AT contents in both species (Table 1).

3.3 Nucleotide Sequence of *Chrysichthys nigrodigitatus* from Lower and Middle Cross River

The length of the sequenced fragment of *C. nigrodigitatus* from Lower and Middle Cross River was 843 bp and 840 bp, respectively. This fragment contains partial sequences of 18S rRNA gene, ITS-1, ITS-2 and 28S rRNA genes and a complete sequence of 5.8S rRNA. The nucleotide sequences have been deposited in the National Centre for Biotechnology Information (NCBI): *Chrysichthys nigrodigitatus* isolate from Lower Cross River CnLCR (Accession # MG902955) and *Chrysichthys nigrodigitatus* isolate from Middle Cross River CnMCR1 (Accession # MG902956.1). The sequence alignments to show the differences in nucleotide sequences from the two regions, using the consensus sequence for *C. nigrodigitatus* in each region, are shown in Supplementary Figs. 1 and 2.

3.4 Nucleotide Sequence of *Chrysichthys auratus* from Lower and Middle Cross River

A fragment size of 755 bp and 741 bp was observed for *C. auratus* samples collected from Lower and Middle Cross River, respectively. As in *C. nigrodigitatus*, this fragment contains partial sequences of 18S rRNA gene, ITS-1, ITS-2 and 28S rRNA genes and complete sequence of 5.8S rRNA. However, the ITS-1 gene is much shorter in *C. auratus* compared to *C. nigrodigitatus*. The nucleotide sequence has been deposited in the National Centre for Biotechnology Information (NCBI): *Chrysichthys auratus* isolate from Lower Cross River CaLCR1 (Accession # MG977440.1) and *Chrysichthys auratus* isolate CaMCR1 from Middle Cross River (Accession # MG977441.1). The sequence alignments to show the differences in the nucleotide sequences from the two regions, using the consensus sequence for *C. auratus* in each region, are shown in Supplementary Figs. 3 and 4.

3.5 Single Nucleotide Polymorphisms (SNPs) in Nucleotide Sequences in the Two *Chrysichthys* Spp

3.5.1 *Chrysichthys nigrodigitatus* from lower and middle cross river

Chrysichthys nigrodigitatus from Lower and Middle sections of the Cross River had a total of 5 and 7 polymorphisms respectively, made up of deletions, single and multiple base insertions as well as single and multiple base substitutions. The most frequent type of polymorphism observed here was the single base transitional substitution (SBST), which constituted about 42% of total polymorphisms recorded for this group. The ratio of transition: transversion polymorphism was 5:1. This was the only group

that recorded deletions (8.3%), (Fig. 1 and Supplementary Table 1).

3.5.2 *Chrysichthys auratus* from lower and middle cross river

A total of 16 polymorphisms were documented for this pair made up of single base insertions (62.5%), multiple base insertions (12.5%), single base substitutions (18.75%) and multiple base substitutions (6.25%). The ratio of transition: transversion mutation was 0.5:1, Fig. 2, Supplementary Table 1.

3.5.3 *C. nigrodigitatus* and *C. auratus* from lower cross river

A total of 133 polymorphisms were documented for this pair, 65% of which were single base substitutions followed sequentially by multiple base insertions (13.5%), multiple base substitutions (12%) and single base insertions (9.8%). The ratio of transition to transversion mutation was 0.95:1 (Fig 3, Supplementary Table 1).

3.5.4 *Chrysichthys nigrodigitatus* and *C. auratus* from middle cross river

This group contained 123 polymorphisms in all, 39% of which were single base transitional substitutions, about 30.9% were single base transversional substitutions, 13% were multiple base insertions, 11.38% were multiple base substitutions and approximately 5.7% were single base insertions, The ratio of transition to transversion polymorphisms was 1.26 : 1 Fig. 4. Supplementary Table 1.

3.5.5 Transition and transversion substitutions

Two types of transition mutations which made up 53% of the total substitutions and four types of transversions (constituting 47%) were observed in the two *Chrysichthys* spp studied. The base substitution C→T was the most frequently encountered followed by A→G while C→G was the least frequent (Fig. 5). The overall ratio of transition to transversion substitution was 96: 85 or 1.12:1.

