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Genetic differentiation and structure of *Chrysichthys nigrodigitatus* populations in some Nigerian coastal waters

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Abstract
The knowledge of genetic structure of a species is a key point for its effective conservation. On this note, studies were carried out to investigate the genetic differentiation and structure of *Chrysichthys nigrodigitatus* populations in some coastal rivers of Nigeria; Itu Bridge River, Badagry, Epe and Igbokoda. A total of 80 DNA samples from four river populations were analyzed at four microsatellite loci. Moderate interpopulation differentiation and sufficient genetic differentiation within individuals were indicated by the four microsatellite loci as evidenced by 3% variation among populations and 89% variation within individuals respectively by AMOVA analysis. This observation was further supported by limited gene flow found among populations. The populations exhibited a positive correlation between genetic and geographical distances which implies that the populations studied fit into isolation by distance model suggesting that structuring in *C. nigrodigitatus* populations is possibly due to isolation by distance.

Keywords: Genetic differentiation, population structure, gene flow, geographical distance, *Chrysichthys nigrodigitatus*

Introduction
The silver catfish (*Chrysichthys nigrodigitatus*) is an economically and nutritionally important fish with a great potential for aquaculture in developing countries like Nigeria and some other West African countries [1]. It belongs to the subfamily claroteinae [2] and can be found in both fresh and brackish water habitats such as rivers, mangrove swamps, lakes, and estuaries and low salinity coastal areas [3]. This species is characterized by spawning migration undertaken in the rainy season from more saline brackish waters to freshwaters where spawning occurs. In the reverse, the juveniles follow the flood water back to the saline environment to feed and grow [4]. Despite the economic importance and aquaculture potentials possess by this species, breeding of the fish is still unsuccessful rather culture of the species still relies on the capture of fry from the wild for stocking. To this effect, the knowledge of genetic differentiation and population structure of this fish species will be useful in developing better breeding and conservation strategy.
It is stated by Changadeya *et al.* [5] that genetic differentiation and population structuring of native species population is an important feature for consideration in breeding, conservation and management programs. Recently, Song *et al.* [1] and Nwafili *et al.* [6] studied the genetic polymorphism and population structure of *C. nigrodigitatus* using microsatellite markers. Furthermore, species of Pacific salmon (*Oncorhynchus spp*.) populations was observed to have a positive correlation between genetic and geographical distances which suggested an Isolation-by-distance effect [7]. Similarly, Li *et al.* [8] reported a significant correlation between genetic distance and geographical distance of *Chiloglanis* populations. A significant correlation was also observed in tagging experiments with anadromous trout, which shows that individuals spawning in a wrong river are most likely to ascend a river in close proximity to the natal river [9]. the present study aims to investigate genetic differentiation and population structuring among populations of *C. nigrodigitatus* from Nigerian coastal waters and examining the geographical and genetic relatedness with a view to provide vital basis for conservation and management of this species.

Materials and Methods

Collection of Fish Samples

Eighty fish samples of *C. nigrodigitatus* were identified and collected from four coastal rivers (twenty per river) in the Niger delta, Nigeria namely Itu Bridge River (Akwa Ibom), Badagry Lagoon (Lagos), Epe (Lagos) and Igbokoda (Ondo). The geographical location in terms of longitudes and latitudes of the sampling stations are presented in Table 1. Experimental fish specimens were obtained from the fishermen at the landing sites after identification.

