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Molecular Evolution and Genetic Analysis of Silver Catfish (*Chrysichthys nigrodigitatus*) in Nigeria

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ABSTRACT

Background and Objective: In a disrupted environment such as the Niger Delta, studies of population genetic diversity and molecular evolution can provide useful information necessary for species conservation and population management. This study was therefore designed to evaluate selection types, demographic expansion, mutation types as well as the maternal lineage of *C. nigrodigitatus*. **Materials and Methods:** Fifty mature Silver catfish were collected from fresh and brackish waters for this study. Muscle tissue was excised from each fish and preserved in 95% ethanol for DNA extraction and analysis. Extraction and purification of mtDNA from fish muscle tissues were carried out using the Quick-gDNA™ MiniPrep kit (Zymo Research, USA). The sequenced fragments were viewed and edited using ChromasPro software and subsequently analyzed using suitable software. **Results:** Tajima's D value was 2.091 ($p<0.05$) in freshwater fish samples and 1.292 ($p>0.10$) in brackish water fish samples, while Fu's Fs value for fresh and brackish water fish samples was 1.667 ($p<0.02$) and 1.108 ($p>0.10$), respectively. Maternal lineage analysis revealed that *C. nigrodigitatus* used in this study may be traced to *Clarias gariepinus* and *Clarias macrocephalus* based on the hypervariable region of the mitochondrial DNA. Positive, negative and neutral selection pressures were detected in both populations, while mutation analysis revealed synonymous and non-synonymous, transversion and transition, deletion and insertion mutations in both populations. **Conclusion:** The findings of this study suggest that there is high genetic polymorphism in the populations of Silver catfish evaluated as well as the molecular similarity between freshwater and brackish water samples. It was also revealed that the origin of Silver catfish is traceable to *Clarias gariepinus* and *Clarias macrocephalus*.

KEYWORDS

Silver catfish, selections, mutation, maternal lineage, demographic expansion, evolution

INTRODUCTION

Chrysichthys nigrodigitatus commonly called Silver catfish are extensively distributed in fresh and brackish waters in Nigeria. They belong to the Claroteidae family and constitute an integral part of the aquatic ecosystem and fisheries in Nigeria. They are benthic omnivores that migrate to freshwater for breeding[1]. Silver catfish are extremely cherished foods throughout West Africa and are among the dominant species of commercial value in the Niger Delta Region of Nigeria.

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Despite the ecological and economic relevance of the aquatic ecosystem in Nigeria especially the Niger Delta, little is known about the molecular evolution and genetic diversity of this economically important fish species inhabiting the said ecosystem. Freshwater, brackish water and marine fishery resources in Nigeria are facing diverse challenges including the destruction of natural habitats by anthropogenic activities[2]. There are concerns in the Niger Delta over hazardous fishing methods, climate change, over-fishing and pollution arising from industries and multinational oil companies, which can impact adversely the genetic diversity of fish and other aquatic organisms[3]. In a disrupted environment such as that in the Niger Delta, studies on population evolution and molecular diversity can provide valuable information that can facilitate the conservation and management of ecosystems and their populations.

Molecular genetic markers have a powerful ability to detect genetic diversity and evolutionary relationships of species or populations[4]. These molecular markers in combination with new statistical tools have revolutionized the analytical power needed to assess genetic diversity and molecular evolution of species. Various molecular markers are currently being used in fisheries management. These markers provide scientific observations which have relevance in species identification, genetic variation and population structure study, comparison between wild and cultured populations, assessment of demographic bottlenecks in a natural population, evolution study and propagation-assisted rehabilitation programmes[5]. This study was therefore, designed to evaluate selection types, demographic expansion, mutation types as well as the maternal lineage of *C. nigrodigitatus* obtained from fresh and brackish waters in the Niger Delta Region of Nigeria.