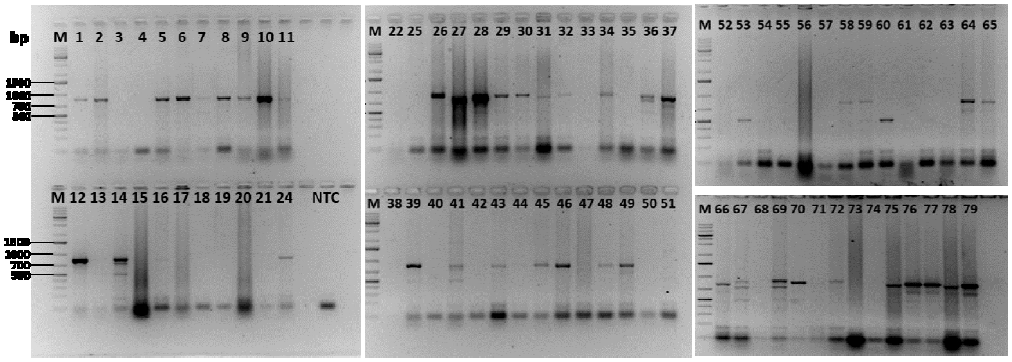

Plate 1. PCR amplification of African catfish rRNAs and ITS genes. PCR was done with primers Cat_5b and Cat_7R (Section 3.1). The amplicon size is about 850 bp. M, GeneRuler 1 kb plus ladder; *C. nigrodigitatus* from Lower Cross River (1-4, 16-24, 27-28, 30-31, 33, 40, 44-46, 53-54, 58-59, 62, 65,75-76); *C. nigrodigitatus* from Middle Cross River (5, 26, 29, 32, 34, 37-38, 43, 55-57, 60-61, 63-64, 66-68, 77-79); *C. auratus* from Lower Cross River (25, 41, 47, 49); *C. auratus* from Middle Cross River (10-15, 35-36; 42, 69-74) *C. spp (unidentified)* from lower Cross River (6-9, 39, 48, 50); *C. auratus* from Middle Cross River (51-52); NTC, non-template control (PCR master mix)

Table 1. GC contents and lengths of ITS1, 5.8S rRNA and ITS2 gene sequences of *C. nigrodigitatus* and *C. auratus*

Species	Location	ITS-1		5.8 rRNA		ITS-2	
		Length	GC (%)	Length	GC (%)	Length	GC (%)
C. nigrodigitatus	LCR	363	65.8	155	54.2	215	61.9
C. nigrodigitatus	MCR	363	66.4	155	54.8	217	62.2
C. auratus	LCR	251	68.5	156	55.1	210	71.9
C. auratus	MCR	251	68.5	156	56.4	203	71.4

