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Morphological and Genetic Diversity Studies of *Chrysichthys nigrodigitatus* from the Cross River, Nigeria, Using Microsatellite Markers

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DAA and EAU designed the experiments. Authors DAA, EAU and CMA conducted field sampling of the fish species. Authors CMA, EAU and VON carried out the molecular analysis. Authors CMA, EAU and DAA wrote the manuscripts. All authors read and approved the final manuscript.

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ABSTRACT

Morphometric and genetic diversity studies were carried out on *Chrysichthys nigrodigitatus* sampled from middle and lower Cross River. The aim was to provide information on variations between these two populations and also augment the limited information currently available on genetic diversity in this species. A total of 79 fish samples were used for the morphometric analysis out of which 30 were used for the genetic study. Genomic DNA was extracted from caudal fin using a modified cetyltrimethylammonium bromide method and amplified using microsatellite markers.Twenty-three morphological features were studied from each fish sample out of which 19 showed significant differences ($P<0.05$) between the two populations. Principal Component Analysis identified head length, head width, caudal peduncle depth, standard length, preventral distance, snout length and anal fin length as key contributors to variation. Genetic analyses indicated low variability in the

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populations studied as evidenced by low Shannon`s information index (mean of 0.944 – 1.034), and positive coefficients of inbreeding (F_{IS}) across both populations suggesting the presence of greater homozygosity in this species. Gene flow of 3.507 was observed between the lower and middle Cross River indicating the possibility of free mating between the two populations. The low levels of genetic diversity call for urgent management and conservation strategies to ensure long term survival of the species.

Keywords: Chrysichthys nigrodigitatus; SSR markers; genetic diversity; morphometrics; catfish; Cross River.

1. INTRODUCTION

Catfish of the genus *Chrysichthys* is a prized food fish in Niger delta region of Nigeria. Its species *Chrysichthys nigrodigitatus* is widely distributed in fresh waters of West Africa where it is highly valued in human nutrition and commercial ventures [1]. According to [2], *Chrysichthys* is found in Nile, West Africa and western coast of Central Africa. In addition, *C. nigrodigitatus* is among the wild commercial catches with all-year-round fishery in the Cross River system and contribute significantly to the economy of the people of the Cross River basin. *Chrysichthys nigrodigitatus* is the third most abundant species in the Cross River Estuary after *Pseudotolithus elongatus* and *Ethmalosa fimbriata* [3]. In recent times, a study by [4] shows a decline in the population of *C. nigrodigitatus* due to over fishing. Such reduction in population size may lead to decreased variation.

Morphological characters have commonly been used to identify fish stock and study variations between fish populations [5,6]. Morphological differences can result from either genetic differences or environmental factors. Environmental factors can produce phenotypic plasticity, which is the capacity of a genotype to produce different phenotypes in response to different environmental conditions [7]. In their study of morphological identification and taxonomic relationship of farmed *Chrysichthys* species, [8] demonstrated that morphological parameters can be utilized for discriminating among *Chrysichthys* species. They however added that the result must be confirmed by genetic analysis.

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.*[9,10, 11,12] Microsatellite markers have been widely used because they are abundant and distributed throughout the genome, are highly polymorphic, suitable for detecting heterozygotes, are bi-parentally inherited and transferable among related taxa. In population genetics, their use has enhanced estimation of genetic diversity, parental relatedness, population structure and recent population history of different fish populations including *Chrysichthys spp.* [13,14]. Genetic diversity is a requirement for adaptation to changing environments by populations [15] and for exploiting the selective breeding of fish species [16]. Unfortunately, despite the economic importance of *C. nigrodigitatus* species occurring in the Cross River Basin, eastern Niger Delta, there is paucity of information about their genetic background. Existing genetic diversity studies of *C. nigrodigitatus* in Nigeria either focused on other regions or were assessed using only a few samples from Cross River or using other marker types [10,12,14,17]. Therefore, the present study was done to document the morphological and genetic variations between two populations of *C. nigrodigitatus* from the Cross River system. Such knowledge will enable informed management decision and facilitate the formulation of effective conservation strategies.

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

The study area was the Cross River which takes its origin from the Cameroon Mountains. Both the middle and the lower portions of the river system are in Nigeria. The study area is found between latitudes 05°00.0797/ N and 05°45.687/ N and longitudes 008°06.438/ and 07°58.248/E. The river bed is characterized by sandy substratum and there are series of underwater mounds that could act as barrier to movement of benthic species.