MATERIALS AND METHODS

Sample collection: This study was carried out between April, 2018 and November, 2019. Fifty Silver catfishes were obtained from Akwa Ibom, River and the Bayelsa States, in the Niger Delta Region of Nigeria. Brackish water fish samples were obtained from the lower reaches of New Calabar River (4°49'4''N, 6°57'24''E), lower reaches of Brass River (4°32'1.46''N, 6°24'14.7''E) and lower reaches of Cross River (4°49'37''N, 8°14'6''E). While, freshwater fish samples were obtained from the middle reaches of New Calabar River (4°53'26''N, 6°54'1''E), middle reaches of river Nun (4°58'88''N, 6°6'22''E) and upper reaches of cross river (4°58'4''N, 8°4'42''E).

Extraction of mitochondrial DNA: Muscle tissues were excised from the left dorsal region of each fish and preserved in 95% ethanol for DNA extraction and analysis. Extraction and purification of mtDNA from fish muscle tissues were conducted in the Molecular Biology Laboratory, Department of Genetics and Biotechnology, University of Calabar, Nigeria. The Quick-gDNA™ MiniPrep kit (Zymo Research, CA, USA) was used to extract the DNA. The eluted DNA was preserved at -20°C for further analysis.

Polymerase Chain Reaction (PCR) amplification: Polymerase chain reaction amplification was conducted at STAB VIDA Laboratory, Portugal. The primers Marinefish-DloopThr-F (AGCACCGGTCTTGTAA ACCG) and Marinefish-Dloop-Phe-R (GGGCTCATCTTAACATCTTCA) were used for this study. The PCR cocktail consisted of 0.2 µM per primer, 5.0 µL 10×Taq buffer, 0.2 mM dNTPs, 2 units Taq DNA polymerase and 1 µL template DNA. The PCR was achieved using the BIO-RAD S1000 thermal cycler (BIO-RAD Laboratories, CA, USA). Amplification conditions were: Pre-denaturation at 94°C for 4 min, 35 cycles denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1.5 min and terminal extension at 72°C for 5 min. Product purification was achieved using the QIAquick kit (Qiagen, MD, USA).

Sequencing of the hypervariable region of the mitochondrial DNA: Sequencing of the hypervariable region of mitochondrial DNA was carried out at STAB VIDA Laboratory, Portugal. The D-loop region of mtDNA was sequenced with Marinefish-Dloop-Thr-F (AGCACCGGTCTTGTAAACCG) and Marinefish-Dloop-Phe-R (GGGCTCATCTTAACATCTTCA) primers. Sequencing was conducted using ABI 3130 genetic analyzer

(Applied Biosystems, CA, USA) and 20 µL mix comprising 20 ng template DNA, 8 µL mix (dNTPs, ddNTPs, buffer, enzyme and $MgCl_2$), 8 µL deionized water, 2 µL primer conditioned at 25 cycles for 10 sec at 96 and 60°C for 5 sec and 60°C for 4 min.

Statistical analysis: ChromasPro software was used to view and edit sequences. The MEGA 6.06 software was used for multiple sequence alignment and estimation of selection types[6]. DnaSP 5.1 software was used to test demographic expansion in the populations[7]. To classify Silver catfish into a maternal lineage based on the hypervariable region of mtDNA, sequences of other species were retrieved from the GenBank database with accession numbers AP012009.1, JN116988.1, EU697148.1, EU625374.1, MF621727.1, KM363317.1 and AF331474.1. Codon Code Aligner version 6.06 was used to analyze the mutation of SNPs in the aligned sequences.

RESULTS

Demographic expansion: The demographic expansion estimate of the two populations is presented in Table 1. The Tajima's D value was 2.091 ($p<0.05$) in freshwater fish samples and 1.292 ($p>0.10$) in brackish water fish samples. The Fu's Fs value for freshwater fish samples was 1.667 ($p<0.02$) and 1.108 ($p>0.10$) in the brackish water fish samples.

Maternal lineage analysis: The maternal lineage analysis shown in Fig. 1 revealed that *C. nigrodigitatus* used in this study may be traceable to *Clarias gariepinus* and *Clarias macrocephalus*. A maximum-likelihood phylogenetic tree was constructed using query sequences of *C. nigrodigitatus* from fresh and brackish water populations with reference sequences obtained from GenBank.