LCR= Lower Cross River; MCR= Middle Cross River

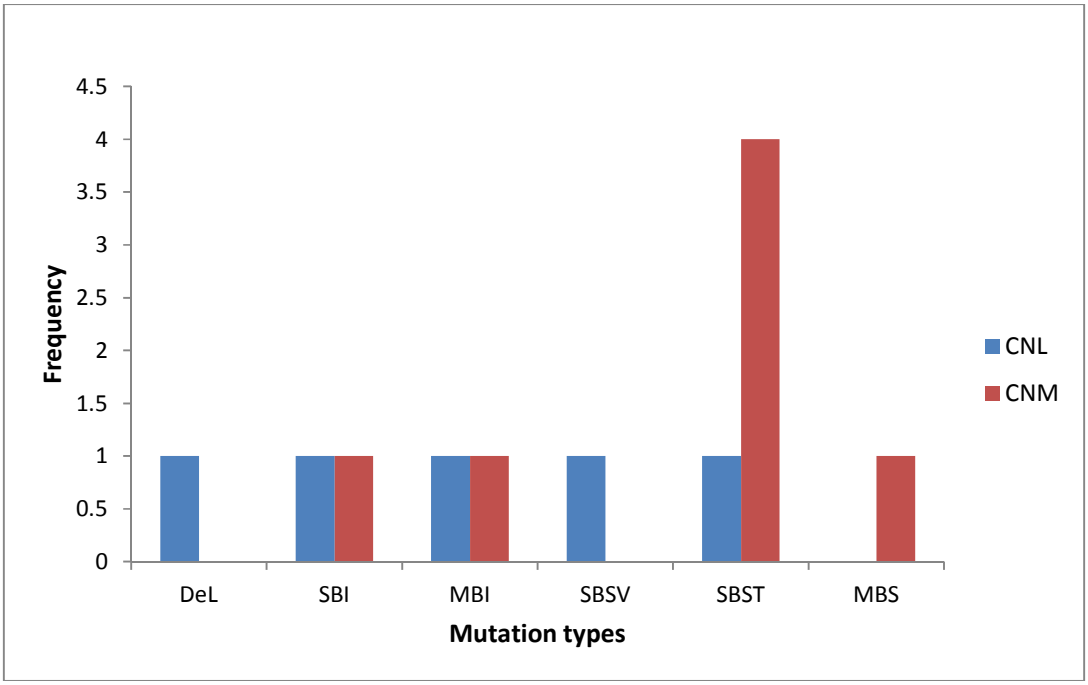

Fig. 1. SNPs analysis of *C. nigrodigitatus* from Lower (CNL) and Middle (CNM) Cross River
Del = Deletion, SBI= Single base insertion, MBI = Multiple base insertion, SBSV=Single base transversion substitution, SBST=Single base transition substitution, MBS = Multiple base substitution

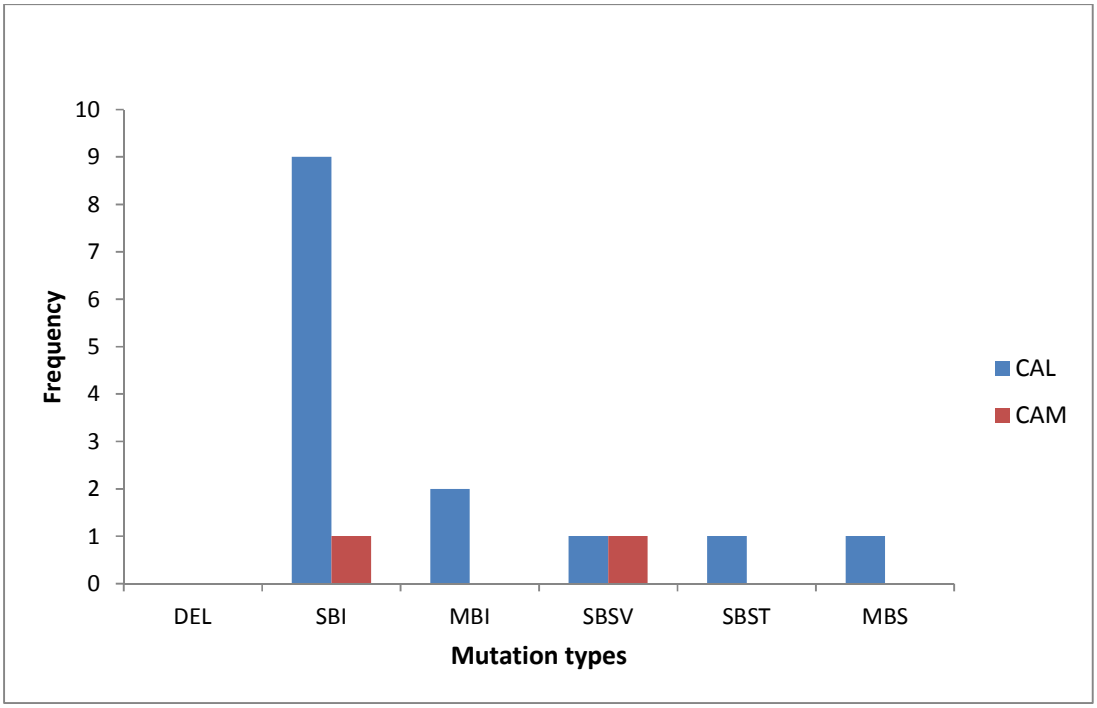

Fig. 2. SNPs analysis of *C. auratus* from lower (CAL) and middle (CAM) Cross River
Del = Deletion, SBI= Single base insertion, MBI = Multiple base insertion, SBSV=Single base transversional substitution, SBST=Single base transitional substitution, MBS = Multiple base substitution

