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MORPHOMETRIC CHARACTERIZATION OF AFRICAN BONYTONGUE (*Heterotis niloticus* Cuvier, 1829) FOUND IN THE LOWER RIVER NIGER OF NIGERIA

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Abstract: This study was conducted to characterize the African bonytongue (*Heterotis niloticus*) found in the lower River Niger of Nigeria at the following locations; Onitsha (6.1329° N, 6.7924°E), Illah (6.4219° N, 6.6491°E) and Ilushi (6.6688° N, 6.6304°E) with the use of morphometric (measured distance) and random amplified polymorphic DNA (RAPD). A total of one hundred and fifty (150) samples were used for the study. Morphometric distances of body parts were subjected to the Principal Component Analysis on Unscramble@ X version 10.4 software. DNA samples were analyzed using muscular portion of the caudal region. Genomic molecular analysis was carried out for the RAPD band pattern. Denodogram was constructed using clustering analysis (UPGMA). PCR was confirmed using gel electrophoreses. Result shows that the Anal fin length at Illah point is statistically significant to Illushi but different from Onitsha, with F value of 3.326 and the P value of 0.039 and standard deviation of 0.1479, snout length of fish sampled at Onitsha is significantly different from those sampled in Illah and Illushi, having F value of 3.837 and P value of 0.024 and standard deviation of 0.0125, Dorsal fin length sampled in Illushi is statistically significant to Illah but different from Onitsha having F value of 4.164, P value 0.017 with standard deviation of 0.0145. A total of 46% of original grouped cases were correctly classified, while 45.3% of cross-validated grouped cases were also classified with overall diversity of 0.67 to 1.00. This gives respective similarity coefficients of 0.45, 0.37 and 0.31.

Keywords: Morphometric, Characterization, Electrophoreses, Molecular, Clustering

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1. Introduction

The African bonytongue (*Heterotis niloticus*) are primarily freshwater fish, they are commonly found in tropical rivers and freshwater lakes in western and central Africa (Leveque et al., 1990). They are characterized by certain distinct feature such as short head with terminal mouth and thick lips, large sensory pith, lateral line extending from operculum to the middle of the caudal peduncle, spineless dorsal and anal fins, a small rounded tail which is relative to body size, lastly the fish possesses scales of a cycloid appearance on the body except the head (Reed et al., 1967; Idodo–Umeh, 2003). The fish can be cultured commercially and readily accepts compounded feeds coupled with its exceptional growth it is of economic interest in inland fisheries (Moreau, 1982; Adite et al., 2006; Odo et al., 2009; Ezekiel and Abowei, 2013). Investigations have been carried out on H. *niloticus* in River Niger at the Pategi area of Kwara State by Adekeye (1989) with the view of establishing its potential in commercial aquaculture as well as providing a basis for its take off.

Studies done by Hockaday et al. (2000) and Nwachi and Irabor (2022) infer that morphometric methods are simple and direct method of assigning species to their population and also could be used for actual species identification. The measuring of body distances morphometric remain a significant method that is used in species differentiation. Hassanien et al. (2011) is of the opinion that morphometric traits are an important trait that can have invariable affects in reproduction, similarly estimate of morphology and genetic variability is essential in other to predict response to selection, during breeding plans and predict breeding values. It is of note that external morphology (body shape) might also serve as economic importance and marketing point of view. Although the extent placed on the use of morphometric traits to determine both the genetic and environmental variation remain poorly understood.

Schwanck and Rana (1996), is of the opinion that mode of inheritance or morphology will contribute positively to the express traits similarly, Gunawickrama (2007), Hassanien et al. (2011), Edema and Osagiede (2011), Kuton and Adeniyi (2014), infer that morphometric could be used in proper identification. However, fish taxonomists used morphology to classify the various types of fish into their respective genre, families and