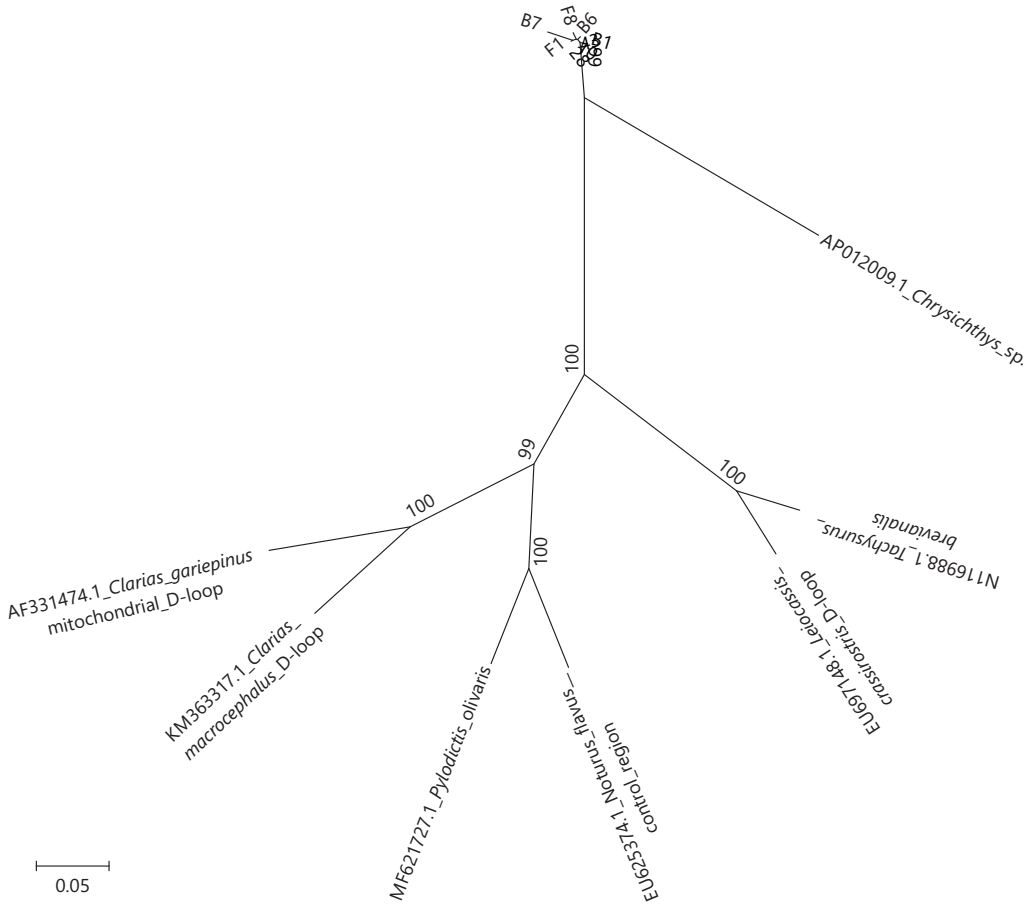

Fig. 1: Maximum likelihood phylogenetic tree showing the maternal lineage of *C. nigrodigitatus* from fresh and brackish waters based on the hypervariable region of the mitochondrial DNA

Table 1: Demographic expansion estimates of *C. nigrodigitatus* from fresh and brackish waters

Demographic expansion parameters	Freshwater	Brackish water
Tajima's D	2.091 (p<0.05)	1.292 (p>0.10)
Fu's F	1.667 (p<0.02)	1.108 (p>0.10)

Table 2: Selection analysis of *C. nigrodigitatus* from fresh and brackish waters

