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ESTIMATION OF GENETIC DIVERSITY IN SILVER CATFISH (Chrysicthys nigrodigitatus) USING RANDOM AMPLIFIED POLYMORPHIC DNA

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ABSTRACT

The Silver catfish (*Chrysicthys nigrodigitatus*) is widely distributed along River Niger. This study was carried out to characterize Silver Catfish found along River Niger, with the help of morphometric and random amplified polymorphic DNA for genetic analysis to determine variation in strain that can be used for identification of the strains and possible allocation. Samples were collected from three stations, namely; Illushi, Illah and Onitsha and total of 1 50 samples (50 each) of silver catfish was collected from landing station. Where 12% of the total sampled fish was used for genetic analysis and 100% of the sample was used for morphometric analysis. Result obtain from the morphometric analysis along River Niger show that dorsal fin length and head length with a value of 0.749 and 0.230 respectively are significantly different (p<0.05) in the sample fish can be used for identification. Result obtained for genetic analysis indicate that samples from the three locations are positively correlated. Value between Illah and Illushi is 0.8991 with a percentage variation of 80%; Onitsha and Illah with a correlated value of 0.79045, a variation of 63% and Onitsha and Illushi with a correlated value of 0.76354 a percentage variation of 50%. Dendogram, Darwin's hierarchy and Darwin's radii graph from the study also show that species are from one ancestry. Identification may be necessary to improve strains because of correlation of samples.

Keywords: Silver catfish, morphometric, amplified, polymorphic, variation

INTRODUCTION

Silver catfish Chrysicthys nigrodigitatus (Lacapade) is highly valued by consumers for its flavor and meat quality, mostly in smoked form, and is used in traditional and continental dishes and is found in most of Nigeria's major rivers (Eyo et al. 2013). The fish is a highly valued food fish that is one of the dominant commercial catches in large rivers in Africa (Okey et al., 2016). Morphometric variance between stocks can be used to aid fisheries management by providing a basis for stock structure and a tool to monitor short-term environmental changes (Mojekwu et al., 2015). According to (Schmieder et al., 2015), the simplest and most direct route to species determination is through morphomeristic measurements, which are often used to identify changes between fish populations (Mojekwu et al., 2015; Dwivedi and Dubey, 2013). According to (Goldstein et al., 2020 and Moroni et al., 2015) a variety of morphological, physiological, behavioral and. Morphometric studies on Nigerian fish have been performed to assess specific variations (González et al., 2016) and related variations (Solomon & Ogbenyikwu, 2015 and Fagbuaro et al., 2015). According to Carvajal-Quintero et al., (2015) environmental variables are attributed to changes in the morphometric characteristics of a species from different locations.

Morphometric studies on Nigerian fish have been performed to assess specific variations (Mojekwu & Anumudu, 2015; González *et al.*, 2016) and related variations (Solomon & Ogbenyikwu, 2015 and Fagbuaro *et al.*, 2015). According to Carvajal-Quintero *et al.*, (2015) environmental variables are attributed to changes in the morphometric characteristics of a species from different locations. Morphometric traits in turn might depend on their genotype (Carvajal-Quintero *et al.*, 2015.), the genetic structure of fish species is also useful for population identification, breeding programs, population improvement, conservation of genetic diversity and management for sustainable yields (Abdul-Muneer, 2014;

Perrier, 2013). Biodiversity encompasses the diversity of living species found in a variety of environments, including terrestrial, marine, and other aquatic ecosystems, as well as ecological complexity (Pérez-Ruzafa *et al.*, 2019; Askari *et al.*, 2013). It encompasses the diversity of all forms along with their genetic makeup and all possible assemblages

This study addresses the problem of identifying silver catfish by location with the use of Morphometric and Molecular marker (RAPD). This study also show the genetic variability and morphometric variation in silver catfish found along the Niger River. Information from this study may be useful in the conservation and management of the Silver catfish both in the wild and for domestication

The objective of this is the characterization and correct identification of silver catfish;

to use morphometric features to identify strains and possible allocation and to use molecular methods /to analyzes with the help of RAPD in the identifying and characterization of the strains based on location.