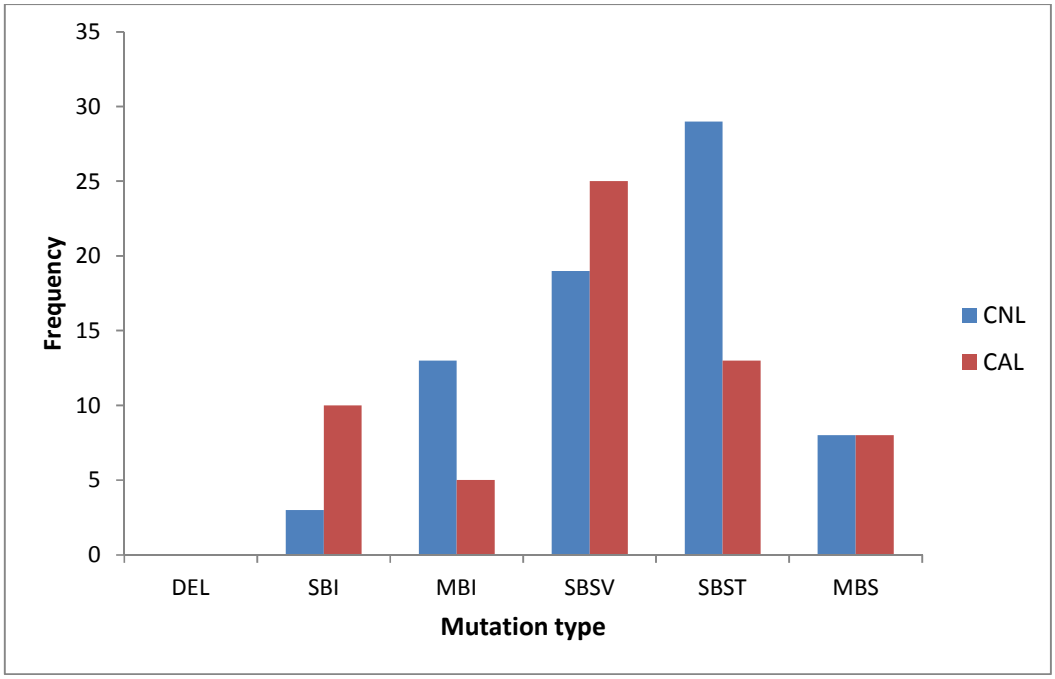

Fig. 3. SNPs analysis of *C. nigrodigitatus* (CNL)and *C. auratus* (CAL) *from* Lower Cross River
Del = Deletion, SBI= Single base insertion, MBI = Multiple base insertion, SBSV=Single base transversional substitution, SBST=Single base transitional substitution, MBS = Multiple base substitution

