SUITABILITY OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS FOR IDENTIFICATION OF SOME FISH SPECIES IN THE NIGER-BENUE CONFLUENCE RIVER, NIGERIA

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Abstract: This study assessed the suitability of Random Amplified Polymorphic DNA (RAPD) technique for the characterization of *Oreochromis niloticus*, *Heterobranchus longifilis*, and *Heterotis niloticus* from the Niger-Benue River confluence in Lokoja, Kogi State, Nigeria. RAPD-Polymerase Chain Reaction amplifications reactions were carried out on DNA extracted fromthe caudal fin tissue of the fish species using five primers (OPA-02, OPA-06, OPA-11, OPAE-10 and OPD-14) that produced consistent bands. It was observed that the intensity and number of amplified bands detected varied depending on the primers used and fish samples. A total of seventy clear, distinct and sharp bands were produced by the five primers. Sixty-two out of the seventy bands were polymorphic while the remaining eight bands were monomorphic. These findings reveal the presence of sufficient genetic variability for the discrimination of the fish species. The 100% polymorphism produced by OPA-02 indicated that the primer is the best for discriminating the three fish species in this study. Similarity coefficient range of 0.33 to 0.45 recorded also indicated presence of genetic diversity and therefore supporting the suitability of the technique for fish species characterization in the Confluence River. The dendrogram puts *Oreochromis niloticus* and *Heterotis niloticus* together in the same group while *Heterobranchus longifilis* was separated into a different group. This indicates that *Oreochromis niloticus* and *Heterotis niloticus* are genetically more related compared to *Heterobranchus longifilis.* This study provided a baseline molecular database for species identification and characterization for conservation and management of fish species population in the Confluence River.

Keywords: RAPD, Primer, polymorphic, monomorphic, characterization

Introduction

Fish surveys at the lower Niger basin have reported the presence of *Oreochromis niloticus* as the most abundant fish species in the area with *Heterotis niloticus* recording a moderate occurrence (Solomon *et al.*, 2012; Okomoda *et al.*, 2013; Adeyemi, 2013). *Heterobranchus longifilis* was not reported in the Confluence River at Lokoja in the previous surveys.

Ichthyologists in Kogi State have concentrated more on morphological diversity and abundance (Adeyemi *et al*., 2010; Solomon *et al*. 2012; Okomoda *et al*., 2013), fish gear survey (Adeyemi *et al*., 2009), fish production estimates (Adeyemi (2013) and fish parasite assessment (Iyaji and Eyo, 2009; Adeyemi and Toluhi, 2014; Iyaji and Yaro, 2016). According to Iyiola *et al.* (2017), the use of morphological characters in species identification is common in Nigeria. The challenges of using morphological methods lie in the difficulties of discriminating closely related species (Iyiola *et al.*, 2017). Morphological characterization of fish is useful in identification of fish species (Asagbra *et al.*, 2014) but it does not offer reliable method of species identification.

However, molecular markers are more reliable and suitable in overcoming the morphological and biochemical characterization of fish species because they can detect variation at the DNA level (Nadeem *et al.*, 2018). Ogbuebunu and Awodiran (2017) reported recently that, genetic assessment using molecular markers have become active area of research for estimating functional variations within and across species of fish and other organisms. Information on the molecular structure of fish species is useful for identification of stocks for breeding programs, fish management for sustainable yield and preservation of genetic diversity (Ali *et al*., 2004; Neekhra *et al*., 2014).

Randomized Amplified Polymorphic DNA (RAPD) is the foremost molecular marker used to generate baseline information for assessing genetic diversity and unraveling species identity. The method offers the advantage of no prior knowledge of genetic makeup of the fish species required to commence study (Almeida *et al.*, 2013; Megbowon, 2019). The relevance of RAPD rests on the fact that it produces clear fingerprints from which fish species could be identified (Asensio *et al*., 2002). The Random Amplified Polymorphic DNA approach is rapid, more reliable and offers the potential to detect mislabeled fish samples during routine fish species authentication. Despite the importance of Random Amplified Polymorphic DNA technique for the delimitation of fish species, there is information dearth on the use of the technique for characterization of fish species from rivers in the study area. Against this background, this study was aimed at investigating the suitability of Random Amplified Polymorphic DNA (RAPD) technique for generating species-specific marker in *Oreochromis niloticus, Heterobranchus longifilis,* and *Heterotis niloticus* from the River Basin in Lokoja, Kogi State, Nigeria since the three fish species are the commonly exploited species at this location. The findings in this study will provide reference database for species identification and characterization for conservation and management of these fish species.