MATERIALS AND METHODS

Area of study

This research study was conducted at different points along the River Niger: Illah, Illushi and Onitsha, as shown in Fig. 3.2. The climate of River Niger is tropical with the dry season lasting from November to April and is usually marked by a cool harmattan haze of the north eastly winds while the wet season last from May to October with a brief dry spell in August known as the August break. The residents of Illushi, Illah and Illushi rely on River Niger as their domestic water supply; and the people are actively engaged in fishing and agriculture. Illushi is located in the Edo State, Illushi being at latitude 640'N and longitude 637'E of the equator ;Illah is located in Delta State with Nigeria at latitude 627'N and longitude 640'E of the equator and Onitsha is located in Anambra State at latitude 68'N and longitude 648E 'of the equator. Three sampling stations were chosen. This study is based on human activity in these areas. Illah is 62.9 Km from Illushi while Onitsha is 104.5 kilometers from Illah. Anthropogenic activities observed in Illah include washing clothes; and bathing at the river bank in Illushi; Bathing, washing clothes, washing harvested rice, and transporting of goods people by boats were the activities observed during the sampling period. Onitsha, by boat, sand dredging on the river bank were the human activities observed this region during the sampling period.



Figure 1: River Niger showing sampling locations

Sample collection

A total of 150 samples of the fish species were collected in batches, 50 per station over a period of six months (September – February). An average of 3 fishes was caught on a daily basis for the six months. Morphometric measurements were made using the Buss-Truss protocols to ensure accuracy of the measurement. The body distances were measured with the Metric Board and Venier Caliper.

Data collection method

Taxonomic keys play an important role in the morphological identification of fish samples as notify by Olaosebikan and Raji (2001) and as such taxonomic keys were used for identification. In this context, one hundred and fifty specimens of silver catfish caught with dragnet and trap were collected by local fishermen at the landing sites of the three locations (Onitsha, Illah and Illusion) for six months. Two samples were taken weekly for twenty-four (24) weeks. Fifty (50) samples were taken from each station during the six (6) month sampling period for a total of 150 samples.

A random sample was carried out regardless of gender because they are monomorphic. The influence of the growth size was masked using logarithmic scale (Portugal and Svaiter, 2011). Eleven morphometric features were measured using Buss-Truss protocols to ensure the accuracy of the measurement. The body distances were measured with the Metric Board and Venier Caliper.

Sample Preparation for Molecular Analysis

A total of eighteen fishes were taken from the sample site of Onitsha, Illushi, and Illah and30 mg of fin tissue from 18 individuals (6 from each section) was preserved in 95% ethanol in a 1.5 ml tube, after which they were taken to the Ibadan African bioscience laboratory for the extraction of the DNA.

Total DNA Extraction Sample dissociation

For the genetic analysis, 18 samples were randomly selected from each sampling station for DNA extraction. The process of extracting DNA from the fish samples was carried out by dissecting part of fins at a previous preservation workstation in order to maintain a strategic distance from crosscontamination (Nugroho, 2011). Each sample was labeled for easy identification and to avoid confusion during the DNA extraction process. The samples were preserved with 95% ethanol and stored in a freezer at temperatures below -20 degrees. Fish tissue genomic DNA was isolated using the CTAB method, Stewart and Laura (1993). The genomic DNA was extracted using the SYNC extraction kit. Each sample was broken into smaller pieces weighing 0.25 milligrams (mg) using a scalpel and placed in 1.5 mg microcentrifuge tubes that already had appropriate labels for the samples. The were then taken individually from the samples microcentrifuge in a mortar, 200 liters of GST buffer were added to each sample in the mortar and macerated evenly. 20 liters of Protenase K were added to the macerated samples and then returned to their respective microcentrifuge. Vortex for 10 seconds to allow for proper mixing to obtain a homogenous solution, then incubated for 1 hour at 60 ° C.

Cell Lyses

Any insoluble material remaining after the incubation was centrifuged at $15,000 \times g$ for 2 minutes. The supernatant was carefully transferred to a new 1.5 ml microcentrifuge tube, 200 liters of GST buffer were added and vortexed for 10 seconds.