species, i.e. they made use of more or less morphological features and characteristics of fishes as tools to identify fishes for their systematic identification, hence such information with respect to fish taxonomic identifications were accumulated. In a similar manner molecular analysis with the aid of researched or optimized primer could also serve as an important tool in understanding traits of strains. Normala et al. (2021) reported the use of Random Amplified Polymorphic DNA Technique (RAPD) in evaluating the traits that could be used in separating triploid from diploid African catfish (*Clarias gariepinus*). Bakar et al. (2018), opined on the use of molecular method in managing wild fish especially those with little or no morphological variations due to technological advancements in the field of genetics, the use of DNA based markers have increasingly being used for identification and unrevealing previously unknown genetic variations in fish species (Isabel et al., 1999). It has been advocated that the RAPD-PCR technique is a simple and straightforward method where the work is based on the amplification of discrete regions of genome by using arbitrary primers to evaluate genetic variability and structure of a variety of species Almeida et al. (2001), Leuzzi et al. (2004) and Matoso et al. (2004). Molecular markers could also be applied for use in identifying different types of populations of fish such as species, hybrid identification, phylogeny and many others (Almeida and Sodre, 2002). Other workers have also used Random Amplified Polymorphic DNA (RAPD) technique for their investigations such as Welsh and McClelland (1990), Hassanien et al. (2004) and Toth et al. (2005). African bonytongue (*Heterotis niloticus*) is an economic fish found in River Niger, recent observation shows an increasing interest for domestication and mass production of fish in controlled environment and fishes from the wild is the major source of most genetic improvement program. To domesticate this fish proper identification is required using both the morphometric and molecular analysis.

2. Materials and Methods

This study was carried out at the teaching and research laboratory of Delta State University Abraka and African Bioscience Laboratory Ibadan Oyo state Nigeria. The fish species used for this research where obtained from three different locations along the river Niger; the stations were marked as A (Onitsha) 6.1329° N, 6.7924° E, B (Illah) 6.4219° N, 6.6491° E and C (Ilushi) 6.6688° N, 6.6304° E as shown on the map in Figures 1 and 2. River Niger is regarded as the principal river of West Africa extending about 4,180 km (2,600 mi).

Figure 1. Map of Nigeria.

Figure 2. Sampling stations of Onitsha, Illushi and Illah.

Its drainage basin is 2,117,700 km² (817,600 sq mi) in area. The river Niger originates from Guinea highlands in the southeastern Guinea. It runs in a crescent through Mali, Niger, on the border with Benin and then through Nigeria, discharging through a massive delta, known as the Niger Delta into the Gulf of Guinea in the Atlantic Ocean (Gleick, 2000).

The water flowing in the river is relatively clear with little silt compared to the river Nile. The river Niger just like the Nile River floods yearly; this begins at September, peaks in November, and finishes by May. During the period of flood, the water is not as clear due to debris washed into the river by runoff water from rains and drainages. The gradient of the river decreases drastically and forms the Inner Niger Delta, the decrease gradient causes the flow of the river to slow down resulting in numerous marches, lakes, and streams. In this area about two thirds of the rivers potential flow is lost, leaking into lakes and marches. When the region floods, during the rainy season, excellent conditions for fishing and farming are created.

The River Niger is important in the livelihood of locals such as Igbuzo, Okpanam, Oko, Okwe, Ugbolu, Illah Imbaka, Odegune, and Umudora Anam. Aside domestic importance which include fishing, irrigation, transportation and agriculture, it is also used for hydropower generation in the Kainji and Jebba dams.

Samples were collected at stations A, B and C as shown on the map, Figure 1 and 2. A total of 25 samples were collected from each station on sampling days totaling 75 samples. Accurate morphometric measurements were done using buss truss protocols as presented in Table 1.

Table 1. Measured distances (Morphometric distances)

Parameters	Distance
Total length (TL)	Distance between the tip of the snout (upper jaw) to the tip of the tail.
Standard length (SL)	Distance between the tip of the snout (upper jaw) to the tip of the anal fin.
Pelvic fin length (PL)	Measured distance from the base to the tip of the pelvic fin.
Head length (HL)	Distance between the tip of the snout to the upper operculum.
Body distance (BD)	Distance between the base of the dorsal fin to the pectoral fin.
Snout length (SL)	Distance between the tip of the upper jaw to the base of the eyes
Pre-dorsal fin length (Pre-DL)	Distance between the tip of the snout to the origin of the dorsal fin.
Dorsal fin length (DL)	Distance between the base of the first dorsal to the last dorsal fin.
Anal spine length (ASL)	Distance from the top of the anal fin to the last of the anal fin ray.
Pectoral fin length (PFL)	Distance from the tip of the pectoral to the base caudal pundicle
Pre-pectoral length (Pre-PL)	Distance from the front of the pectoral base to the snout.