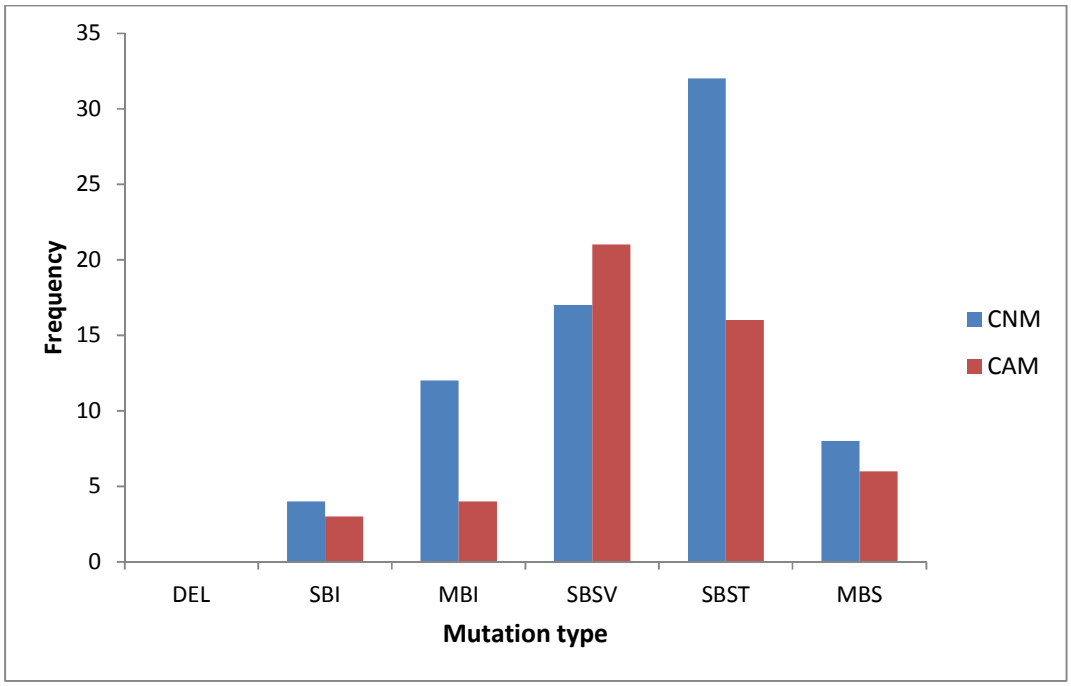

Fig. 4. SNPs analysis of *C. nigrodigitatus (CNM)* and *C. auratus (CAM)* from Middle Cross River
Del = Deletion, SBI= Single base insertion, MBI = Multiple base insertion, SBSV=Single base transversional substitution, SBST=Single base transitional substitution, MBS = Multiple base substitution

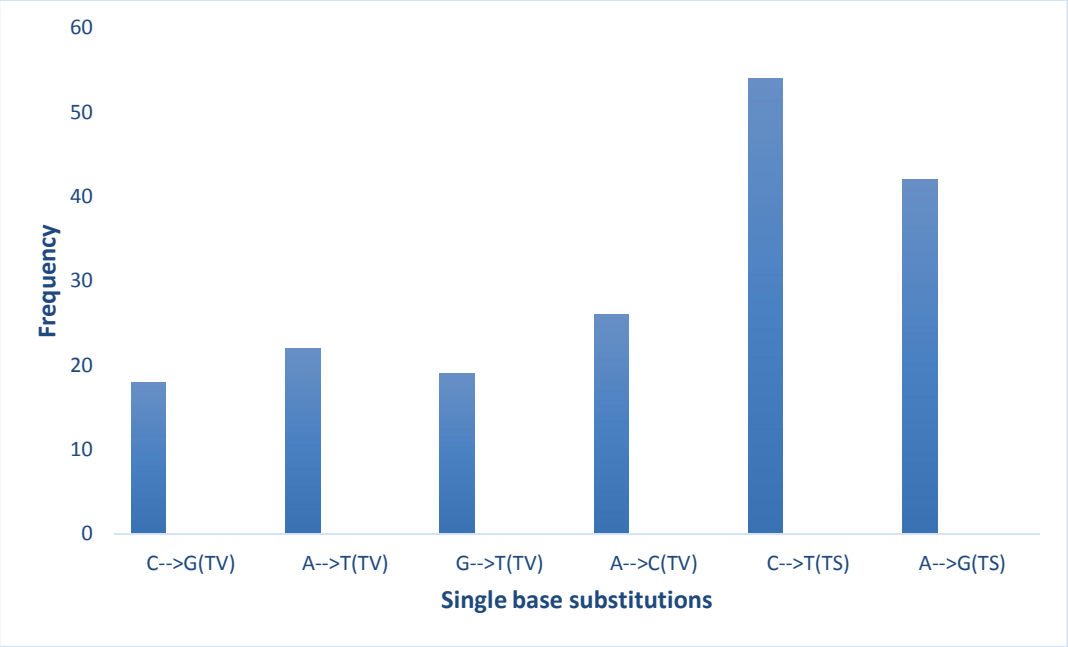

Fig. 5. Frequency of Transition (TS) and Transversion (TV) Substitutions in the 2 *Chrysichthys* spp from Middle and Lower Cross River

3.6 Polymorphism Sites and Single Nucleotide Polymorphism (Snp) Analysis in *Chrysichthys Spp*

Single nucleotide polymorphisms and the corresponding amino acid changes for *C. nigrodigitatus* from lower and middle cross river are presented in Tables 2 a and b while those for *C. auratus* are given in Tables 3 a and b respectively.