Materials and Methods

Study area

The area of study is the Kpata River side of the Niger-Benue Confluence River. The climate of this study area is characterized by wet and dry season while the annual rainfall is between 1016 mm and 1524 mm with the mean annual temperature of 27.7°C (Suleiman *et al*., 2015).

Sample collection

Ten samples each were obtained for *Oreochromis niloticus, Heterotis niloticus* and *Heterobranchus longifilis* from the Lokoja axis of River Niger with the help of local fishermen. The geographical coordinates of Kpata is latitude 7°48' N and longitude 6°46' E. The caudal fins of the samples were removed and stored in sample bottles containing absolute ethanol. The samples were then preserved in ice for transportation to the Laboratory of Molecular Biology and Biotechnology at the Nigerian Institute of Medical Research,

Lagos. At the laboratory, the caudal fin muscle of the ten selected samples for each fish species were ground together and further preserved in ice below -20°C for DNA extraction.

Genomic DNA extraction

The DNA extract was prepared from the caudal fin tissue according to the methods of Ausubel *et al.* (2001), using modified Cetyl Trimethyl Ammonium Bromide (CTAB) method.

A tissue (0.5 g) was homogenized in an eppendorf tube and 600 µl of 2 X CTAB buffer solutions was added. The sample was incubated at 65^0C for 30 min and allowed to cool to room temperature before the addition of chloroform. The sample was then mixed by gently inverting the tube several times. Thereafter, the sample was spun at 14,000 rpm for 15 min and the supernatant transferred into a new eppendorf tube where equal volume of cold Isopropanol was added to precipitate the DNA. The sample was kept in the freezer for one hour and later spun at 14,000 rpm for ten minutes. The supernatant was discarded while the pellet was washed with 70% ethanol. The air-dried pellet was re-suspended in 100 µl of sterile distilled water. The genomic DNA was kept at -20^0C till when needed. Each genomic DNA of 10 µl sample was taken into eppendorf tube and 990µl sterile distilled water was added to make 1000 µl. The final concentration became about 3 ng/µl.

PCR reaction mix

The reaction mix was carried out in 20 µl final volume containing about 60 ng genomic DNA, 0.1 µM of the primers, 2 mM $MgCl_2$, 125µM of each dATP, dTTP, dGTP, dCTP and 1 unit of Taq DNA polymerase. The thermo-cycler profiles starting with an initial denaturation temperature of 94^0C for 3 min, followed by 45 cycles of denaturation temperature of 94^0C for 20 seconds, annealing temperature of 37^0C for 40 seconds and primer extension temperature of 72^0C for 40 seconds, followed by final extension temperature at 72^0C for 5 min was added.

In order to ensure that the amplified DNA bands actually came from the genomic DNA and not from primer artefacts, negative control was carried out for three of the primers. The absence of amplification in the negative control reaction confirmed DNA amplification in samples. Each reaction was repeated twice to ensure consistency of result. There was reproducibility of amplified products when reactions were repeated using the same conditions. RAPD-PCR amplifications reactions were carried out on the extracted DNA sample using five RAPD primers that produced consistent bands as shown in Table 1.

Table 1: Base sequences of the primers considered for the study

Primer	Base Sequence
OPA-02	5-'TGCCGAGCTG-3'
OPA-06	5'- GGTCCCTGAC- 3'
OPA-11	5'- CAATCGCCGT- 3'
OPAE-10	5'- CTGAAGCGCA- 3'
OPD-14	5'- CTTCCCCAAG- 3'

Agarose gel electrophoresis

PCR amplicon electrophoresis was carried out by size fractionation on 1.4% agarose gels. This was done at 100V for 2 h. The DNA was visualized and photographed on Polaroid 667 film under UV light source.