Populations	Selection types	d_N	d_S	d_N-d_S	Site index	p-value
Freshwater	Positive	17.899	0.000	17.899	32	0.230
	Negative	4.282	47.917	-43.636	26	0.248
	Neutral	0.000	0.000	0.000	675	0.000
Brackish	Positive	15.892	0.000	15.892	30	0.230
	Negative	7.727	52.032	-44.305	36	0.340
	Neutral	0.000	0.000	0.000	677	0.000

d_N: Non-synonymous and d_S: Synonymous

Table 3: Mutation analysis of Single Nucleotide Polymorphisms (SNPs) in the hypervariable region of the mitochondrial DNA of *C. nigrodigitatus* from fresh and brackish waters

S/N	Freshwater SNP	Amino acid change	d_S/d_N	Mutation types	S/N	SNP	Brackish water	d_S/d_N	Mutation types
1	5A>T	Asp2Val	d_N	Transversion	1	6delT	Coding region	-	Deletion
2	20G>A	Ser7Asn	d_N	Transition	2	20G>A	Ser7Asn	d_N	Transition
3	34A>G	Asn12Asp	d_N	Transition	3	34A>G	Asn12Asp	d_N	Transition
4	63A>T	Leu21Phe	d_N	Transversion	4	63A>T	Leu21Phe	d_N	Transversion
5	66T>C	Asn22Asn	d_S	Transition	5	66T>C	Asn22Asn	d_S	Transition
6	71delT	Coding region	-	Deletion	6	71delT	Coding region	-	Deletion
7	80A>G	Try27Lys	d_N	Transition	7	80A>G	Tyr27Cys	d_N	Transition
8	82T>C	Ser28Pro	d_N	Transition	8	82T>C	Ser28Pro	d_N	Transition
9	84A>C	Ser28Pro	d_N	Transversion	9	84A>C	Ser28Pro	d_N	Transversion
10	87G>A	MET29Ile	d_N	Transition	10	87G>A	MET29Ile	d_N	Transition
11	95C>T	Ala32Val	d_N	Transition	11	95C>T	Ala32Val	d_N	Transition
12	117T>C	Asn39Asn	d_S	Transition	12	117T>C	Asn39Asn	d_S	Transition
13	119A>G	Thr40Ile	d_N	Transition	13	119C>T	Thr40Ile	d_N	Transition
14	121A>G	Thr41Ala	d_N	Transition	14	185C>G	Ser62Cys	d_N	Transversion
15	185C>G	Ser62cys	d_N	Transversion	15	187C>A	Pro63Thr	d_N	Transversion
16	187C>A	Pro63Thr	d_N	Transversion	16	187C>G	Pro63Ala	d_N	Transversion
17	187C>G	Pro63Ala	d_N	Transversion	17	190delG	Coding region	-	Deletion
18	190delG	Coding region	-	Deletion	18	190G>T	Glu64STP	d_N	Transversion
19	193A>G	Asn65Asp	d_N	Transition	19	193A>G	Asn65Asp	d_N	Transition
20	198T>C	Ile66Ile	d_S	Transition	20	198T>C	Ile66Ile	d_S	Transition
21	199A>C	Lys67Gln	d_N	Transversion	21	199A>C	Lys67Gln	d_N	Transversion
22	211G>A	Val71Lys	d_N	Transition	22	211G>A	Val71Lys	d_N	Transition
23	212T>A	Val71Lys	d_N	Transversion	23	212T>A	Val71Lys	d_N	Transversion
24	213A>G	Val71Val	d_S	Transition	24	213A>G	Val71Val	d_S	Transition
25	217T>A	Try73Asn	d_N	Transversion	25	217T>A	Try73Asn	d_N	Transversion
26	222C>T	Thr74Thr	d_S	Transition	26	222T>C	Thr74Thr	d_S	Transition
27	226A>G	Lys76Glu	d_N	Transition	27	226A>G	Lys76Glu	d_N	Transition
28	229A>G	Ile77Ala	d_N	Transition	28	227A>G	Lys76Gly	d_N	Transition
29	230insA	Coding region	-	Insertion	29	228A>G	Lys76Lys	d_S	Transition
30	230T>C	Ile77Thr	d_N	Transition	30	230insA	Coding region	-	Insertion
31	230T>C	Ile77Ala	d_N	Transition	31	230T>C	Ile77Thr	d_N	Transition
32	251A>G	STP84STP	d_S	Transition	32	251A>G	STP84STP	d_S	Transition
33	252G>A	STP84STP	d_S	Transition	33	252G>A	STP84STP	d_S	Transition
34	260G>A	Cys87STP	d_N	Transition	34	260G>A	Cyp87STP	d_N	Transition
35	261C>A	Cys87STP	d_N	Transversion	35	261C>A	Cys87STP	d_N	Transversion
36	287T>C	Leu96Ser	d_N	Transition	36	287T>C	Leu96Ser	d_N	Transition
37	312C>T	Cys104Cys	d_S	Transition	37	312C>T	Cys104Cys	d_S	Transition
38	329C>T	Pro110Leu	d_N	Transition	38	334T>A	Ser112Thr	d_N	Transversion
39	334T>A	Ser112Thr	d_N	Transversion	39	335C>T	Ser112Thr	d_N	Transition
40	335C>T	Ser112Leu	d_N	Transition	40	341T>A	Ile114Asn	d_N	Transversion
41	337A>G	Thr113Alg	d_N	Transition	41	351C>G	Pro117Pro	d_S	Transversion
42	341T>A	Ile114Asn	d_N	Transversion	42	338insA	Coding region	-	Insertion
43	351C>G	Pro117Pro	d_S	Transversion	43	359A>T	Tyr120Phe	d_N	Transversion