The specific sampling points were Ikot Okpara (IK) in middle Cross River, Ayadehe (AH), and Akani Obio Uruan (AU) in lower Cross River (Fig. 1). The river bank morphology of the middle

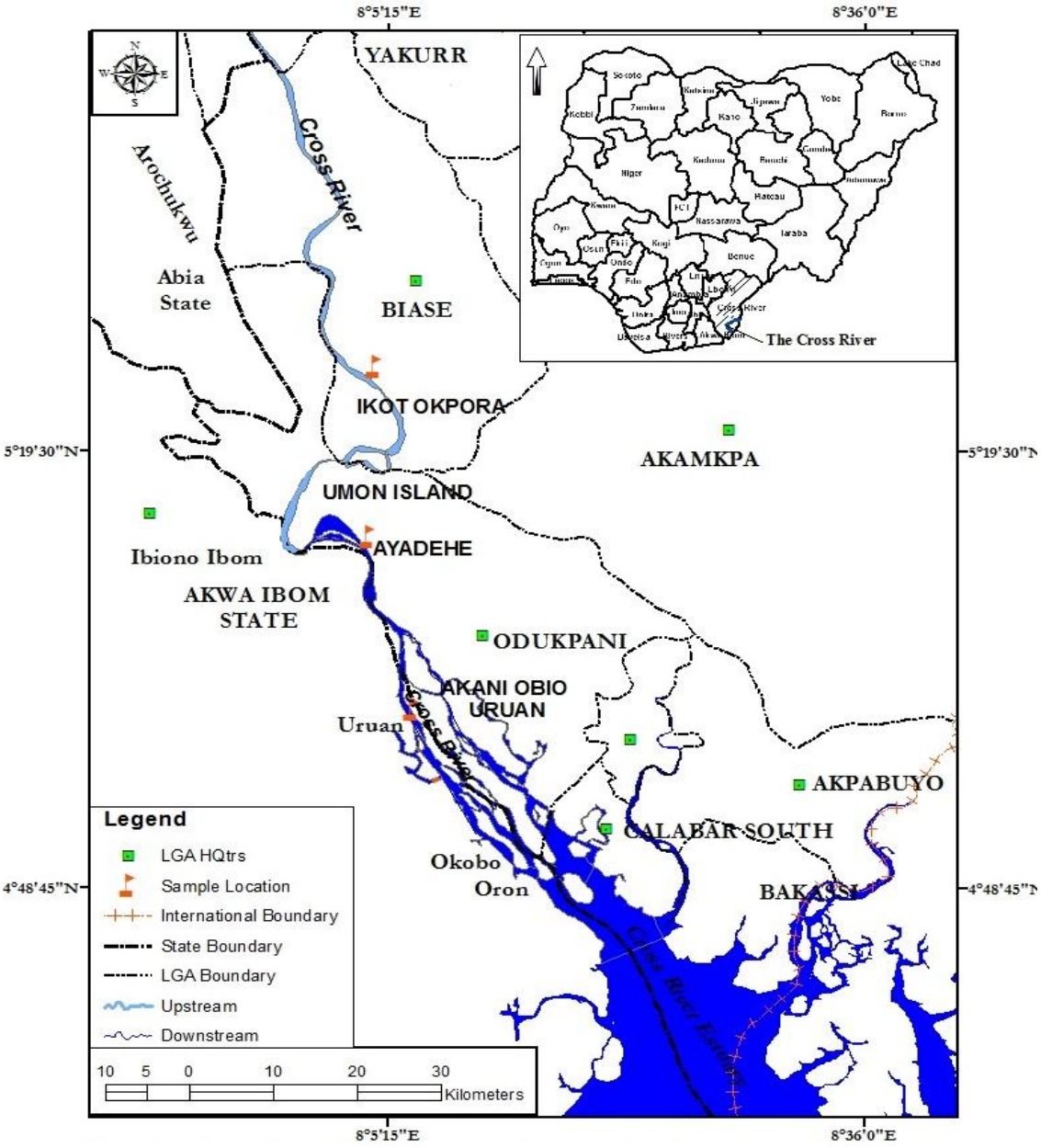

Fig. 1. Map of the Cross River, showing the sampling locations (Deeper blue colour indicates the lower Cross river while the lighter blue colour indicates the middle Cross River)