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
Igbokoda	$N06^1\ 21.\ 001^1$	$E04^1\ 48.220^{11}$	Ondo

Extraction of DNA and PCR Amplification

Fish muscle tissue (1 cm^2) was used for the analysis and was collected from each fish sample; it was preserved with 95% ethanol inside eppendorf tubes until analysis. Genomic DNA was extracted from the tissue using DNA Prep kit. The extracted DNA quality was checked using a Nano-drop spectrophotometer (Shimadzu corporation Japan, MODEL UV-1800,2000 series) at absorbance of 260/280nm. Four microsatellite primers originally developed for *C. nigrodigitatus* was used for the amplification. A total volume of 25 µl of the PCR ingredients that consisted of 2.5 µl buffer, 2.0 µl dNTP, 2.0 µl of 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: 30 cycle of denaturation at 94 ^oC, 1min; annealing at 45-55 ^oC, 1min and extension at 72 ^oC, 4sec. The samples were stored at -20 ^oC until polyacrylamide gel electrophoresis (6% polyacrylamide gel, at 80 V for 2 h in a 1x TBE buffer) was run for separation. The gel was stained with ethidium bromide and visualized in a UV transilluminator. Two researchers scored the gel bands independently to reduce or rule out error due to improper scoring.

Retracted genomic DNA of Ondo samples (20 samples) were degraded due to power alteration and were excluded during PCR amplification in the present study.

Data Analysis

Population genetic data generated was analysed using GenAlEx 6.51b program (Genetic Analysis in Excel) to obtain gene flow; and F-statistics. The genetic differentiation among populations was also measured by *F*ST and analyzed by analysis of molecular variance (AMOVA). Genetic relationship among populations was estimated by constructing a dendrogram using UPGMA (unweighted pair-group method of analysis).

Results

Genetic structure and Population Differentiation

The mean *F*ST value was estimated at 0.029. Thus, about 98% of total genetic variation resides within each population (Table 2). Pairwise FST values ranged from 0.009 to 0.032 among all three geographic locations (Table 3). These values represented moderate levels of population differentiation. The lowest genetic differentiation was between Badagry and Epe (FST = 0.009) reflecting close populations while the largest genetic differentiation was between Itu and Epe (FST = 0.032), reflecting distinct populations indicating that they were quite isolated populations (Table 3). The AMOVA analysis revealed that a small (3%) but significant (p<0.01) level of genetic differentiation was observed among populations within groups while a large and significant genetic differentiation (89%, p<0.01) was found within individuals among populations (Table 4). Wright, 1978; Hart and Clark 1997 stated that population differentiations of more than 15% are considered high rather than moderate and are associated with low gene flow among the populations. The study registered an overall limited gene flow of more than one migrant per generation among the three populations. The highest number of migrants per generation (Nm = 28.38) was observed between Badagry and Epe populations and the lowest gene flow was between Itu and Epe populations (Nm=7.55) (Table 3).

Cluster analysis and genetic relationships among populations

Cluster analysis dendrogram indicated that Badagry and Epe populations were the most genetically close and Itu population was genetically isolated from the rest of the populations (Fig 1). Geographical result also showed that Badagry and Epe populations are closer than Itu population (Table 3). It could be deduced therefore that populations from the same region (Badagry and Epe) tended to cluster with high levels of gene flow hence there is a positive correlation between genetic and geographical distances among the populations studied. Thus, the structuring in *C. nigrodigitatus* populations is necessarily due to isolation by geographical distances.

Table 2: F-statistics and Gene flow for all loci

Locus	Fis	Fit	Fst	Nm
CN1	-0.339	-0.305	0.025	9.74
CN2	-0,228	-0.177	0.041	5.85
CN3	-0.679	-0.658	0.013	19.29
CN4	-0.628	-0.569	0.036	6.69
Mean	-0.460	-0.417	0.029	8.26

Legend: Fis- inbreeding coefficient, Fst- genetic differentiation, Nm- gene flow

Table 3: Pairwise population Differentiation, estimates of Gene flow, Genetic distance and Geographical distance

Population Pair	Genetic Distance	FST	Nm	Geographical Location (Km)
Badagry and Itu	0.02	0.025	9.821	617.48
Epe and Itu	0.04	0.032	7.550	675.51
Badagry and Epe	0.00	0.009	28.380	226.34

Table 4: Percentages of variation among populations, among individuals and within individuals.