Table 3: Continued

S/N	Freshwater SNP	Amino acid change	d_S/d_N	Mutation types	S/N	SNP	Brackish water	d_S/d_N	Mutation types
44	358InsA	Coding region	-	Insertion	44	363C>T	Asn121Asn	d_S	Transition
45	359A>T	Try120Phe	d_N	Transversion	45	364C>G	Pro121Val	d_N	Transversion
46	363C>T	Ans121Asn	d_S	Transition	46	365C>T	Pro121Val	d_N	Transition
47	364C>G	Pro122Val	d_N	Transversion	47	367A>G	Ile122Val	d_N	Transition
48	365C>T	Pro122Val	d_N	Transition	48	369insC	Coding region	-	Insertion
49	367A>G	Ile123Val	d_N	Transition	49	432T>C	Asn144Asn	d_S	Transition
50	395insC	Coding region	-	Insertion	50	434C>T	Ser145Leu	d_N	Transition
51	432T>C	Ans144Asn	d_S	Transition	51	494C>T	Thr165Ile	d_N	Transition
52	434C>T	Ser145Leu	d_N	Transition	52	497C>T	Ala166Val	d_N	Transition
53	494C>T	Thr165Ile	d_N	Transition	53	516T>C	Ile172Ile	d_S	Transition
54	497C>T	Ala166Val	d_N	Transition	54	518G>A	Cys173Tyr	d_N	Transition
55	516T>C	Ile172Ile	d_S	Transition	55	522C>T	Pro174Pro	d_S	Transition
56	518G>A	Cys173Tyr	d_N	Transition	56	575G>A	Arg192His	d_N	Transition
57	522C>T	Pro174Pro	d_S	Transition	57	580T>C	Phe194Leu	d_N	Transition
58	558C>T	Ser186Ser	d_S	Transition	58	596A>T	Tyr199Phe	d_N	Transversion
59	572A>G	Lys191Arg	d_N	Transition	59	608C>T	Leu203Gln	d_N	Transition
60	575A>G	Arg192His	d_N	Transition	60	613C>T	Leu205Phe	d_N	Transition
61	580T>C	Phe194Leu	d_N	Transition	61	621T>G	Ser207Ser	d_S	Transversion
62	596A>T	Try199Phe	d_N	Transversion	62	628C>T	Leu210Phe	d_N	Transition
63	613C>T	Leu205Phe	d_N	Transition	63	630T>C	Leu210Leu	d_S	Transition
64	621T>G	Ser207Ser	d_S	Transversion	64	632T>G	Pro211Leu	d_N	Transversion
65	624C>A	Gly208Gly	d_S	Transvertion	65	633T>G	Pro211Leu	d_N	Transversion
66	628C>T	Leu210Phe	d_N	Transition	66	633T>G	Pro211Pro	d_S	Transversion
67	630T>C	Leu210Phe	d_N	Transition	67	640T>A	Tyr214Asn	d_N	Transversion
68	633T>G	Pro211Xaa	d_N	Transversion	68	646T>G	Trp216Gly	d_N	Transversion
69	633T>G	Pro211Leu	d_N	Transversion	69	653T>C	Val218Xaa	d_N	Transition
70	640T>A	Tyr214Asn	d_N	Transversion	70	656A>G	Lys219Arg	d_N	Transition
71	646T>G	Trp216Gly	d_N	Transvertion	71	661G>A	Glu221Lys	d_N	Transition
72	653T>C	Val218Xaa	d_N	Transition	72	688T>C	Phe233Ser	d_N	Transition