Cross River is characterized by rocky banks and high riparian vegetation, while the lower Cross River is characterized by low lying muddy banks with grassy vegetation cover. These sites were chosen because they are the major landing sites for *C. nigrodigitatus* fishery along the river system. A total of 79 fish samples of *C. nigrodigitatus* were collected from the above sampling points using nets, with the help of local fishermen. Some were obtained from the artisanal fishers. The specimens were transported in ice-cold boxes to the Biotechnology Laboratory in the University of Calabar, Calabar, for morphometric measurements the same day before preservation.

2.2 Morphometric Studies of the Fish Samples

Morphological measurements were taken from each of the 79 fish samples. A total of 18 morphological measurements were taken namely:

Standard length (SL); Predorsal distance (PDD); Dorsal fin length (DFL); Distance between dorsal and caudal fin (DDCF); Distance between occipital process and dorsal fin (DODF); Body depth at anus (BDA); Prepectoral distance (PPD); Prepelvic distance (PVD); Preanal distance (PAD); Pelvic fin length (PFL); Pelvic spine length (PSL); Anal fin length (AFL); Caudal peduncle depth (CPD); Head width (HW); Head length (HL); Snout length (SnL); Eye diameter (ED); and Inter-orbital distance (ID). Measurements were taken using a meter rule and a vernier caliper to the nearest 0.01 cm.

Meristic measurements such as the number of Dorsal fin rays (DFR), Pectoral fin rays (PTFR), Anal fin rays (AFR), Pelvic fin rays (PVFR) and Gill raker (GRN) were also taken from the fish samples collected. After morphometric measurements, fin samples were cut off from the caudal fin and preserved in 95 percent ethanol (analytical grade) for DNA analysis.

2.3 Extraction of Genomic DNA and Primer Selection

Total DNA was extracted from fins of the collected fish samples using a modified cetyltrimethylammonium bromide (CTAB) method [18]. The DNA concentration was evaluated using a JenWay Genova nano system. Approximately 1.5µl of the DNA was tested on the nanodrop using 1.5µl of TE buffer as a blank. The concentrations were adjusted by diluting the DNA with TE buffer to bring to 250ng/µl concentration.

Four microsatellite primers Cn13, Cn25, Cn45 and Cn67 (Table 1) reported by [19] to be useful in studying genetic diversity in *C. nigrodigitatus* were procured from Inqaba Biotech West Africa. The primers were first tested on randomly selected fish from each population in order to select primers that were of high resolution, repeatability and intensity.

2.4 Polymerase Chain Reaction (PCR) Amplification with Microsatellites

PCR amplifications for microsatellite analysis were done in a 40 µl reaction volume containing 2 µl genomic DNA (100 ng/µl), 4µl of 10X PCR buffer, 0.8µl of dNTP,1 µl of 10 µM of each primer, 0.2µl Taq polymerase and 31µl 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 30 seconds, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes. After amplification, 10µl of PCR product was loaded on 1% agarose gel, electrophoresed at 100 Volts for 45 minutes and detected by gel red. A one Kilobyte DNA ladder was used as a reference marker to enable the determination of allele sizes.

2.5 Statistical Analysis

For morphometric data analysis, the mean, standard deviation, analysis of variance (ANOVA) principal component analysis (PCA) and cluster analysis were performed using the computer software (Predictive analytical software "PASW") version 18.0. Prior to morphological analysis, data were transformed with an allometric formula as given by [20] in order to remove length effects in the samples as there was significant linear correlation between all morphometric characters and standard length of fish. The efficiency of size adjustment transformations was assessed by testing the significance of correlations between transformed variables and standard length.