DNA Binding

Here, 200 liters of absolute ethanol were added to the sample and vortexed immediately for 10 seconds. GS column was placed in a collection tube and all of the mixture was transferred to the GS column, centrifuge for 1 minute, then the 2 ml collection tube was discarded with the flow-through. GS column was transferred to a new 2 ml collection tube.

Wash

400 liters of WI buffer was added to the GS column and centrifuged at 15,000 x g for 30 seconds. The flow through was discarded and the GS column was returned to the 2 ml collection tube, washing buffer was added to the GS column and centrifuged again at 15,000 xg for 30 seconds. The process was repeated by returning the GS column to the 2 ml collection tube and centrifuging it again for 3 minutes at 15,000 xg to dry the column matrix.

DNA Elusion

The GS column was then transferred to a dry 1.5 ml microcentrifuge tube, 100 liters of preheated elution buffer was added to the center of the column matrix and allowed to stand for 20 minutes. Centrifugation was performed at 15,000 x g for 30 seconds to remove purified DNA. The ReliaPrepTM Tm binding column was discarded while the genomic DNA was stored at -20 ° C for further analysis. The resulting sample was diluted to 100 ng, while the resulting genomic DNA was stored at temperatures below -20 ° C until further analysis. Test genomic DNA with a nano-droplet to determine the DNA concentration and view it in gel electrophoresis under ultraviolet light with the Gel doc XR system Pc and Mac from the USA to determine the DNA quality. 3.7.0 Screening of primers and PCR amplification. A total of 7 random primers (OPA2, OPA10, OPA7, OPCO2, OPA6, OPA17 and OPA13) were used to determine using the following protocol from Azrita et al (2014); Kusmini et al., (2011). The PCR amplification was screened with the Veriti 96 Thermal Cycler in the African Bioscience Ibadan laboratory, with a total reaction volume of 25 I, containing 50 ng of genomic DNA, 10X PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCL and 0.01% gelatin), 2.5 mM of each dNTP, 5 pmol ; Primer followed by 25 cycles at 940 ° C for 1 min, 40° C for 1 min and 720 ° C for 2 min with a final extension at 720 ° C for 10 min.

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Agarose gel electrophoresis and visualization of bands

The agarose gel was prepared by mixing $1 \times \text{TBE}$ buffer in a ratio of 1.1 (1%). A total of 5 1 l GelRedTM (Nucleic Acid Gel Stain). The hardened gel was then placed in such a way that indentations are formed. The PCR products were loaded

into the wells. The molecular weight of each band was estimated using a standard marker. The PCR products are viewed as Gel doc XR System Pc and Mac from the USA.

Statistical Analysis

The morphometric data obtained were analyzed using SPSS (version 26). The independent variables (Y) were grouped as sampling stations (Onitsha, Illushi and Illah), while the fish morphology (morphometric) were grouped as dependent variables (X). The molecular data were also analyzed using SPSS, Darwin 6.01 and NTSYS PC (2.01) software. The RAPD band pattern was used to visualize and score from photographs. For this purpose only district and well separated bands was selected for the comparative analysis. In the molecular analysis, the patterns of the generated band were converted into numerical values, whereby the presence of a band was rated 1 and the absence of a band was rated 0. The binary data were transmitted for analysis using UPGMA. Cluster analysis was performed by the unweighted pair group method using arithmetic means (Sneath and Sokal, 1973). The dendrograms were created with NTSYS PC software in order to obtain the similarity coefficient between the genotypes (Rohlf, 1993). Canonical correlation was obtained using SPSS, which was used to determine the correlation between the sample station (Onitsha, Illah and Illushi). On the other hand, the Darwin 6.01 software was used to draw the Darwin hierarchy and the Darwin radii to see if samples in the three locations (Onitsha, Illah, and Illushi) are related.