Data analysis

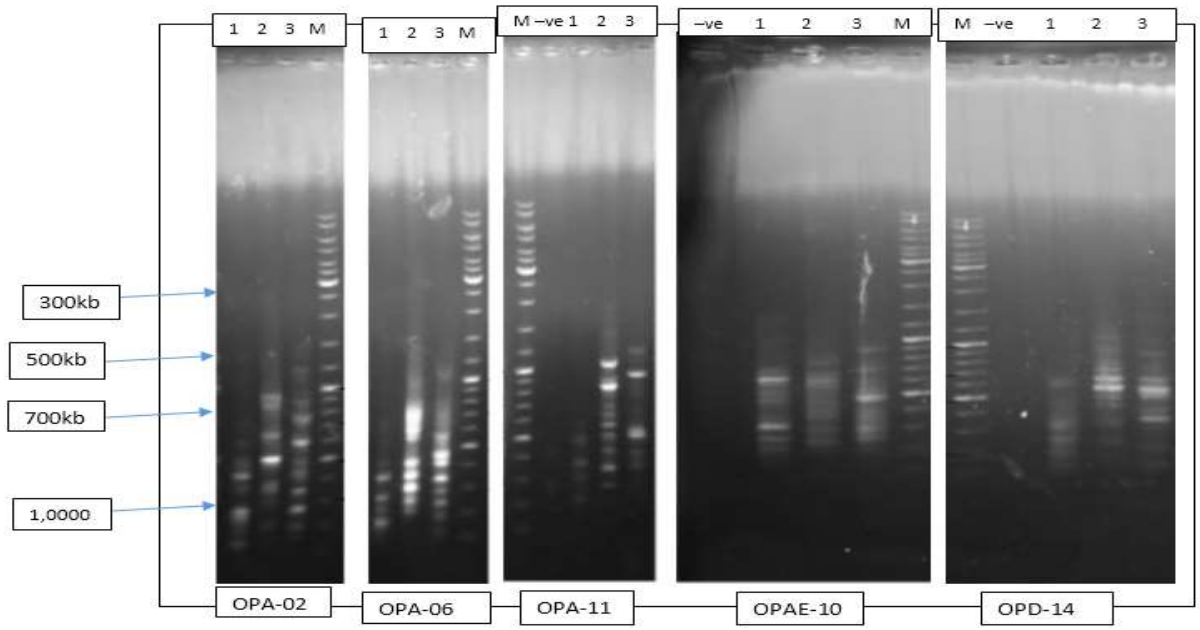
Bands clearly visible in at least one genotype were scored '1' for present '0' for absent and entered into a data matrix. Similarity Matrix (coefficient) proposed by Nei and Li (1979) was used to calculate the degree of similarity (S_{ab}), between two species a & b according to the formula:

$$S_{ab} = 2N_{ab} / (N_a + N_b)$$

Where: $N_{ab=}$ number of bands common to both species a and b; $N_{a=}$ number of bands in species a; $N_{b=}$ number of bands in species b. Hierarchical clustering (dendogram) was constructed using Unweighted Pair Group Method with arithmetic Average (UPGMA) with SPSS version 23 window software.

Results and Discussion

Plate 1 shows the protein banding pattern of the five primers (OPA-02, OPA-06, OPA-11, OPAE-10 and OPD-14) generated by amplification with the three fish species studied (*Oreochromis niloticus*,*Heterobranchus longifilis* and *Heterosis niloticus*). It was observed that the intensity and number of amplified bands detected varied depending on the primers used and fish samples.



M = Marker; –ve = Negative control; 1 = *Oreochromis niloticus;* 2 = *Heterobranchus longifilis;* 3 = *Heterotis niloticus*

Plate 1: Banding pattern of the samples detected by Agarose gel electrophoresis

Table 2: Polymorphic and monomorphic bands in *Oreochromis niloticus, Heterobranchus longifilis* and *Heterotis niloticus* generated by the five different primers

Primer	Total No. of Bands Present	Number of Polymorphic Bands	Number of Monomorphic Bands	Percentage of Polymorphic Bands (%)
OPA-02	13	13	0	100
OPA-06	15	13	02	86.67
OPA-11	15	14	01	93.33
OPAE-10	12	08	04	66.67
OPD-14	15	14	01	93.33
Total	70	62	08	88.57

A total of seventy clear, distinct and sharp bands were produced by the selected five primers (Table 2). Sixty-two out of the seventy bands were polymorphic in the samples while eight monomorphic bands were produced by the five primers. Three primers (OPA-06, OPA-11 and OPD-14) produced the highest number of bands (15) while the least number of bands (12) was produced by OPAE-10. The highest number of polymorphic bands (14) was recorded for Primer OPD-14 while OPAE-10 produced the least number of polymorphic bands (8). The highest number of monomorphic bands was recorded for OPAE-10 (4) while no monomorphic band was recorded for OPA-02. Also, primer (OPA-02) produced the highest percentage of polymorphic bands (100%) while OPAE-10 produced the least percentage of polymorphic bands (66.67%). In this study, 88.57% of all the bands showed polymorphism.