2.1. Total DNA Extraction

Extracting DNA from the fish samples was done by dissecting part of the strong muscular portion of the caudal region, at an earlier conservation workstation to maintain a strategic distance from cross contamination (Nugroho, 2011). Each sample was tagged for easy identification and also to avoid mix up during the DNA extraction process. Preservation of the samples was done using 95% ethanol and stored in temperatures below -20 degree freezer. The genomic DNA of fish tissues was isolated using CTAB method (Stewart and Laura, 1993). Genomic DNA was extracted utilizing (gSYNC) extraction kit. Each sample was dissected into smaller pieces of weight 0.25 mg using a scalpel and placed into a 1.5 mg micro centrifuge tubes which already had corresponding tags of the samples. The samples were then individually removed from the microcentrifuge into mortars, 200 µl of GST Buffer was added to each sample in the mortar and macerated evenly. 20 µl of proteinase K was added to the macerated samples and then returned to their respective microcentrifuge. Vortex for 10 seconds to allow proper mixing to yield a homologous solution and then incubated at 60 °C for 1 hour. Insoluble material which remained after incubation was centrifuged for 2 min at 15000xg. Carefully the supernatant was transferred to a new 1.5 ml microcentrifuge tube 200 µl of GST Buffer was added and vortexed for 10 sec. Here 200 µl of absolute ethanol was added to the sample and vortexed immediately for 10 sec. Placing a GS column in a collecting tube and transfer all the mixture to the GS column, centrifuge for 1mimute then discard the 2 ml collecting tube containing the flow through. Transfer the GS column to a new 2 ml collecting tube. A total of 400 µl of WI buffer was added to the GS column and centrifuge for 30 sec at 15000xg. The flow through was discarded and placing the GS column back in the 2ml collecting tube, wash buffer was added to the GS column and centrifuged again for 30 sec at 15000xg. The process was repeated by placing the GS column back in the 2 ml collecting tube and again centrifuging for 3 min at 15000xg to dry the column matrix. The GS column was then transferred to a dry 1.5 ml microcentrifuge tube, 100µl of pre heated elution buffer was added into the center of the column matrix and left to stand for 20 min. Centrifuge was done at 15000xg for 30 sec to elude purified DNA. The ReliaPrepTM ™ Binding column was disposed while the genomic DNA was stored in -20 °C for further analysis. The resulting sample was diluted to 100 ng while the resulting genomic DNA was stored in temperatures below -20 °C until further analysis. Test Genomic DNA using a nanodrop to ascertain DNA concentration and viewed in gel electrophoresis under ultraviolet light using Gel doc XR system Pc and Mac form USA to ascertained DNA quality.

2.2. Screening of Primers and PCR Amplification

A total of 15 arbitrary primers of OPA series (Operon Technologies Ltd. USA) with random sequence was used to screen using the following protocol of Azrita et al. (2014) and Kusmini et al. (2011). The PCR amplication was screened using Veriti 96 thermal cycler at the laboratory of African Bioscience Ibadan. At a total reaction volume of 25 µI containing 50 ng 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; of primer followed by 25 cycles at 94 °C, for 1 min, 40 °C for 1min and 72 °C for 2min with a final extension at 72 °C for 10 min.

2.3. Agarose Gel Electrophoresis and Visualization of Bands

Preparation of the agarose gel will be carried out by mixing 1 xTBE buffer at a ratio of 1.1 (1%). A total of 5µl of µl of GelRed™ (Nucleic Acid Gel Stain). The harden gel will be placed in such a way that wells will be created. The PCR products will be load inside the wells. The molecular weight of each band will be estimated using a standard marker. The PCR products will be viewed Gel doc XR system Pc and Mac form USA.