RESULTS AND DISCUSSION

Result of Morphometric Analysis

The table 1 shows that head length of silver catfish sampled in Illah is statistically significant to that in Illushi, but differs from the Onitsha variety, which has an F value of 3.326 and a P value of 0.039 and a standard deviation of 0.1479902 as shown in Table 4.4. Similarly, the snout length of the fish sampled in Onitsha differed significantly from those in Illah and Illushi, although they had an F-value of 3.837 and a Pvalue of 0.024 with a standard deviation of 0.0125637 however, the length of the dorsal fins sampled in Illushi is statistically significant to Illah, but different from Onitsha with the F-value of 4.164, P-value of 0.017 with a standard deviation of 0..0145544. The anal fin length of Illushi and Illah is statistically significant but is different from Onitsha, which has an F-value of 4.719 and a P-value of 0.010. Illushi and Illah are also statistically significant with respect to the pectoral fin, but differ from Onitsha with the F-value of 3.099, the P-value of 0.48 and the standard deviation of 0.0122627. The length of the pre-caudal fin of the specimens at the Illah and Illushi point is statistically significant, but differs from Onitsha, which has an F-value of 3.230 and a P-value of 0.042, similarly, the lower jaw length in Onitsha is significantly different from Illushi and Illush, even though they are F. has a value of 3.730 and a P-value of 0.026 with a standard deviation of 0.0192956, however the sample of the length of the pre-dorsal fin in Illushi is statistically significant for Illah, but differs from Onitsha with an F-value of 3.843, a P. Value of 0.024 and a standard deviation of 0.017662.

Parameters		Illah (IL)	Illushi	Onitsha	Std.	Std. Error	F value	P value
(cm)			(SH)	(ON)	Deviation			
Total Length		1.2930	1.2824	1.3514	0.1782478	0.0145539	2.204	0.114
Pelvic	Fin	0.4448	0.3986	0.4769	0.2131796	0.0174060	1.725	0.182
Length								
Head Length		0.8945 ^a	0.9038 ^a	0.9638 ^b	0.1479902	0.0120833	3.326	0.039
Body Length	1	0.5803	0.5826	0.6273	0.1162875	0.0094948	2.654	0.074
Snout Length	1	0.4103 ^b	0.4112 ^b	0.4832 ^a	0.1538727	0.0125637	3.837	0.024
Dorsal	Fin	0.5353 ^b	0.5466 ^b	0.6425 ^a	0.1782545	0.0145544	4.164	0.017
Length								
Anal Fin Length		0.7648 ^b	0.7699 ^b	0.8425 ^a	0.1546949	0.0126308	4.719	0.010
Pectoral	Fin	0.3681 ^b	0.3602 ^b	0.4277 ^a	0.1501870	0.0122627	3.099	0.048
Length								
Caudal	Fin	0.4019	0.3967	0.4608	0.1574591	0.0128565	2.612	0.077
Length								
Pre Caudal	Fin	0.6802 ^b	0.6787 ^b	0.7625 ^a	0.1913995	0.0156277	3.230	0.042
Length								
Lower	Jaw	0.1163 ^b	0.0988 ^b	0.2163 ^a	0.2363215	0.0192956	3.730	0.026
Length								
Standard Ler	ıgth	-0.1769	-0.2412	-0.1299	0.2805856	0.0229097	2.007	0.138
Pre Dorsal	Fin	0.5353 ^b	0.5466 ^b	0.6425 ^a	0.216309	0.017662	3.843	0.024
Length								

Table 1. Mornhometric relationship between measured predictors

Means without superscript on the same row indicates no statistical difference by the Duncan test $p \le 0.05$ for the physical character/features.