73	656A>G	Lys219Arg	d_N	Transition	73	669T>C	Phe223Ser	d_N	Transition
74	659T>A	Val220Glu	d_N	Transversion	74	673T>G	Cys225Gly	d_N	Transversion
75	661G>A	Glu221Lys	d_N	Transition	75	676C>T	Leu226Ser	d_N	Transition
76	668T>C	Phe223Ser	d_N	Transition	76	676C>T	Leu266Xaa	d_N	Transition
77	669T>C	Phe223Ser	d_N	Transition	79	677T>C	Leu226leu	d_S	Transition
78	669T>C	Phe223Phe	d_S	Transition	80	681A>C	Gln227His	d_N	Transversion
79	673T>G	Cys225Gly	d_N	Transversion	81	684G>A	Pro228Pro	d_S	Transition
80	676C>T	Leu226Phe	d_N	Transition	82	687G>A	Ala229Ala	d_S	Transition
81	676C>T	Leu226Ser	d_N	Transition	83	690T>G	Asn230Lys	d_N	Transversion
82	677C>T	Leu226Ser	d_N	Transition	84	692T>G	Val232Glu	d_N	Transversion
83	678C>T	Leu226Leu	d_S	Transition	85	694G>A	Val232Ile	d_N	Transition
84	678C>T	Leu226Phe	d_N	Transition	86	695T>C	Val232Xaa	d_N	Transition
85	684A>G	Pro228Pro	d_S	Transition	87	696T>C	Val232Xaa	d_N	Transition
86	687G>A	Ala229Ala	d_S	Transition	88	704G>C	Ser235Thr	d_N	Transversion
87	692T>A	Val231Glu	d_N	Transversion	89	707A>G	Try236Cys	d_N	Transition
88	694G>A	Val232Ile	d_N	Transition	90	707A>G	Try236Xaa	d_N	Transition
89	695T>C	Val232Xaa	d_N	Transition	91	709T>C	Ser237Pro	d_N	Transition
90	696T>C	Val232Xaa	d_N	Transition	92	711T>A	Ser237Ser	d_N	Transversion
91	696T>C	Val232Val	d_N	Transition	93	715T>C	Phe239Leu	d_N	Transition
92	696T>C	Val232Val	d_S	Transition	94	722T>A	Ile241Asn	d_N	Transversion
93	702delT	Coding region	-	Deletion	95	722T>G	Ile241Ser	d_N	Transversion
94	704G>C	Ser235Thr	d_N	Transversion	96	723A>T	Ile241Asn	d_N	Transversion
95	707A>G	Try236Xaa	d_N	Transition	97	724A>T	Ile241Xaa	d_N	Transversion
96	709T>C	Ser237Pro	d_N	Transition	98	723A>T	Ile241Ser	d_N	Transversion
97	711T>A	Ser237Pro	d_N	Transversion	99	724G>A	Ala242Thr	d_N	Transition
98	711T>A	Ser237Ser	d_S	Transversion	100	724G>T	Ala242Ser	d_N	Transversion
99	722T>A	Ile241Asn	d_N	Transversion	101	733A>G	Ser245Gly	d_N	Transition
100	722T>G	Ile241Ser	d_N	Transversion	102	739delT	Coding region	-	Deletion
101	723A>T	Ile241Ser	d_N	Transversion	103	749T>G	Leu250Trp	d_N	Transversion
102	723T>A	Ile241Asn	d_N	Transversion	104	759A>C	Pro253Pro	d_S	Transversion
103	724T>A	Ser242Thr	d_N	Transversion	105	761C>T	Pro254Leu	d_N	Transition