Table 1. Primer sequences and annealing temperature of microsatellite markers tested in this study

Primer	Sequence	Annealing temperature
Cn13	F: AAGCACAGATTTGGCCCTAC R: TTCGTGTGTACAGGCTTAG	64°C
Cn25	F: TCAGCACAGAATACAGCATG R: GGTTATCACCAGTTATTCTATTGTG	62°C
Cn45	F: GCATGCCGACTCCCACTC R: CATTTTCCGGGAAAAGCC	65°C
Cn67	F: TGAGTGAGGAGGTTATTCTCACC R: AGTAAATGCCAAAAATGTACATGC	63°C

Microsatellite loci scoring for each locus was performed using presence /absence of each allele. A single genotypic matrix was constructed for all loci of *C. nigrodigitatus* species. Number of alleles, observed heterozygosity (*H*O), expected heterozygosity (*H*E), Shannon`s information index, and Wright's F-statistics were calculated using the GENEPOP programme Version 3.3 [21]. Polymorphic information content (PIC) was calculated using FSTAT v.2.9.3. Genetic distances between populations and gene flow between populations were obtained using POPGENE programme Version 1.32 [22]. Hardy Weinberg Equilibrium Test was based on Chi square analysis using STATA software.

3. RESULTS

3.1 Morphological Analysis of *C. nigrodigitatus*

In the present study, 23 morphometric parameters were studied in the 79 samples of *C. nigrodigitatus* obtained from Cross River. Results obtained showed significant differences(P<0.05) in 19 of these morphometric features, some of which are standard length, predorsal distance, preventral distance, dorsal fin length, anal fin length, head width and gill raker (Table 2). From the table, *C. nigrodigitatus* samples from Akani Obio Uruan (AU) had significantly higher (P<0.05) values in head length, snout length, dorsal fin length, body depth at anus, anal fin ray and pelvic fin lengths (P<0.05) than the rest. *C. nigrodigitatus* from Ikot Okpara (IK) had significantly lower (P<0.05) values in standard length, predorsal distance, preanal distance, preventral distance, pelvic fin length and caudal peduncle depth compared with the rest. Samples from AH were significantly higher (P< 0.05) than the rest in number of pelvic fin rays and dorsal fin rays.

The dendrogram showing the relationship based on morphometric features of *C. nigrodigitatus* gave three major clusters. Cluster one consists of samples of *C. nigrodigitatus* from Ikot Okpara. On the other hand, clusters two and three consist of samples of *C. nigrodigitatus* across the three locations (Fig. 2).

Table 2. Means and standard errors for pooled morphometric traits obtained from the sampled *C. nigrodigitatus* species

Parameters/Locations	AH	AU	IK
Standard length	1.482^{a}±0.036	1.573^{a}±0.018	1.348^{b}±0.031
Predorsal distance	1.017^{a}±0.042	1.089^{a}±0.029	0.859^{b}±0.034
Preanal distance	1.351^{a}±0.064	1.389^{a}±0.031	1.191^{b}±0.050
Preventral distance	1.189^{a}±0.036	1.269^{a}±0.025	1.052^{b}±0.030
Prepectoral distance	0.845±0.045	0.912±0.044	0.763±0.014
Dorsal fin length	0.451^{b}±0.455	0.601^{a}±0.029	0.390^{b}±0.039
Anal fin length	0.532±0.037	0.638±0.020	0.414±0.041
Pelvic fin length	0.142^{b}±0.061	0.385^{a}±0.034	0.064^{c}±0.031
Pelvic spine length	0.559±0.059	0.646±0.043	0.528±0.035
Distance between dorsal and caudal fin	1.222±0.034	1.091±0.031	1.079±0.035
Distance occipital and dorsal fin	0.089^{b}±0.018	0.381^{a}±0.043	0.435^{a}±0.066
Caudal peduncle depth	0.585^{a}±0.035	0.660^{a}±0.017	0.447^{b}±0.322
Body depth at anus	0.717^{b}±0.043	0.865^{a}±0.038	0.618^{b}±0.026
Head length	0.595^{c}±0.041	0.994^{a}±0.026	0.789^{b}±0.029
Head width	0.697^{ab}±0.051	0.796^{a}±0.027	0.659^{b}±0.034
Snout length	0.411^{b}±0.051	0.578^{a}±0.023	0.279^{c}±0.061
Inter-orbital distance	0.409^{ab}±0.051	0.572^{a}±0.034	0.362^{b}±0.031
Eye diameter	0.099±0.003	0.175±0.038	0.043±0.017
Dorsal fin ray number	7.360^{a}±0.360	6.900^{b}±0.580	7.030^{b}±0.270
Anal fin ray number	11.140^{b}±0.350	12.100^{a}±0.230	11.030^{b}±0.260
Pelvic fin ray number	7.790^{a}±0.350	6.240^{b}±0.120	6.580^{b}±0.150
Pectoral fin ray number	9.710^{a}±1.290	9.140^{b}±0.220	9.500^{a}±0.190
Gill raker number	25.500$^{a\pm}$0.420	24.210^{ab}±0.560	23.540^{b}±0.450