Results of Genetic Analysis

The dendrogram, is shown in Figure 2 reflects the clustering observed by the three sampling stations. The ON1 and ON4; IL2 and IL6, SH2 and SH5 are most similar because the height of the link that connects them is the smallest. Similarly, clusters ON2 and ON5, ON3 and ON6, are similar from their observed height. The smaller the height, the more strongly the cluster correlates. The dendogram also shows the hierarchical relationship between the samples. The dendogram shows that SH2 and SH5 are related to the clusters IL1 and IL4 and form a main correlation cluster. In addition, the sample IL3 forms a cluster with the clusters IL2 and IL6. This also forms another ancestor cluster with cluster samples from SH1 and SH4 and it also show that the samples SH3 and SH6 are closely connected and form another main cluster with the clusters ON3 and ON6. Figure 3 shows that all of these closely related clusters come from the same family tree or from the same ancestors. The result of the silver catfish sampled in Onitsha, Illah and Illushi shows an overall diversity of 0.58 to 0.98. These values were calculated using Nei's genetic similarity matrix of data scoring for the electrophoresis gel. Similarity coefficient and Darwin trees



ON1-ON6- Silver catfish in Onitsha IL1-IL6 - Silver Catfish in Illah SH1 - SH6 - Silver Catfish in Illushi

presence of a band was rated 1 and the absence of a band was rated 0, of the six RAPD markers used (OPA2, OPA10,

Table 2 showing table scoring from electrophoresis gel. The OPCO2, OPA6, OPA17 and OPA13), five were informative on agarose gel with 54% polymorphisms.

Differential function analyzes of Onitsha, Illushi and Illha were analyzed for the eigen values, and the wilks-lambda vaue was shown in Table 4.3, which were carried out to know whether the populations were of the same base species or not. The value recorded between Illushi and Illah from root 1 and 2 are: Eigen values; 4.25880 and 1.52694, canonical correlation; 0.8991 and 0.77734 with percentage variance of

80 and 60 respectively and that of Onitsha and Illah are: Eigen values; 1.66542 and 0.72904, canonical correlation; 0.79046 and 0.64934 with percentage variance of 63 and 42 respectively while the recorded values between Onitsha and Illushi from root 1 and 2 are: Eigen values; 1.39803 and 0.93665, canonical correlation value; 0.76354 and 0.69549 with percentage variation of 58 and 48 respectively.

Table 2: Eigen	Values and	Canonical	Correlations	between s	sample	stations

Deet	Illah and Illushi									
ROOL	EigenValues	Canon Cor.	% of variance							
1	4.25880	0.8991	80%							
2	1.52694	0.77734	60%							
		Onitsha and Il	lah							
	EigenValues	Canon Cor.	% of variance.							
1	1.66542	0.79046	63%							
2	0.72904	0.64934	42%							
		Onitsha and ill	ushi							
	EigenValues	Canon Cor.	% variation							
1	1.39803	0.76354	58%							
2	0.93665	0.69545	48%							



Figure 5: Darwin's hierarchy

Fig 5 shows that all samples from Onitsha, Illushi and Illah alongs River Niger have a common ancestry. That is, all samples are connected to one node.



Figure 3: Darwin's radii graph

Fig. 3 shows that all samples from Onitsha, Illushi, and Illah radiates from a common node, indicating that all samples have the same ancestry regardless of their individual differences in relation.

The predicted value shows how related the predictors (body part) are from table 3. The original value recorded at Onitsha is at 31%, although Illah and Illushi recorded 24% and 22%

respectively, to predict the value of 62.0 recorded at Onitsha, 48 recorded at Illah and 44 recorded at Illushi A total of 40% is recorded as the original group cases what where correctly predicted. This indicate the success rate in the population allocation of sampled fish at the sampled locations (Onitsha, Illah and Illushi).

	Physical	Onitsha (ON)	Illah (IL)	Ilushi (SH)	Total
	character/features				
Original Value	Onitsha (ON)	31	12	7	50
	Illah (IL)	24	9	17	50
	Ilushi (SH)	22	8	20	50
Predicted Value	Onitsha (ON)	62.0	24.0	14.0	100
	Illah (IL)	48.0	18.0	34.0	100
	Ilushi (SH)	44.0	16.0	40.0	100

40.0% of original grouped cases correctly classified

In table 5 Dorsal fin length recorded the highest value in function 1 while in function 2 Pectoral fin length. Dorsal fin length and head length shows significant different in function

1 while in function 2 only dorsal fin length and pectoral fin length show no significant difference