2.4. Statistical Analysis

Body part values was analyzed using multivariate, specifically Principal Component Analysis (PCA). PCA was chosen due to its ability to handle high-dimensional data, quantify differences among observed components, and reveal innate relationships between data points. The Unscramble@ X version 10.4 software aided in this process. To eliminate variation resulting from allometric growth, the researchers standardized and normalized the

morphology data. Next, molecular analysis was conducted using RAPD (Random Amplified Polymorphic DNA) band patterns from sample test fish across various stations. The most prominent primer was used for molecular characterization of the *H. niloticus* population in the sample station. The RAPD band patterns were visualized and scored from photographs. Only distinct and well-separated bands were selected for comparative analysis. Gene assignment was determined by scoring "1" for presence and "0" for absence, excluding weak and unresolved bands. Finally, a dendrogram was constructed using genetic distance values through clustering analysis (UPGMA) to reveal the relationship between different *H. niloticus* populations.

3. Results

The Anal fin length of *H. niloticus* that was sampled in rable 2 at Illah point is statistically significant to Illushi but different from Onitsha, which has the F value of 3.326 and the P value of 0.039 and standard deviation of 0.1479902, similarly the snout length of fish sampled at Onitsha is significantly different from those sampled in Illah and Illushi, although it has F value of 3.837 and P value of 0.024 and standard deviation of 0.0125637, however dorsal fin length sampled in Illushi is statistically significant to Illah but different from Onitsha and the F value is 4.164, the P value is 0.017 with standard deviation of 0.0145544 the anal fin length of Illushi and Illah is statistically significant but different from Onitsha, which has the F value of 4.719 and P value of 0.010. Illushi and Illah are also statistically significant in terms of pectoral fin but different from Onitsha with the F value of 3.099, P value of 0.48 and standard deviation of 0.0122627.

Table 2. Morphometric characteristics of the Predictors

Parameters	Onitsha (NI)	Illah (LA)	Illushi (IL)	Std. deviation	Std. error	F value	P value
Total length	1.4559	1.4700	1.4775	0.0425	0.0035	1.650	0.195
Pelvic length	0.0303	0.0873	0.0255	0.2481	0.0201	0.967	0.383
Head length	0.6725	0.5993	0.6725	0.3427	0.0278	0.904	0.407
Body length	0.7520	0.7573	0.7684	0.1869	0.0152	0.102	0.903
Snout length	0.1652	0.1511	0.1698	0.2056	0.0167	0.112	0.894
Dorsal fin length	0.0645[b]	0.1640[a]	0.0853[b]	0.1579	0.0128	5.904	0.003
Anal fin length	0.0137[b]	0.0476[a]	0.0084[a]	0.2590	0.0210	0.337	0.714
Pectoral fin length	0.3995	0.3755	0.3114	0.1941	0.0157	2.889	0.059
Caudal fin length	0.2862	0.2822	0.2736	0.1408	0.0114	0.106	0.899
Lower jaw length	1.1791[a,b]	0.2015[a]	0.0421[b]	0.2803	0.0227	4.365	0.014
Standard length	1.4191[a,b]	1.4360[a]	1.4129[b]	0.0448	0.0036	3.746	0.026
Pre-dorsal length	1.2309	1.2286	1.2208	0.0738	0.0060	0.262	0.770
Pre-anal length	1.1399	1.1928	1.1775	0.1328	0.0108	2.136	0.122
Pre--pectoral fin length	0.3521[a,b]	0.3706[a]	0.2660[b]	0.1966	0.0159	4.318	0.015
Pre-dorsal fin length	0.7043	0.7288	0.7405	0.1231	0.0010	1.140	0.323

Means without superscript on the same row indicates no statistical difference by the Duncan test P≤0.05 for the water quality parameter.

The pre-caudal fin length of the samples at Illah and Illushi point is statistically significant but different from Onitsha which has the F value of 3.230 and P value of 0.042, similarly the lower jaw length at Onitsha is significantly different from Illushi and Illah although it has F value of 3.730 and P value of 0.026 and standard deviation of 0.0192956, however, pre-dorsal fin length sample at Illushi is statistically significant to Illah but different from Onitsha with F value of 3.843, P value of 0.024 and standard deviation of 0.017662.