Table 3: Continued

S/N	Freshwater SNP	Amino acid change	d_S/d_N	Mutation types	S/N	SNP	Brackish water	d_S/d_N	Mutation types
104	724T>G	Ser242Ala	d_N	Tranversion	106	761T>G	Pro254Leu	d_N	Transversion
104	733A>G	Ser245Gly	d_N	Transition	107	762G>C	Ala255His	d_N	Transversion
105	739delT	Coding region	-	Deletion	108	764C>A	Ala255His	d_N	Transversion
106	746T>A	Leu249STP	d_N	Transversion	109	765A>T	Ala255His	d_N	Transversion
107	761delT	Coding region	-	Deletion	110	766T>C	Ser259Pro	d_N	Transition
108	778T>C	Ser260Arg	d_N	Transition	111	775T>C	Ser259Pro	d_N	Transition
109	778T>C	Ser260Xaa	d_N	Transition	112	777C>T	Ser259Pro	d_N	Transition
110	779C>G	Ser260Arg	d_N	Transversion	113	778T>C	Phe260Xaa	d_N	Transition
111	780G>C	Ser260Arg	d_N	Transversion	114	778T>C	Phe260Ser	d_N	Transition
112	780G>C	Ser260Xaa	d_N	Transversion	115	780C>G	Phe260Ser	d_N	Transversion
113	781delC	Coding region	-	Deletion	116	781G>C	Ala261Arg	d_N	Transversion
114	784T>G	STP262Val	d_N	Transversion	117	781G>C	ALa261del	d_N	Transversion
115	785A>T	STP262Val	d_N	Transversion	118	782C>G	Ala261Arg	d_N	Transversion
116	794C>A	Pro265His	d_N	Transversion	119	782delC	Coding region	-	Deletion
117	794delC	Coding region	-	Deletion	120	785T>G	Val262Gly	d_N	Transversion
					121	786A>T	Val262Gly	d_N	Transversion
					122	788delA	Coding region	-	Deletion
					123	794C>T	Pro265Leu	d_N	Transition
					124	795C>A	Pro265Pro	d_S	Transversion
Total	117	107	86/21	66/41	Total	124	115	92/23	68/47

Selection analysis: The result obtained from the assessment of selection types in the two populations of *C. nigrodigitatus* is presented in Table 2. The ratio of non-synonymous to synonymous substitution (d_N-d_S) in freshwater fish samples was higher (17.88) with the positive selection occurring at 32 sites compared to negative selection which occurred at 26 sites with d_N-d_S substitution of -43.636. In the brackish water fish samples, positive selection occurred at 30 sites with a d_N-d_S substitution of 15.892 and negative selection occurred at 36 sites with a d_N-d_S substitution of -44.305.

Mutation analysis of SNPs: The results showing mutation types associated with all the SNPs detected in the hypervariable region of the mtDNA sequences of *C. nigrodigitatus* from the two populations are presented in Table 3. In freshwater fish samples, the hypervariable region sequences revealed 117 SNPs, the different SNPs resulted in 66 transition and 41 transversion mutations. The 107 amino acid changes were leading to 86 non-synonymous and 21 synonymous mutations. There were more SNPs in the brackish water fish samples giving a total of 124. The SNPs associated with purine-purine and pyrimidine-pyrimidine substitutions resulted in 68 transition mutations while the SNPs associated with purine-pyrimidine and pyrimidine-purine substitutions resulted in 47 transversion mutations. There were 92 non-synonymous and 23 synonymous amino acid changes. A few deletions and insertions were also recorded in SNPs of both fresh and brackish water fish samples.

