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MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY OF *Chrysichthys nigrodigitatus* FROM SOME COASTAL RIVERS IN NIGERIA

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ABSTRACT

Chrysichthys nigrodigitatus is an economically and nutritionally important fish commonly found in both fresh and brackish waters in Nigeria. The purpose of the study was to assess the genetic diversity *C. nigrodigitaus* using microsatellites with a view to ascertaining the necessity for breeding and conservation of the species. A random sample of 60 fish samples were obtained from three coastal populations in Nigeria for genetic diversity assessment by 4 microsatellite loci. All investigated populations demostrated high polymorphism in all loci. High genetic vatiation was indicated by the four microsatellite loci in all the three populations with the number of alleles (Na) ranging from 2 – 3 alleles per locus while the effective number of alleles (Ne) ranged from 2.12 – 2.41. Badagry population had the highest genetic diversity as was revealed by heterozygosity (observed and expected) and shanon index. Clustering based on the genetic distance gave two major clusters indicating some level of genetic variability between the studied populations which may be explored for appropriate breeding and conservation of *C. nigrodigitatus* in Nigeria.

Keywords: Genetic variation; microsatellite analysis; *Chrysichthys nigrodigitatus;* coastal rivers

INTRODUCTION

Chrysichthys nigrodigitatus (Silver catfish) is a euryhaline fish of tropical Africa which supports successful commercial fisheries with great potentials for aquaculture in West Africa. It is an important source of animal protein and income throughout the world especially in developing countries like Nigeria. It is found in both fresh and brackish water habitats such as rivers, mangrove swamps, lakes, and estuaries and low salinity coastal areas (Moses, 2001). The silver catfish is a benthic omnivorous fish that migrates to freshwater to breed (Nwafili *et al*. 2012). According to Nwafili *et al*. (2015), the wild population of *C. nigrodigitaus* is declining due to destructive fishing methods, environmental pollution and overfishing. Furthermore, culturing the fish still relies on capture of fry from the wild for stocking which could affect the genetic diversity of the natural populations. Considering the nutritional and economic importance of *C. nigrodigitaus* species, understanding the current

level of genetic diversity and patterns of population genetic structure are paramount for sustainable aquaculture practices, conservation, and improvement through selective breeding.

Nigerian coastal waters are facing serious climate change challenges of which flooding is an important consequence which can impact negatively on genetic diversity. It was suggested by Bickham *et al*. (2000) that reduced genetic diversity stemming from population bottlenecks and inbreeding could result from environmental alterations. To this effect, studies of population genetic diversity can provide baseline information that can aid in undertaking conservation measures and management of ecosystems and their populations.

For a better understanding of the genetic diversity and evolutionary divergence of different populations of *C. nigrodigitatus*, microsatellite markers were employed to give more insight into the diversity and population genetic structure of this species. Information about the genetic diversity of the wild fish populations through microsatellite analysis is essential in breeding for heterosis and effective fish management (Bo-young Lee *et al*., 2005). Nwafili *et al*. (2015) confirmed that studying the genetic polymorphism and population structure of *C. nigrodigitatus* with a limited number of primer combinations prevents drawing more robust conclusions. Therefore, investigating the genetic diversity and structure of *C. nigrodigitatus* populations along the coast of Nigeria using microsatellite markers to determine the genetic status of the species for conservation and breeding purposes necessitated this study.

MATERIALS AND METHODS

Study Area

The sampling locations for the study were Itu Bridge River in Akwa Ibom state and Badagry Lagoon and Epe in Lagos state. The sampling locations and their coordinates are shown in Table 1. All the locations are along the coast of Nigeria.

Collection of Fish Samples

A total of n = 60 of *C. nigrodigitatus* fish were identified and collected from four coastal rivers (twenty from each river) in the Niger delta, Nigeria, namely Itu Bridge River (Akwa Ibom), Badagry Lagoon (Lagos) and Epe (Lagos). The geographical locations of the sampling stations are presented in Table 1. Experimental fish samples were identified to be *C. nigrodigitatus* by a fish taxonomist from Nigerian Institute for Oceanography and Marine Research Lagos, Nigeria using a field guide to Nigerian fresh water fishes. The fish samples were obtained from the fishermen at the landing sites.