Table 3. Structure matrix

Parameters	Functions	
	1	2
Dorsal fin length	0.721*	0.693
Head length	0.222*	0.187
Pectoral length	0.255	0.967*
Standard length	0.284	0.710*
Total length	0.309	0.704*
Caudal fin length	0.220	0.671*
Body length	0.024	0.208*
Pelvic fin length	0.327	0.493*
Snout length	0.320	0.480*
Anal fin length	0.131	0.473*
Lower jaw length	0.106	0.453*
Pre-dorsal fin length	0.226	0.379*
Pre-anal fin length	0.123	0.338*

In Table 3 the structure matrix indicates the loadings at two functions; dorsal fin length loaded at 0.721 and 0.693, the head length load at 0.222 and 0.187 respectively. The caudal fin length is at 0.220 and 0.671 while the snout length loads at 0.320 and 0.493. In the function evaluated the pre-dorsal fin length was at 0.226 and 0.379, total length at 0.309 and 0.704 although the pre-anal fin length is at 0.226 and 0.379. The standard length recorded a loading of 0.284 and 0.710. A total of 46% of original grouped cases were correctly classified, the cross-validation is done only for those cases in the analysis, in cross-validation each case is classified by the functions derived from all cases other than that case. A total of 45.3% of cross-validated grouped cases were correctly classified in Table 4.

Table 4. Predicted group membership

	Site	SHA	LAH	ILU	TOTAL
	SHA	21	17	12	50
Original count	LAH	16	23	11	50
	ILU	8	17	25	50
	SHA	42	34	24	100
%	LAH	32	46	22	100
	ILU	16	34	50	100
	SHA	21	46	12	50
Cross-validated count	LAH	17	34	11	50
	ILU	8	17	25	50
	SHA	42	34	24	100
%	LAH	34	44	22	100
	ILU	16	34	50	100

Function analyses of predictors from the sampling station of Illah and Illushi, for the Eigen values and the Wilk's Lamda at Table 5, that was carried out to evaluate the origin of the sampled population showed no significant difference. In Table 6 while the Wilk's Lamba diversity within Illah and Illushi was at 0.27303 with significance value of 0,009 at 0.94 degree of variation. The differential function between Onitsha and Illah infer that the Eigenvalues and Wilk's Lamba diversity gives a value of 29958, while on analysis between Onitsha and Illushi a value of .35622 with significance value of .107 at 0.94 degree of variation in Table 7. The Correlations of predictors loading indicate that head length load negatively (-077) likewise body length (-022) on the plot and could not be used for proper identification of strain. However, anal fin length, Pectoral fin length and lower jaw length has a positive loading of 034, 027 and 032 respectively on Figure 3. It is of note that in Figure 4 the result of the *H. niloticus* shows overall diversity of 0.67 to 1.00. This gives respective similarity coefficients of 0.45, 0.37 and 0.31. These values were the calculated Nei's genetic similarity matric of the data scoring for the electrophoresis gel.

Table 5. Eigenvalues and canonical correlations for Illah and Illushi

Root	Eigenvalues	Canon. cor.	% of variance
1	1.40784	0.76465	58%
2	0.31352	0.48856	24%

Table 6. Eigenvalues and canonical correlations for Onitsha and Illah

Root	Eigenvalues	Canon. cor.	% of variance
1	1.56352	0.78097	60%
2	0.15303	0.36431	13%

Table 7. Eigenvalues and canonical correlations for Onitsha and illushi

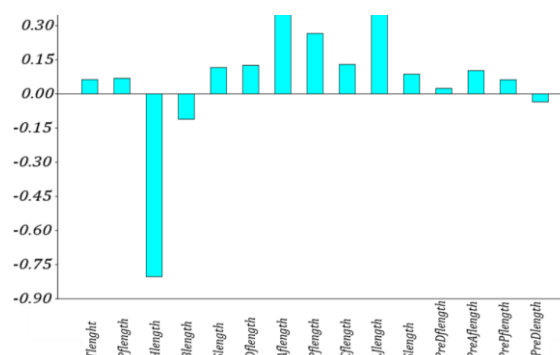| Root	Eigenvalues	Canon. cor.	% variation
1	0.89476	0.68719	47%
2	0.24438	0.44316	19%