Among the three fish species, *Heterobranchus longifilis* produced the highest number of bands (46) and number of unique bands (25). *Heterotis niloticus* had the highest number of polymorphic bands of 15. The least total number of bands, number of unique bands and number of polymorphic bands of 21, five (5) and eight (8) were recorded for *Oreochromis niloticus* (Table 3). Eight monomorphic (common) bands were recorded among the three fish species studied.

Table 3: The total number of bands, unique bands, polymorphic bands, monomorphic bands in each fish species

Fish species	Total Number of bands present	Number of unique bands	Number of polymorphic bands	Number of monomorphic bands
Oreochromis niloticus	21	05	08	08
Heterobranchus longifilis	46	25	13	08
Heterotis niloticus	37	14	15	08

Table 4: Similarity Index for the Three Fish Species Studied

Species	*Oreochromis niloticus*	*Heterobranchus longifilis*	*Heterotis niloticus*
Oreochromis niloticus	-		
Heterobranchus longifilis	0.33	-	
Heterotis niloticus	0.45	0.43	-

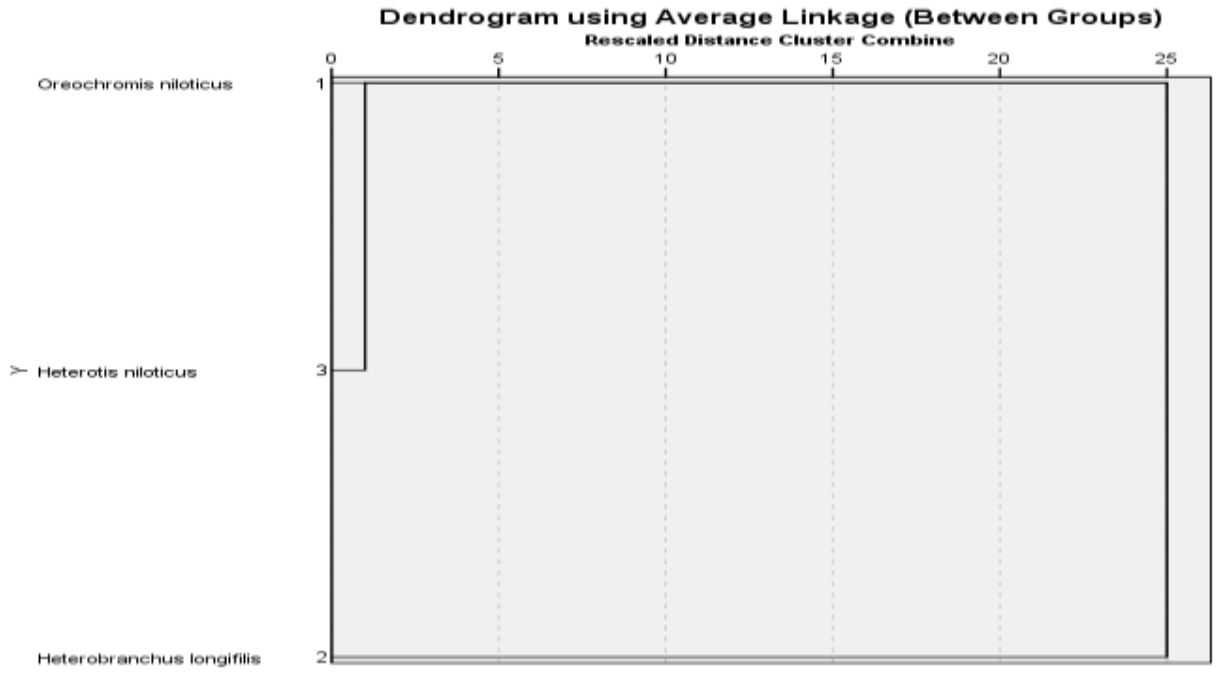


Fig. 1: Dendogram showing the relationship among the three fish species studied

Table 4 showed the similarity index (coefficient) for the three fish species studied. The similarity index range of 0.33 to 0.45 was recorded for the three fish samples. The highest similarity coefficient of 0.45 was observed between *Oreochromis niloticus* and *Heterotis niloticus* while the least similarity coefficient of 0.33 was observed between *Oreochromis niloticus* and *Heterobranchus longifilis.*

Hierarchical cluster analysis (dendrogram) result in Fig. 1 showed that *Oreochromis niloticus* and *Heterotis niloticus* clustered together in the same group while *Heterobranchus longifilis* occupied a separate cluster.