Table 1. Geographical location of the sampling stations

Location	Latitude	Longitude	State
Itu	$N05^0\ 10^1\ 44.0^{11}$	$E008^0\ 03^1 57.3^{11}$	Akwa Ibom
Badagry	$N04^o\ 25.012^1$	$E02^o\ 52.988^1$	Lagos
Epe	$N06^0\ 35^1\ 0.2^{11}$	$E02^o\ 59.096^1$	Lagos

Extraction of DNA and PCR Amplification

Fish muscle tissue (1 cm^3) was collected from each individual and placed in 95% ethanol for preservation until analysis. Genomic DNA was extracted from the tissue using DNA Prep kit. The quality of extracted DNA was checked using a Nano-drop spectrophotometer (Shimadzu corporation Japan, MODEL UV-1800, 2000 series) at absorbance of 260/280nm. Amplification was carried out using four microsatellite primers (Table 2) originally developed for *C. nigrodigitatus*. A total volume of 25 µl of the PCR mix which consisted of 2.5 µl buffer, 2.0 µl dNTP, 2.0 µl 25M $Mgcl_2$, 1.0 µl forward primer, 1.0 µl reverse primer, 0.06 µl Taq,13.44 µl H_2O and 3 µl of template DNA (10-100 ng) was run on a Thermocycler (Biorad, module 170 – 8731). The program for PCR amplification was: 30cycles of denuturation at 94°C, 1min; annealing at 45-55°C, 1min and extension at 72°C, 45s (Table 2). The samples were stored at -20°C until separation on polyacrylamide gels (6% polyacrylamide gel, at 80 V for 2 h in a 1 x TBE buffer). The gel was stained with ethidium bromide and visualized in a UV transilluminator. Two researchers independently scored the gel bands to reduce or rule out error due to improper scoring.

Table 2: SSR primer code, sequences, annealing temperature and band size

Primer code	Sequence	Annealing temperature (°C)	Molecular size (bp)
CN13	F:aagcacagatttggccctac R:ttcgtgtgtacaggcttag	52	150-250
CN25	F: tcagcacagaatacagcatg R:ggttatcaccagttattctattgtg	52 53	100-250
CN45	F:gcatgccgactcccactc R:cattttctccgggaaaagcc		100-150
CN67	F:tgagtgaggaggttattctcacc R:agtaaatgccaaaaatgtacatgc	53	100-200

Data Analysis

Population genetic data generated were analyzed using GenAlEx 6.51b program (Genetic Analysis in Excel) to obtain the number of different alleles, number of effective alleles, shannon's information index, observed heterozygosity, expected heterozygosity, fixation index, percentage polymorphic loci. Genetic relationship among populations was estimated by constructing a dendrogram using a UPGMA (unweighted pair-group method of analysis). Marker characteristics which include polymorphic information content (PIC) and Gene diversity were determined using PowerMarker v. 3.25. Fstat program was used for allelic richness and inbreeding coefficient while Nei's Pairwise genetic distance was analyzed with NTSYSpc 2.02 program.

RESULTS

Genetic Variability among Microsatellite Loci

Primers CN13, CN25, CN45 and CN67 reported by Kotoulas *et al*. (1998) as useful for amplification studies in *C. nigrodigitatus* were used to determine genetic diversity at four

microsatellite loci in *C. nigrodigitatus*. The four microsatellite loci used in this study exhibited high polymorphism in the three populations investigated (Table 2). A total of 9 alleles were found in the study. The mean number of alleles per locus was 3.0. All the loci gave the same number of alleles (3 alleles respectively). The allelic richness showed that across the 4 loci, CN25 had the highest value of 3.00. The level of diversity revealed by the studied loci ranged from 0.51 to 0.62 with an average of 0.67 (Table 3). All four microsatellite loci were polymorphic in all populations with polymorphic information content (PIC) values ranging from 0.41 at locus CN45 to 0.55 at locus CN25 with an average of 0.48 (Table 3). The highest expected heterozygosity was obtained by locus CN67 (0.907) while locus CN13 had the lowest (0.733). The inbreeding coefficient (Fis) was negative for all populations across all the loci (Table 4).