Means with different alphabetical letters along the same horizontal array are statistically different (P<0.05) based on Least Significant Difference tests. AH= Ayadehe; AU= Akani Obio Uruan; IK= Ikot Okpara

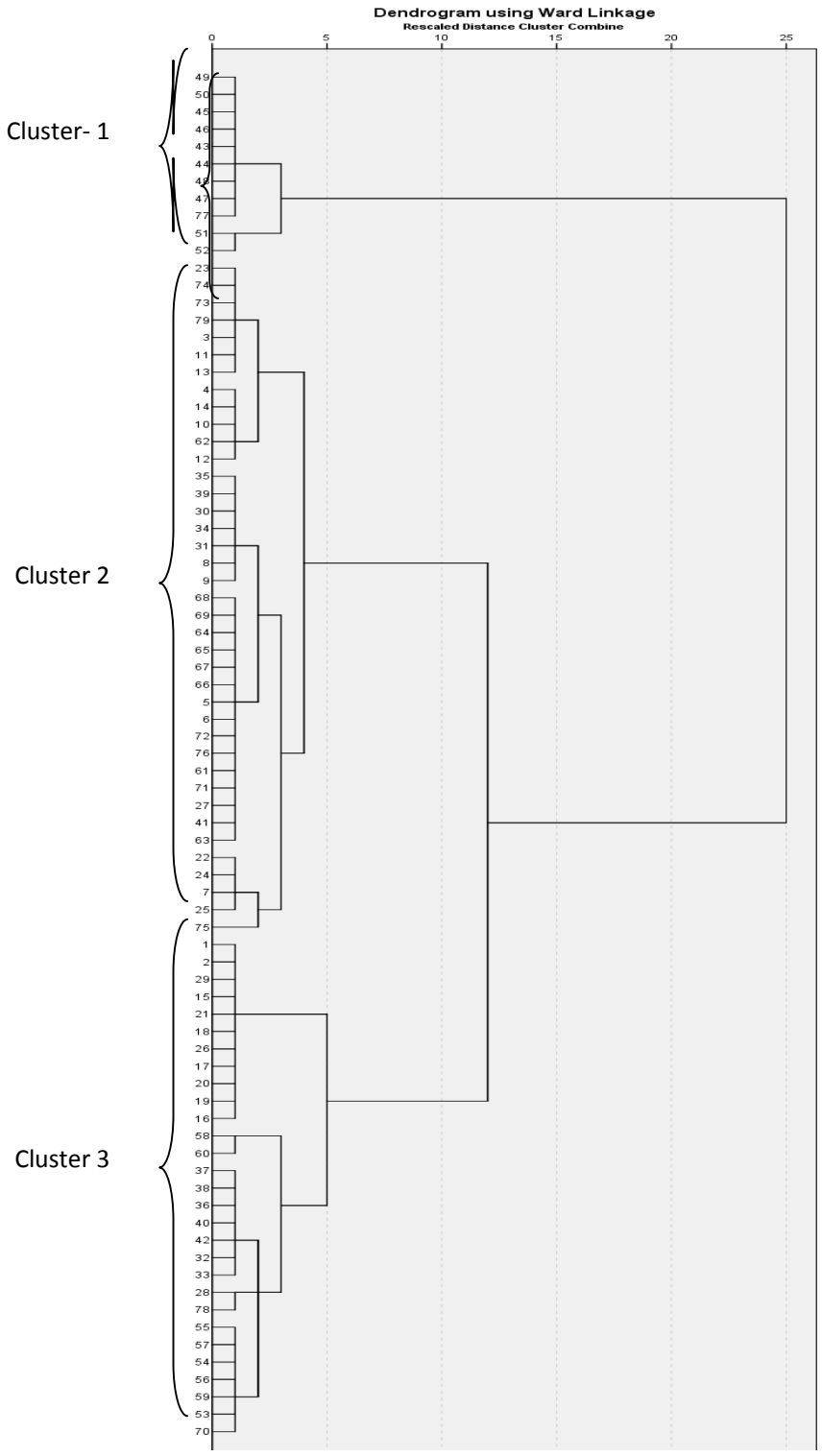

Fig. 2. Dendrogram based on Ward`s linkage cluster analysis of transformed morphometric data in *C. nigrodigitatus* population; Ayadehe (samples 1-14); Ikot Okpara (samples 28-54;70-79); Akani Obio Uruan (samples 15-27 ; 55-69).The Predictive Analytical Software was used