Figure 3. Correlations of predictors loading plot. Tlenght= total length; Pflength= pectoral fin length; Hlength= head length; Blength= body length; Slength= snout length; Dflength= dosal fin length; Aflength= anal fin length; Pflength= pectoral fin length; Cflength= caudal fin length; LJlength= lower jaw length; Slength= standard length; PreDflength= pre-dorsal length; PreAflength= pre-Anal fin length; PrePflength= pre-Pectoral fin length; PreDlength= pre-Dorsal fin length.
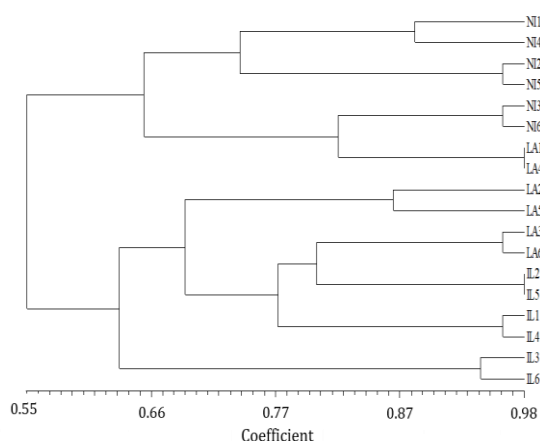
Figure 4. Dendogram for fish samples. NI1-NI3– *H. niloticus* in Onitsha; LA1-LA3 – *H. niloticus* in Illah; IL1 – IL3 – *H. niloticus* in Illushi.
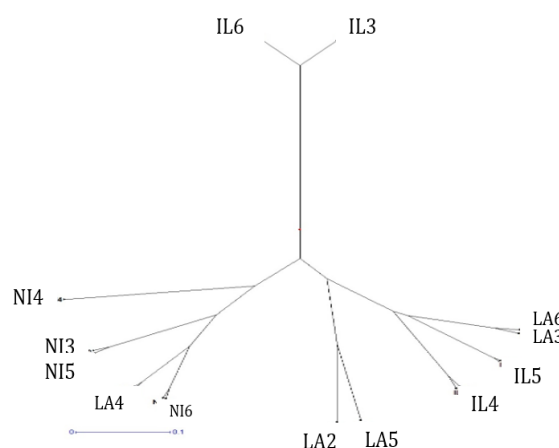

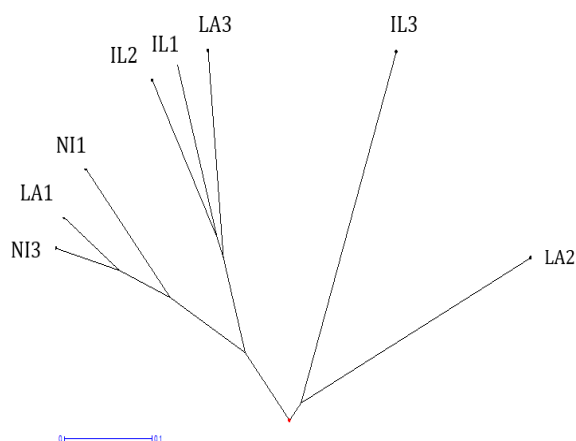**Figure 5.** Darwin's hierarchy of the RAPD analysis of sampling station.

Figure 6. Darwin's radii graph of the sampling stations.

The relationship between the test fish as shown in Figures 5 and 6 Darwin's hierarchy and radii graph infer that all the fish sampled could come from the same family as shown in the graph.

4. Discussion

Differences have been recorded between species and strains of fish because of their ability to respond to environmental changes. Water quality parameters such as temperature and available food could affect both the growth and over all well-being of fish. Morphology is useful in distinguishing differences among fishes of same strain but obtained from different sampling stations. In this study discriminant analysis was used to evaluate differences that could be used in assigning the correct strain to the sample population of *H. niloticus* that is sampled from Onitsha, Illah and Ilushin point of the River Niger.

Observation from studies indicated that *H. niloticus* found in the three locations of (Onitsha, Illah and Illushi) show higher levels of similarities, but samples at Illushi and Illah exhibited the most similarities across the strains. However, in the analyses done to know if the samples were sufficiently different, the Eigenvalues and Wilk's Lambda shows no significant differences at 0.95. This indicated that the genus may be descended from a single ancestor. On further examination of the morphological trait of *H. niloticus* in Table 4 there is variation on the values from measured distances of; head length, snout length, dorsal fin length, anal fin length, pectoral fin length, pre-caudal fin length, lower jaw length and pre-dorsal fin length among fish sampled at Illushi and Illah.