Source of Variation	df	Sum of Square	MS	Est. Var.	% Variation
Among Populations	2	6.325	3.163	0.042	3%
Among Individuals	57	83.400	1.463	0.111	8%
Within Individuals	60	74.500	1.242	1.242	89%
Total	119	164.225	-	1.395	100%

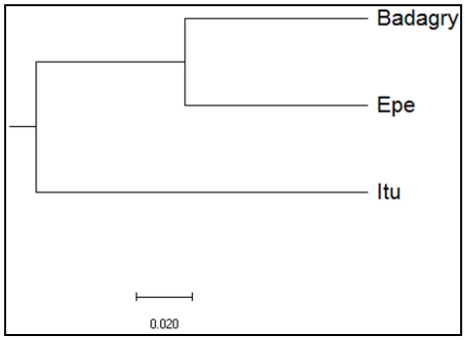

Fig 1: Genetic relationships among the populations based on Nei' genetic distances

Discussion

Genetic structure and differentiation within and among population

The present study revealed a moderate level of genetic differentiation among wild populations of *C. nigrodigitatus* which may suggest limited dispersal between collection sites. Similarly, limited gene flow found among populations is the plausible reason for the observed moderate genetic differentiation among the populations. Yongfeng *et al.* [10] made a similar observation in a genetic structure study of *Gobiocypris rarus* populations. The Significant (p<0.05) population differentiation (FST) obtained in this study hence signify that the populations are distinct indicating that the populations were genetically structured hence, requiring independent conservation management for each river system. This finding is in agreement with Nwafili *et al.* [11] who made a similar observation in the populations of *C. nigrodigitatus* studied. According to Dewoody and Avise [12], fish which distribute in freshwater and brackish water environment may show genetic differentiation somewhat in between those that inhabit barrier free marine environment and barrier isolated freshwater.

However, the pairwise FST population between Badagry and Epe was high due to the closeness of the river systems which attributed to an increase in the level of gene flow among the populations. The 89% of genetic variation resides among individuals as revealed by AMOVA results possibly suggest reasonable random mating due to the existence of large individuals within populations. However, a small (3%) but significant amount of genetic variation that was found among populations reflects a moderate interpopulation differentiation. Overall, the obtained results showed that *C. nigrodigitatus* retains enough genetic differentiation within individuals and a moderate interpopulation differentiation in the studied sites. Nwafili *et al.* [11] made a similar observation in *C. nigrodigitatus* with microsatellite markers.

Genetic relationships and cluster analysis of the populations

Badagry and Epe populations are the most genetically similar and geographically close in the present study. This similarity may be due to common founding population which is reflected in form of more shared alleles (highest gene flow) among the population pairs resulting in the least genetic differentiation among them. The high relationship between genetic and geographical distance indicated that the populations fit into the isolation by distance model as revealed by Mantel's test. It was stated by this model that gene flow is highest between close populations and it is expected that close populations reflect similar genetic composition. Similarly, Michael and Karen [13] observed a significant correlation between geographical and genetic distances of sea trout (*Salmo trutta* L.). On contrary, findings of this study did not concur with other studies of *Lethrinops* species flock by Changadeya *et al.* [14] and Duponchelle *et al.* [15] which reported fish flocks not fitting into the isolation by distance model.

Geographical distance yielded significant results with genetic distance. This suggests that geographical distance between populations within the coastal states studied is of much importance to the genetic structure of populations. It could be deduced that the existence of correspondence between gene flow and geographical distances between populations could be the result of hybridization. Thus, Proximity may be a significant factor favoring gene flow between these populations.

Conclusion

The current study revealed moderate genetic differentiation among the studied populations. However, *C. nigrodigitatus* populations from the same river system are genetically related than population from other river system. Thus, there is a positive correlation between genetic and geographical distances suggesting that structuring in *C. nigrodigitatus* populations is possibly due to isolation by distance.

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