Table 3. Principal components for 23 morphometric traits in *C. nigrodigitatus*

Morphological traits	PC1	PC2	PC3	PC4	Communality
Standard length	0.986	0.047	-0.023	-0.040	0.976
Predorsal distance	0.955	0.042	0.025	-0.023	0.915
Preanal distance	0.749	0.091	-0.139	0.098	0.599
Preventral distance	0.977	0.045	-0.012	0.011	0.958
Prepectoral distance	0.488	-0.123	-0.264	0.394	0.478
Dorsal fin length	0.887	-0.037	-0.035	-0.02	0.797
Anal fin length	0.871	0.025	-0.104	-0.002	0.769
Pelvic fin length	0.787	-0.075	-0.031	-0.109	0.638
Pelvic spine length	0.616	0.061	0.250	-0.341	0.562
Distance between dorsal and caudal fin.	0.641	0.274	0.068	-0.394	0.646
Distance between occipital process and dorsal fin	0.103	-0.572	0.636	0.028	0.744
Caudal peduncle depth	0.920	0.054	-0.018	-0.017	0.849
Body depth at anus	0.819	-0.067	-0.054	-0.008	0.678
Head length	0.882	0.035	0.067	0.057	0.786
Head width	0.749	-0.051	0.034	0.248	0.627
Snout length	0.842	0.039	0.016	-0.242	0.770
Interorbital distance	0.787	-0.145	0.110	-0.098	0.662
Eye diameter	0.458	-0.345	0.526	0.306	0.699
Dorsal fin rays no.	-0.025	0.775	0.385	0.363	0.881
Anal fin rays no.	0.411	-0.488	-0.296	0.366	0.628
Pelvic fin ray no.	0.153	0.647	-0.346	-0.071	0.567
Pectoral fin ray no.	0.116	0.792	0.398	0.222	0.848
Gill raker no.	0.436	0.130	-0.233	0.420	0.438
Component matrix					
Eigen Value	11.391	2.496	1.442	1.182	_
Proportion of variance (%)	49.526	10.854	6.267	5.140	_
Cumulative variance (%)	49.526	60.380	66.647	71.787	_

Principal Components Analysis indicated that the first two principal components (PC_1 and PC_2) contributed 60.376 percent of the total variation (Table 3). The first principal component contributed 49.526 percent of the total variance with standard length (0.986), preventral distance (0.977), predorsal distance (0.955), anal fin length (0.871), snout length (0.842), body depth at anus (0.819), head length (0.882) and caudal peduncle depth as key players. Metric parameters such as number of dorsal fin rays (0.775), number of pelvic fin rays (0.647) and number of pectoral fin rays (0.792) contributed high variability in PC_2 accounting for 10.85 percent of the total variation (Table 3).

3.2 Microsatellite Profiles and Genetic Diversity Analyses of the Sampled *C. nigrodigitatus*

Out of the four available microsatellite markers (Cn13, Cn25, CN45 and Cn65) tested for PCR amplification, two (Cn25and Cn65) failed to amplify probably due to lack of priming sites in the genome. Primers Cn45 and Cn13 showed polymorphism and better banding patterns for *C. nigrodigitatus* with varied molecular weight ranging from 94 to 370 bp. The number of alleles were low (3) across both loci (Cn45 and Cn13). Frequencies of major alleles for both populations are given in Table 4.Genetic diversity analysis revealed the effective number of alleles (Ne) to be 2.528 (with primer Cn45) and 2.113 (with primer Cn13) for middle Cross River while the values for lower Cross River were 2.761 (Cn45) and 2.571 (Cn13). Shannon`s information index ranged from 0.898 (Cn13) to 1.056 (Cn45) for middle and lower Cross River samples of *C. nigrodigitatus.* The observed heterozygosity (Ho) and expected heterozygosity (He) had mean values of 0.433±0.141 and 0.5851±0.057 respectively for samples of *C. nigrodigitatus* from middle Cross River while the values for samples from lower Cross River were 0.467±0.187 and 0.646 ±0.020 respectively. Polymorphic information content ranged from 0.521 to 0.567 for middle and lower Cross River samples of *C. nigrodigitatus.* The chi square values from Hardy Weinberg Equilibrium test ranged from 7.384 to 20.007 with largely insignificant P-values ranging