4. DISCUSSION

This study is the first molecular characterization of the two species of *Chrysichthys* in Nigeria using ribosomal RNA and ITS genes. The range of lengths of ITS-1 were longer than those of ITS-2 for each species. These findings are similar to those reported on African anguilliform catfish (Siluriformes: Clariidae) [15]. However, in comparison to African anguilliform catfish, the ITS-1 and ITS-2 regions of *Chrysichthys* are much shorter. The G+C contents were higher than A+T contents and fall within the range of GC content observed in other catfish species [15,20]. The high G+C content could be due to the fact that the fishes dwell in the tropics and could be adaptation to warmer climates [15]. A frequent characteristic of species is a comparable GC% between ITS-1 and ITS-2. This phenomenon was observed in the two species studied here, which could indicate a relic of shared evolutionary history.

Nwafili et al. [11] reported that *C. nigrodigitatus* of the Cross River was characterized by less genetic variation, compared to species from Imo River, New Calabar river and Lagos Lagoon, all of which are Nigerian coastal rivers, thus corroborating our > 99% similarity from sequence alignment. The general belief has been that these species move from the lower river to the huge flood plains which provide a breeding ground that enables high effective population size to interbreed, thereby increasing genetic variation within populations and reducing variation between populations [21]. Ama-Abasi et al. [6] reported that *Chrysichthys nigrodigitatus* engages both in local wanderings and directed migration. This will result in high level of gene flow, thus reducing genetic variation between the populations. Moreover [22] in their morphological and genetic studies of *Chrysichthys* species in Bia River of Cote D'evoire observed that there was high genetic diversity in *Chrysichthys* population and concluded that the population from Bia River had a long evolutionary history in a large stable population. This is in contrast to our findings in this study. Our study however confirms the work of [16] on the low genetic diversity of *Chrysichthys* species of the Cross River system.

The implication of low genetic diversity in the *Chrysichthys* populations of the Cross River is that any spontaneous environmental variability can lead to a wipe out of the population. Consequently, conservation measures should be put in place for sustainable exploitation of the fishery. This also underscores the need for domestication of the species.

From the pairwise alignment of the consensus sequence of *C. nigrodigitatus* and *C. auratus* in the MEGA software, the consensus base at each site was used to infer the original state. Observed nucleotide substitutions between 2 sequences may be classified as: synonymous transitions, nonsynonymous transitions, synonymous transversions, nonsynonymous transversions. The ratio of transitional to transversional polymorphisms in this study varied greatly in the different groups examined but the overall ratio, pooling all the 4 groups examined stood at 1.12:1. Transition/transversion ratios between homologous strands of DNA is generally 2:1 but is typically elevated in coding regions where transversions are more likely to change the underlying amino acid which may lead to fatal mutations in the translated proteins. Unequal transition and transversion mutation ratios are believed to be features of DNA sequence evolution [23] and variable non-synonymous/synonymous rate ratios among lineages may indicate adaptive evolution [24,25]. However, the overall ratio reported in the present study shows an almost equal rate of transitional to transversional mutations. This is not surprising, since there is low genetic diversity in the studied populations as a result of possible gene flow between them. The most frequently observed single base substitution in this study was a transition of C→T while the least was the transversion C→G.

Table 2(a). Polymorphism analysis of *C. nigrodigitatus* from lower Cross River

Mutation site	SNP	Amino acid change	d_S-d_N	Mutation type
1	ins (T)	coding region	-	Insertion
2	ins (G)	coding region	-	Insertion
3	ins (G)	coding region	-	Insertion
28	del (G)	coding region	-	Deletion
57	C>G	Gly (GGC) - Gly (GGG)	dS	Transversion
112	ins (C)	coding region	-	Insertion
659	del (T)	coding region	-	Deletion
660	Del (C)	coding region	-	Deletion
728	T>C	Leu (CUU) – Pro (CCU	dN	Transition
845	ins (T)	coding region	-	Insertion
846	ins (T)	coding region	-	Insertion