Genetic Differences among Populations

The Badagry population had the highest mean number of alleles (3.00) while Itu and Epe had same and lowest mean number of alleles (2.25) respectively. The mean effective alleles varied from 2.12 to 2.41. In all populations, the mean effective number of alleles was lower than the mean number of alleles. Shannon information index was observed highest in Badagry population (0.94) followed by Epe (0.84) and then Itu (0.77) populations. All populations showed high average observed heterozygosity. Badagry was the most variable (Ho = 0.87) followed by Epe (Ho = 0.79) and then Itu (Ho = 0.74) while the average expected heterozygosity was high in Badagry (0.60), Epe (0.56) and Itu (0.54) populations following the same trend with observed heterozygosity as shown in Table 5.

According to Table 6, Nei's genetic distance between the populations ranged from 0.00 to 0.04. The highest genetic dissimilarity was between Epe and Itu with a genetic distance of 0.04 while Badagry and Epe had no genetic dissimilarity (0.00) reflecting 100% similarity. The UPGMA dendrogram based on the genetic distances revealed two clusters: Cluster-1 consists of Badagry and Epe and cluster-2 consists of Itu that clustered separately (Figure 1).

Table 3: Characteristics of SSR Loci analysed

Marker	Na	GD	He	PIC
CN1	3	0.566	0.733	0.471
CN2	3	0.622	0.738	0.551
CN3	3	0.516	0.857	0.405
CN4	3	0.575	0.907	0.494
Mean	3	0.570	0.809	0.480

Na- number of alleles, GD - gene diversity, He - heterozygosity, PIC - polymorphic information content

Table 4: Inbreeding coefficient (Fis) per population (Fstat) of *C. nigrodigitatus*

	Akwa Ibom	Badagry	Epe
CN13	-0.091	-0.611	-0.467
CN25	-0.111	-0.133	-0.305
CN45	-0.571	-0.667	-0.733
CN67	-0.895	-0.554	-0.463
Mean	-0.393	-0.477	-0.478

Table 5: Summary of the genetic diversity level in the studied populations

Population	Na	Ne	I	Ho	He	F	%P
Itu	2.25±0.25	2.12±0.20	0.77±0.09	0.74±0.08	0.54±0.04	-0.45±0.19	100
Badagry	3.00±0.00	2.41±0.14	0.94±0.05	0.87±0.05	0.60±0.02	-0.51±0.12	100
Epe	2.50±0.29	2.24±0.20	0.84±0.10	0.79±0.06	0.56±0.04	-0.46±0.10	100

N - number of different alleles, Na - Number of effective alleles, I - shannon's information index, Ho - observed heterozygosity, He - expected heterozygosity, F - fixation index, %P - percentage polymorphic loci

Table 6: Genetic similarity (below the diagonal) and genetic distance (above the diagonal) between *C. nigrodigitatus* populations studied

Population	Itu	Badagry	Epe
Itu	***	0.02	0.04
Badagry	0.98	***	0.00
Epe	0.96	1.00	***

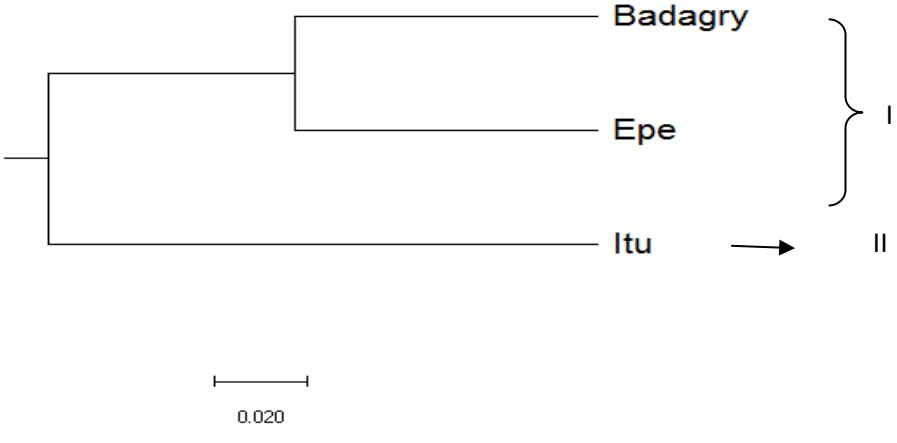

Figure 1: UPGMA dendrogram showing the genetic relationships among 3 populations based on Nei's genetic distance