from 0.000 to 0.06 (Table 4). Results of inter population genetic structure F- statistics (F_{ST}) for *C. nigrodigitatus* averaged 0.066 (Table 5). Wright`s (1965) threshold for F_{ST} were adopted: little genetic differentiation (0 - 0.05); moderate genetic differentiation (0.05 – 0.25); high level of genetic differentiation (> 0.25). The mean value of F_{IS} was positive in all sample locations. Sufficient gene flow was observed in *C. nigrodigitatus* (3.523) samples from middle and lower Cross River (Table 5).

4. DISCUSSION

Out of the 23 morphometric characters studied, 19 showed significant differences (P<0.05) among the populations of *C. nigrodigitatus*. Causes of morphological differences among species are often quite difficult to explain [23] but it is well known that morphometric characters may show a high degree of phenotypic plasticity in response to different environmental factors [7,24]. The morphological differences observed between samples from middle and lower Cross River could be traced to differences in habitat factors. The lower Cross River sampling points could have provided an environment with plenty of food, leading to larger fish sizes in samples from Ayadehe (AH) and Akani Obio Uruan (AU). Similar to our findings, [25] concluded that the observed morphometric differences in *C. nigrodigitatus* samples from Lagos Lekki and Badagry Lagoon were related to environmental and climatic differences and were therefore phenotypic rather than genetic. This argument by [25] is concurred by [26] who reported that there is greater than 99 per cent homology between specimens of *Chrysichthys nigrodigitatus* from the Cross River in both lower and middle Cross River which constitute the sampling area of the present study. Cluster analysis of morphometric data of *C. nigrodigitatus* in this study also showed good differentiation between sampling locations. Similar results were reported by [8]. In their study, a total of 18 morphometric characters were used to differentiate *C. nigrodigitatus* samples. They noted that the morphometric variability within specimens of *C. nigrodigitatus* showed a good level of intra-population variation, but added that genetic assessment was required to corroborate the results.

Principal component analysis is used to identify parameters that contribute greatly to observed variation and thus useful in differentiating populations. In this study, several features which facilitated morphometric differentiation between samples were located in the head region such as snout length, head length and head width. Parameters from other parts of the body were, however, equally important and included standard length, predorsal distance, preanal distance, preventral distance, dorsal fin length, anal fin length and body depth at anus. Such characters would be useful in selection for breeding. Turan et al. [5] observed that morphological characters that differentiated *Clarias gariepinus* populations were mainly from head measurements.

Genetic information on *C. nigrodigitatus* is limited and only few microsatellite markers have been developed. The two microsatellite markers used in the present study had good polymorphic information contents (PIC) ranging from 0.521 to 0.567. PIC measures the informativeness of a genetic marker and values higher than 0.5 demonstrate high polymorphic information content for the microsatellite loci [27]. Nwafili et al. [14] reported that the two loci used in this study were good for genetic diversity study of *C. nigrodigitatus* but they recorded slightly higher mean PIC values of 0.812 for Cn45 and 0.774 for Cn13.

Table 4. Summary of genetic diversity parameters and HWE values for *C. nigrodigitatus*

Population	Locus	Maf	Na	Ne	I*	Ho	He	PIC	HWE
C. nigrodigitatus (Middle Cross River)	Cn45	0.465	3	2.528	0.991	0.533	0.625	0.521	20.007 (0.000)
	Cn13	0.633	3	2.113	0.898	0.333	0.425	0.567	8.173 (0.042)
C. nigrodigitatus (Lower Cross River)	Cn45	0.467	3	2.761	1.056	0.600	0.660	0.565	7.384 (0.060)
	Cn13	0.500	3	2.571	1.011	0.333	0.632	0.536	12.785 (0.005)

Maf, major allele frequency; Na, number of alleles; Ne, number of effective alleles; I, Shannon`s information index; Ho, observed heterozygosity; He, expected heterozygosity; PIC, polymorphic information content; HWE, Hardy Weinberg Equilibrium. Values in brackets are the P values for HWE.*