DISCUSSION

Studies of population genetic diversity and molecular evolution can provide useful information necessary for species conservation and population management. So, maternal lineage analysis was conducted using query sequences of *C. nigrodigitatus* with reference sequences obtained from GenBank, the phylogenetic tree generated revealed that the origin of *C. nigrodigitatus* could be traced to *Clarias gariepinus* and *Clarias macrocephalus*.

Tajima's D and Fu's Fs values were used to determining demographic expansion in *C. nigrodigitatus*. Tajima's D value was 2.091 ($p<0.05$) in freshwater fish samples and 1.292 ($p>0.10$) in brackish water fish samples, while Fu's Fs value was 1.667 ($p<0.02$) in freshwater fish samples and 1.108 ($p>0.10$) in brackish water fish. Tajima's D values reveal the presence of high polymorphism perhaps arising from recent population bottlenecks. Fu's Fs are considered to be more sensitive in detecting population expansion and Fu's Fs values obtained reveal that genetic drift may have occurred in the populations. These findings are

consistent with the reports of Nwafili *et al.*[1], Nwafili and Gao[3], Sita *et al.*[8] and therefore suggest that the brackish water fish population was not subjected to demographic expansion[9].

This study also revealed that the rate of synonymous to non-synonymous (d_N-d_S) substitution for positive selection site index was high in fresh and brackish water fish populations indicating the presence of positive selection pressure in the populations. According to Li *et al.*[10], positive selection takes place when populations experience new environmental pressures as a result of migration from one environment to another leading to rapid changes in allelic frequency and speciation. The positive selection pressure identified in *C. nigrodigitatus* populations may be linked to a high rate of d_N-d_S substitution for positive site index considering that this fish is known to be migratory[1,11]. This is therefore an indication that many alleles in the population are under positive selection advantage of perpetuity which may eventually lead to population structuring and speciation over time. It may also be an indication that certain haplotypes in the populations are having selective advantage which could positively influence the adaptation of *C. nigrodigitatus* in the advent of environmental hazards[12-14]. Negative selection pressure was also recorded in both populations with brackish water having a high negative site index. Perhaps the high negative selection pressure observed in the brackish water fish population acted to remove the effects of deleterious mutations in the habitat[15].

Mutation analysis of Single Nucleotide Polymorphism (SNP), revealed a high rate of non-synonymous amino acid substitution and transition mutations. The high rate of non-synonymous mutation could be a strong indication of the observed polymorphic haplotype and nucleotide diversities reported by Ikpeme *et al.*[16]. Non-synonymous mutation involves the substitution of one amino acid by another in a protein polypeptide chain, while transition mutation occurs when purine is substituted by purine or pyrimidine substituted by pyrimidine. These mutation types are often involved in creating genetic variations in populations and may be associated with the genetic polymorphism observed in *C. nigrodigitatus* populations studied. These variations are vital in population studies and have some significant biological implications[3,12]. It implies that despite the reported cases of pollution and other anthropogenic activities that could disrupt the aquatic habitat, there is high genetic diversity within the populations of Silver catfish studied and molecular similarity between fresh and brackish water populations. This study also provides useful information for the culturing and genetic improvement of Silver catfish in the Niger Delta Region of Nigeria. This study was limited to wild populations, so cultured populations should be evaluated to compare the level of genetic diversity in this fish species.

CONCLUSION

The results of this study suggest that there is high genetic polymorphism in the populations of Silver catfish evaluated as well as the molecular similarity between freshwater and brackish water samples. It was also revealed that the origin of Silver catfish is traceable to *Clarias gariepinus* and *Clarias macrocephalus*. This study, therefore, provides baseline information for the conservation and genetic improvement of this fish species.

SIGNIFICANCE STATEMENT

This study discovered high genetic polymorphism, the molecular similarity between freshwater and brackish water samples and the origin of Silver catfish. Therefore, this study will help researchers to uncover the critical areas of molecular evolution, genetic improvement and conservation of Silver catfish that many researchers were not able to explore.

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