They were also statistically different to samples from Onitsha despite the fact that they were from the same strain. This could be attribu Table to the fact that some of them had the same parent stock, which demonstrates why they are so similar. Senay et al. (2017), evaluated the morphological differentiation in northern pike and found out that elongated heads, deeper bodies and caudal peduncles and dorsal fin could be used for proper identification of same strain from the sampling stations. Hassanien et al. (2011), Pathak et al. (2013), reported that measured distances (morphometric) could be used in actual differentiation of strain. Similarly, total length, carapace length, standard length and abdominal lengths were used by Amin et al. (2010), to evaluate variation among the species of genus acetes.

In the study variation was recorded from the measured distances of the sampled stations, some of the measured distances seem similar to each other while element of dissimilarities also takes place between the sampled stations. Statistical significant were recorded among the sampled stations with variable p value. Wilk's Lamba analysis infers that all the test fish were from same strain although variation was recorded in the sampling stations. In Figure 3 the Correlations loading of predictors in the study could be used to explain the fact that the anal fin length, pectoral fin length and lower jaw length could be used to place the strain to their base population indicating that one can actually make positive identification of strain based on their location by examining these predictors. In general, structure matrix in Table 3 showed the loadings of predictors in two

functions, the dorsal fin length; pectoral length anal fin length and lower jaw length load positively in either function which is an indication the sampled fish could be link to their sampled station with the aid of these predictors. In the study although a total of 46% of the sampled strains were correctly assigned to their population with the aid of the predictors, the cross-validation gives 45.3% indicating that predictors could actually serve as a mean of identification.

Standard characterization and evaluation could be carried out by using different methods including traditional practices for more information (Barletta et al. 2015), hence molecular analysis was carried out for the study with the aid of Random Polymorphic Primers (RAPD) Characterization' is the description of a character or quality of an individual it could also be referred to as the distinguishing factor. In genetic terms, characterization is referred as the detection of variation that could result from differences in modification (Novroski et al., 2015). A total of 15 arbitrary primers of OPA series (Operon Technology LtD USA) was used in screening sampled fish from the landing points of Onitsha, Illah and Ilushi. The hierarchical clustering of the sampled fish gives a diversity of 0.67 to 1.00, although similarity coefficients of 0.45, 0.37 and 0.31for Onitsha, Illah and Ilushi were observed

In Onitsha, Illah, and Illushi, genetic diversity among species was minimal, with matrix index of 1. According to Sousa et al. (2020), genetic variability in a population is important for biodiversity because without variability if a population's ability to adapt to environmental changes becomes more complicated, it becomes more vulnerable to extinction. Species in Onitsha shows more variability difference from species in Illah and Illushi. The distribution of species or populations and their genetic structure depends not only on biological and environmental but also on historical factors (Agnèse et al., 1997), these climatic variations could explain the genetic structure of some fish populations in West Africa (Giddelo et al., 2002).

The study discovered a low degree of genetic diversity at the sampled station, analysis among the species found along the Niger River in Onitsha, Illah, and Illushi. In Figures 5 and 6 Darwin's hierarchy indicate the relationship between the sampled station and the strain, the relationship explained the relatedness of the strain if the stations were used as base line. Sousa et al. (2020), opined that genetic diversity in a population is critical for biodiversity because without it, a population's ability to adapt to environmental changes is compromised, making it more vulnerable to extinction. Morphometric and genetic analysis is an efficient and accurate method of evaluating fish populations.

5. Conclusion

The study evaluated the use of morphometric traits (measured distances) and Random Amplified Polymorphic DNA (RAPD) in characterizing African bonytongue fish that was sampled in three stations of Onitsha, Illah and Ilush along the lower region of River Niger. Anal fin length, pectoral fin length and lower jaw length were found to be traits that could be used in assigning the fish to their base population. The uniqueness of sampled fish based on gene analysis was also examined with the aid of polymerized chain reaction and the products evaluated with gel electrophoresis. Results infer that despite the similarities as indicated by Darwin's hierarchy each strain from the sampled location has its own unique traits.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	O.F.N.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

The experimental procedures were approved by the local ethics committee of Faculty of Agriculture Delta State University (approval date: 07 May, 2020, protocol code: S179/037).

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