Table 4: Structure matrix of predictors

S/N	Physical characters/features	Function 1	Function 2	
1	Dorsal fin length	0.749*	0.662	
2	Head length	0.230^{*}	0.180	
3	Pectoral fin length	-0.222	0.975^{*}	
4	Standard length	0.319	0.699*	
5	Total length	0.340	0.692^{*}	
6	Caudal fin length	0.254	0.665^{*}	
7	Body length	0.045	0.608^{*}	
8	Pre-Dorsal fin length	0.038	0.498^{*}	
9	Pelvic length	0.354	0.483*	
10	Anal fin length	0.139	0.471^{*}	
11	Snout length	0.333	0.470^{*}	
12	Lower Jaw length	0.117	0.449^{*}	
13	Pre-Pectoral fin length	0.250	0.374*	
14	Pre-Dorsal length	0.138	0.333*	
15	Pre-Anal length	0.000	0.219*	

Physical character/features with asterisk values indicates significant difference

In Table 4 above, the dorsal fin length recorded the highest value in function 1, while in function 2 pectoral fin length,

standard length, total length, caudal fin length and body length were recorded at 0.975, 0.699, 0.692, 00.665 and 0.608 in the structural matrix, respectively. Similarly, the canonical discrimination function of the reliable variables

Table 5: Data scoring for electrophoresis gel

	ON 2	ON 2	ON 4	ON 5	ON		IL 2		IL 4	IL 5	IL ć	SH	SH	SH	SH	SH	SH
<u> </u>	<u>2</u> 1	<u> </u>	4	<u>5</u> 1	0 1	0	<u>2</u> 1	<u> </u>	<u>4</u> 0	<u> </u>	0 1	1 1	<u>2</u> 1	<u> </u>	4	<u>5</u> 1	<u> </u>
0	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	1	1	0	1	1	1	0	0	1	0	0
0	0	0	0	0	1	0	1	1	0	1	1	1	0	1	1	0	1
1	1	1	1	1	1	0	1	1	0	1	1	1	0	1	1	0	1
1	1	1	1	1	1	0	1	1	0	1	1	1	0	1	1	0	1
1	1	1	1	1	1	0	1	1	0	1	1	1	0	1	1	0	1
1	1	1	1	1	1	0	1	1	0	1	1	1	0	1	1	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0
0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1
1	1	1	1	1	1	0	0	0	1	1	0	1	1	0	1	0	0
1	1	0	1	1	0	1	0	1	1	0	0	0	0	0	0	0	0
1	1	0	1	1	0	1	0	1	1	0	0	0	0	0	0	0	0
1	1	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0
0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1
0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1
0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1
1	1	0	1	1	0	0	0	0	0	0	0	1	0	1	1	0	1
1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
0	0	1	0	0	1	1	0	0	1	1	0	0	1	0	0	1	0
0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1
0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	1	0	1	1	1	1	0	1	1	0
0	0	0	0	0	0	0	1	1	0	1	1	1	0	0	1	0	0
0	1	1	0	1	1	0	1	1	0	1	1	0	1	0	0	1	0
1	1	1	1	1	1	0	0	0	0	0	0	1	0	0	1	0	0
0	1	0	0	1	0	0	1	1	0	1	1	1	1	0	1	1	0
0	0	0	0	1	0	0	1	1	0	1	1	1	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5 shows the correlated value between individual sample from ON1 - ON6, IL1 - IL6 and SH1 - SH6. All of the above individual samples are highly correlated and no negative correlation was observed. The lowest correlated value exists between IL4 and SH4 with a value of 0.3864 and the highest correlated value exists between ON1 and ON4 with a value of 0.9773.

Discussion

Morphometric and Morphology Structures

Of the 15 morphometric characters examined, 9 showed no significant differences (p > 0.05) between species of Illah and Illushi, while the population of Illah and Illushi showed significant differences (p < 0.05) from that of Onitsha among the population of C nigrodigitatus as shown in Table 1. The causes of morphological differences between species are often quite difficult to explain (Poulet et al., 2004), but it is known that morphometric traits can exhibit a high degree of phenotypic plasticity in response to different environmental factors and food abundance (Murta, 2000; Banerjee et al 2017; Hossain et al 2010). This claim was collaborating with the report by Neelam et al. (2013) on tilapia from two different ponds show variations in their extreme environments. This also agrees with the observation of Ramler et al. (2014) who concluded that variation is possible based on the strain sampled in the same body of water.