dS = Synonymous substitution. dN = Non synonymous substitution

Table 2(b). Polymorphism analysis of *C. nigrodigitatus* from middle Cross River

Mutation site	SNP	Amino acid change	d_S-d_N	Mutation type
1	del (T)	coding region	-	Deletion
2	del (G)	coding region	-	Deletion
3	del (G)	coding region	-	Deletion
28	ins (G)	coding region	-	Insertion
86	A>G	Lys (AAG) – Arg (AGG)	d_N	Transition
112	del (C)	coding region	-	Deletion
215	A > G	His (CAC) – Arg (CGC)	d_N	Transition
438	A > G	Leu (CUA) – Leu (CUG)	d_S	Transition
484	A > T	Lys (AAG) - Leu (UUG)	d_N	Transversion
485	A > T	Lys (AAG) - Leu (UUG)	d_N	Transversion
659	ins (T)	coding region	-	Insertion
660	ins (C)	coding region	-	Insertion
805	C>T	Pro (CCU) – Ser (UCU)	d_N	Transition
845	del (T)	coding region	-	Deletion
846	del (T)	coding region	-	Deletion

dS = Synonymous substitution. dN = Non synonymous substitution

Table 3(a). Polymorphism analysis of *C. auratus* from lower Cross River

Mutation site	SNP	Amino acid change	d_S-d_N	Mutation type
1	ins (C)	coding region	-	Insertion
2	ins (G)	coding region	-	Insertion
3	ins (C)	coding region	-	Insertion
4	ins (C)	coding region	-	Insertion
10	ins (A)	coding region	-	Insertion
12	A > G	Ile (AUA) – Met (AUG)	d_N	Transition
321	G>T	Ala (GCG) – Ala (GCU)	d_S	Transversion
322	A>T	Arg (AGA) – Leu (UUA)	d_N	Transversion
323	G>T	Arg (AGA) – Leu (UUA)	d_N	Transversion
329	A>G	Asn (AAU) – Ile (AUU)	d_N	Transversion
523	ins (T)	coding region	-	Insertion
534	ins (C)	coding region	-	Insertion
563	ins (G)	coding region	-	Insertion
564	ins (C)	coding region	-	Insertion
590	ins (C)	coding region	-	Insertion
606	ins (G)	coding region	-	Insertion
628	ins (C)	coding region	-	Insertion
640	ins (G)	coding region	-	Insertion
659	ins (G)	coding region	-	Insertion
691	del (A)	coding region	-	Deletion
712	ins (T)	coding region	-	Insertion

dS = Synonymous substitution. dN = Non-synonymous substitution

Table 3(b). Polymorphism analysis of *C. auratus* from middle Cross River

Mutation site	SNP	Amino acid change	d_S-d_N	Mutation type
1	del (C)	coding region	-	Deletion
2	del (C)	coding region	-	Deletion
3	del (C)	coding region	-	Deletion
4	del (C)	coding region	-	Deletion
10	del (A)	coding region	-	Deletion
562	C>G	Arg (CGC) – Ala (GCC)	d_N	Transition
563	del (G)	coding region	-	Deletion
564	del (C)	coding region	-	Deletion
590	del (C)	coding region	-	Deletion
606	del (G)	coding region	-	Deletion
628	del (C)	coding region	-	Deletion
640	del (C)	coding region	-	Deletion
659	del (C)	coding region	-	Deletion
691	ins (A)	coding region	-	Insertion
712	del (T)	coding region	-	Deletion

dS = Synonymous substitution. dN = Non-synonymous substitution

Mutations that produce a desired trait such as increased survival and fecundity are positively selected and its genotype increases in frequency while deleterious mutations are negatively selected thereby maintaining the long-term stability of biological structures. Since evolution is ultimately dependent on such nucleotide changes, information provided in this study would form a baseline for further studies on molecular evolution of *Chrysichthys* spp.

5. CONCLUSION

Pairwise alignment of the consensus sequences of *Chrysichthys nigrodigitatus and C. auratus* from the Cross River, Nigeria showed that the rRNA and ITS genes are highly conserved within the region with >99% nucleotide identity. However, 284 polymorphisms were recorded in these genes in the two species, in the form of deletions, single base insertions, multiple base insertions, single base transition substitutions,

single base transversion substitutions and multiple base substitutions. The almost equal overall ratio of transition: transversion polymorphisms in the different groups examined indicate low level of genetic instability in this genus. This study has thus provided insight into the mutation patterns of rRNA and ITS genes in *Chrysichthys* spp across the four populations studied which could form the basis for further genomic characterization and mapping of these species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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