DISCUSSION

Four microsatellite markers were utilized to characterize and investigate genetic variation in some coastal populations of *C. nigrodigitatus* in Nigeria with a view to assessing the need for breeding and conservation programme. Microsatellite analysis of three populations of *C. nigrodigitatus* revealed high levels of genetic variability similar to marine species as suggested by Kotoulas *et al*. (1991). The pattern of genetic polymorphism in the *C. nigrodigitatus* is high and similar to those described for *Plecoglossus altivelis* (Iguchi *et al*., 2002). Our results in which a total of 9 alleles were revealed is in contrary to the study of Kotoulas *et al*. (1998) who detected 29-30 alleles in four natural populations of *C. nigrodigitatus* at three microsatellite loci. The detection limited number of alleles could be

attributed to small samples in the present study. The observed number of alleles (Na) and the effective number of alleles (Ne) varied among *C. nigrodigitatus* populations in the present study. The average number of alleles observed in Badagry was higher than that of other populations indicating more allelic polymorphism in Badagry population than others.

Electrophoresis of PCR–amplified DNA gave one or two bands. This is expected in microsatellite analysis where one band represents homozygosity and two bands represent heterozygosity. The mean polymorphic information content (PIC) of 0.48 obtained in our study suggests that the microsatellite loci considered were moderately informative with good discriminating power in accordance with the view of Botstein *et al*. (1980). Thus, these markers had good merits for detecting DNA identity and diversity in these populations and are therefore suitable for use in the characterization of natural populations and determination of genetic differentiation in *C. nigrodigitatus*. All the investigated populations demonstrated polymorphism for all loci. This result is in agreement with the result obtained by Corujo *et al*. (2004) in 9 populations of brown trout in Spain with as many as 7 populations having all loci polymorphic. Similarly, Agbebi *et al*. (2013) observed 100% polymorphic loci in a Preliminary characterization of genetic strains in clariid species.

All the populations investigated were identified as having high biodiversity. This was based on Shannon's information index and heterozygosity (observed and expected) which were high in these populations. This finding is in agreement with Nwafili *et al.* (2015) who obtained high values of heterozygosity in all the populations of *C. nigrodigitatus* studied. This genetically diverse nature of *C. nigrodigitatus* from these populations could be attributed to the reduced inbreeding. Higher heterozygosity implies greater genetic variability according to Mu *et al*. (2011) who stated that heterozygosity is an important measure of population diversity at the genetic level. Thus, in order to embark on a meaningful breeding and conservation programme for *C. nigrodigitatus* in Nigerian coastal waters, the studied populations (i.e. Badagry, Epe and Itu) should be considered as sources of fish for breeding and improvement programmes. Capture of fry for aquaculture and overexploitation could affect the genetic diversity of this species and should be a major concern for the future of *C. nigrodigitatus.*

The inbreeding coefficient (Fis) defined as the probability that two homologous alleles present in the same individual are identical by descent (Nwafili *et al.,* 2015). It is expressed as a deficiency in heterozygotes, the theoretical value ranges from -1 to +1, where by positive values indicate heterozygote deficiency possibly due to inbreeding (Brinez *et al*, 2011). In the present study, all the loci showed non-significant negative Fis values for all populations, indicating the presence of more heterozygous individuals than expected in these populations. This reflects excess of heterozygotes relative to Hardy Weinberg expectations. A similar result was obtained by Nwafili *et al.* (2015) who observed negative Fis values in all *C. nigrodigitatus* populations investigated. According to him, Overall, *C. nigrodigitatus* populations were found to be generally out crossing with little or no inbreeding. Clustering based on the genetic distance gave two clusters indicating genetic variability between the studied populations.

CONCLUSION

In the current study, *C. nigrodigitatus* maintains moderate genetic differentiation among populations studied. All populations contain sufficient genetic diversity that can be exploited for breeding. However, due to the poor representation of *C. nigrodigitatus* populations from Nigerian coastal waters as a result of flood and degraded DNA in the

present study, an extensive study comprising more populations and increased number of microsatellite loci is needed to have a strong conclusion on the genetic diversity and population structure of *C. nigrodigitatus* for fish improvement in Nigeria.

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