SPSS analysis was used to identify parameters that contribute strongly to the observed variation and are therefore useful to differentiate populations. Researchers like Amin *et al.* (2010) examined variations that might occur in three species of the genus Acetes. Similarly, Azua *et al.* (2017) Differences in the morphometric measurement of two tilapia fish in relation to their body weight. However, this study examined several features that facilitated a morphometric distinction between specimens located in the head region, such as: B. Snout length, head length. However, parameters from other body parts were also important and included: predorsal fin length, anal fin length, mandible length. Such traits would be useful when selecting for breeding.

Turan et al. (2005) observed that morphological features distinguishing Clarias gariepinus populations mainly arose from head measurements. Significant differences (p>0.05) in dorsal fin length and head length of C. nigrodigitatus along the Niger River, as shown in Table 3 and Table 4, could be used as a means of identifying C. nigrodigitatus. This is related to the work of Portillo et al. (2017) on the most important predictor that could be used in population mapping of Glossogobiuscelebius population attribution of the sampled fish, since morphological characteristics have been commonly used to identify fish stocks and study variations between fish populations (Turan et al. 2005). The use of morphometric characters in taxonomy, especially for species with extremely similar external characters and high phenotypic plasticity, could hamper the discovery and identification of cryptic intraspecific diversity (Bingpeng et al. 2018; Conte-Grand et al., 2017 and Ward et al., 2005), while morphometric taxonomy might potentially overlook diversity, molecular markers with genetic identification have been suggested as a complementary taxonomic tool for species identification and deciphering cryptic diversity (Remigio et al., 2003).

Turan and Karan (2019) reported the use of mtDNA to determine the molecular structure of turbot. Random amplify polymorphic DNA (RAPD) primers as molecular markers were used to characterize the genetic variation of silver catfish from the sampling station along the Niger River (Onitsha,

Illah and Illushi) congruently to Sousa et al. (2011) that genetic variability in a population is important for biodiversity since without variability it becomes difficult for a population to adapt to environmental changes and therefore makes them more vulnerable to extinction. This study, as shown in Table 2, shows a high correlation between sampling stations and a high percentage of variability, which contradicts the studies by Prado et al. (2018) who conclude that the Scophthalmus maximum was reached in the Atlantic, Mediterranean, Black Sea and Baltic Sea with a genetic distance of 0.090 between sampling stations. From the study, Figure 2, and Figure 3, show that species originate from the same root, which is consistent with the study by Agense et al. (1997) that the distribution of species or populations and their genetic structure depends not only on biological and environmental factors, but also on historical factors

CONCLUSION

The study examined the variability that might occur when sampling silver catfish at different stations in Onitsha, Illah and Illushi. When evaluating using morphometric measurement (body distances) and molecular analysis using the RAPD primer. Morphometric distances: pectoral fin length, standard length, overall length, tail length and body length could be used to identify the location where the test fish were sampled. This suggests that these measured distances could be used to identify the tribes from the sampled sites of Onitsha, Illah, and Ilush. Similarities in the sample strain were observed at the three stations. However, Illushi and Illah showed the greatest genetic proximity. The Random Amplification of Polymorphic DNA (RAPD) primer provides the genetic distances, the hierarchy based on relatedness, the graphical dendogram and data suggesting that the fish studied descended from a common ancestor. From the research study, breeding between Illushi and Illah could produce good species of fish as according to the research, both species have high variation and are highly correlated.. Primer OPA13 did not indicate polymorphism for Silver catfish could be used only as a means of identification. Dorsal fin length and head length for Silver catfish and could be used as a means of identification. Silver catfish obtained from Illah and Illushi has similar traits future studies for stock improvement in line breeding could be carried out based on the discovery and Silver catfish could be classified genetically based on location

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