Table 5. F-statistics and gene flow for the two loci studied in *Chrysichthys nigrodigitatus*

Population	Locus	F_{ST}	F_{IS}	N_m
C. nigrodigitatus	Cn45	0.066	0.088	3.538
	Cn13	0.067	0.414	3.507
	Mean	0.066	0.244	3.523

F_{IS}, Inbreeding coefficient; F_{ST}, Fixation index; N_m, Gene flow

Low genetic diversity among the *C. nigrodigitatus* populations studied was evident in the present study from the low heterozygosity levels observed, the low Shannon`s information index (mean of 0.944 – 1.034) recorded across all loci as well as the positive inbreeding coefficients (F_{IS}). Shannon's information index is a measure of species diversity and an index value of ≤ 1 indicates less diversity. The inbreeding coefficients (F_{IS}) were positive for all populations studied, indicating the absence of many heterozygous individuals in these populations. This further confirms the low diversity level of these populations. Ajang et al. [4] reported on the exploitation rate of this species in the lower Cross River and concluded that the stock was overfished. Such reduction in the size of a population can reduce variations in a gene pool, leaving a limited number of species to pass on genes to offspring through sexual reproduction. Earlier studies of *C. nigrodigitatus* from Cross River region of the Niger Delta using mtDNA and AFLP analyses reached similar conclusions of low genetic diversity [9,10,26].

The populations` genetic differentiation (F_{ST}) values obtained in this study also confirmed that the samples were genetically poorly differentiated from each other or almost genetically similar to each other as values of 0.25 and above are said to indicatehigh level of genetic differentiation [28]. The high genetic similarity could have resulted from lack of geographical barriers between the middle and lowers sections of the Cross River, allowing potamodromous migration and mating to occur freely between fish species from both locations. Ama-Abasi et al. [29] studied the migration pattern in the lower and middle Cross River and concluded that the species engages in potamodromous migration within the river. This point is further strengthened by relatively high gene flow values of 3.507-5.400 obtained in the present study which indicates sufficient gene flow among samples from both sampling locations allowing little or no genetic differentiation. These results are similar to the findings of [30] which recorded a gene flow of 3.87 – 5.54 in a study of *Heteropneustes fossilis*. On the contrary, [17] reported moderate genetic differentiation and limited gene flow in wild *C. nigrodigitatus* from the Lagos Lagoon complex. In another study of *C. nigrodigitatus* species from the Niger Delta, the number of migrants was 0.618 which is less than 1, indicating moderate levels of gene flow [14]. Nwafili and Gao [17] remarked in their study that all populations of *C. nigrodigitatus* from the Niger Delta, with the exception of Cross River, exhibited high genetic diversity.

It is worth noting that different methods applied by different authors, AFLP [9], mt DNA [10], Microsatellite [12,14] and ribosomal RNA and Internal transcribed spacers [26] all revealed the low genetic diversity of *Chrysichthys nigrodigitatus* of the Cross River. This therefore lends credence to the reliability of this study and its findings. Furthermore, in most of the loci and populations analyzed in the present study, data obtained showed no significant deviation from Hardy Weinberg Equilibrium (HWE) except for *C. nigrodigitatus* from lower Cross river at locus CN 45 where P=0.06. Significant deviations from HWE in most loci would suggest the presence of population bottlenecks.

The low genetic diversity observed in this study implies that the *Chrysichthys* population of the Cross River is fragile. Any drastic and sudden change in the environmental condition of the River system can lead to instantaneous wipe out of the population with grave consequences on the socio-economic life of the people of the Cross River basin and the ecology of the river system. An immediate conservation and management strategy including domestication of the species to shield it from any environmental perturbation like climate change, pollution and overfishing is thus recommended.

5. CONCLUSION

Morphological and genetic diversity studies of *Chrysichthysnigrodigitatus* species reveal that although this species from Cross River region of the Niger Delta showed significant morphometric variability, the genetic diversity assessment revealed low diversity in the studied *C. nigrodigitatus* species. Such low genetic diversity

implies that any spontaneous disruption in the environmental parameters can lead to a wipe out of the population. The study can serve as preliminary information regarding the genetic variation and population structure of *Chrysichthys* species from Cross River.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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