This study revealed the suitability of Random Amplified Polymorphic DNA marker technique for the assessment of genetic diversity among the three fish species (*Heterotis niloticus, Oreochromis niloticus* and *Heterobranchus longifilis)* studied. All the five primers considered in this study amplified perfectly with the samples but the intensity and number of bands detected varied with primers and fish species. This finding indicated that genetic diversity existed among the sample for their identification.Abu-Almaaty *et al.* (2018) reported that RAPD technique is a useful tool for identifying DNA polymorphism, estimation of genetic diversity and differentiating related fish species.

The fact that a total of seventy distinct bands were produced by the five primers considered indicated that the primers will be suitable for assessing genetic diversity as well as taxonomic delimitation of fish species from the river. The seventy bands and sixty-two polymorphic bands observed in this study is higher than total number of fifty-five bands and forty polymorphic bands reported by Neekhra *et al.* (2014) who used six primers. This also supported the suitability of the five primers for generating genetic diversities among fish species.

The range of 12 (OPAE-10) to 15 bands (OPA-06, OPA-11 and OPD-14) observed could be attributed to differences in binding sites of the markers throughout the genome of the studied fish species. The high percentage of polymorphism (100%) reported for OPA 02 in this study revealedthat primer is the best among previously studied primers for identifyingthe three fish species. Also, the presence of more unique bands (25) in *Heterobranchus longifilis* and less unique bands (5) in *Oreochromis niloticus* further supported the suitability of the selected primers for identification of fish species from the studied river. This finding also suggested that the studied primers may be more relevant for the delineation of *Heterobranchus longifilis* than the other two fish species studied. It could also be infer that *Heterobranchus longifilis* is genetically different from the remaining two species studied.

The presence of eight monomorphic (common) bands among the three fish species studied revealed that although the three fish species are from different families, they have common ancestry. Monomorphic bands expressed the degree of genetic relatedness among the three fish species. These monomorphic (common) bands may be common to all the fish families over evolutionary time and could therefore be used to discriminate fish samples from other organisms. Alege (2019) reported that the presence of monomorphic (common) bands strongly suggested that the genes coding for the bands are fixed in the samples under study. The presence of common bands according to Neekhra *et al.* (2014) indicated close genetic affinity and common evolutionary relationship.

The highest similarity coefficient of 0.45 observed between *Oreochromis niloticus* and *Heterotis niloticus* suggested close affinity between the two species while the low similarity coefficient of 0.33 observed between *Oreochromis niloticus* and *Heterobranchus longifilis* indicated that the two species are phylogenetically diverse. The similarity index range of 0.33 to 0.45 observed in this study was similar to the 0.23 to 0.53 reported by Megbowon (2019) on four tilapiine species obtained from Epe Lagoon in Lagos State, Nigeria.

The dendrogram puts the three fish species into two groups with *Oreochromis niloticus* and *Heterotis niloticus* clusteredtogether while *Heterobranchus longifilis* was separated into a different group. This finding revealed that *Oreochromis niloticus* and *Heterotis niloticus* are genetically more related compared to *Heterobranchus longifilis.* This finding supported the earlier reported absence of *Heterobranchus longifilis* along the River basin (Solomon *et al.,* 2012; Okomoda *et al.* 2013; Adeyemi 2013). It is therefore obvious that *Heterobranchus longifilis* is a recent introduction at Niger-Benue Confluence River, Lokoja, Nigeria.

Conclusion and Recommendation

The characterization of three fish species (*Oreochromis niloticus, Heterobranchus longifilis* and *Heterotis niloticus*) obtained from Lokoja axis of the Confluence River using RAPD revealed sufficient level of genetic diversity for the identification of fish species from the river. The five molecular markers were also able to characterize the three fish species and establish their successful use as viable tools for studying the genetic structure of fish species. This study has generated baseline molecular data which has been lacking for fish species identification and characterization for conservation and management of fish species from the Confluence River. Since the choice of selection of the three fish species considered in this study was based on the fact that they are the commonly exploited species in the river, further studies using more RAPD primers and other fish species from the river is recommended to compliment the findings of this study.

Conflict of Interest

Authors declare that there is no conflict of interest related to this work.

References

Abu-Almaaty AH, Abdel-Basset ME & Mohammad A 2018. Genetic characterization of four fish species of Genus Synodontis using RAPD Marker. *Indian J. Geo-Mar. Sci.,* 47(12): 2395-2406.

Adeyemi SO, Akombu PM & Adikwu IA 2010. Diversity and abundance of fish species in Gbedikere Lake, Bassa, Kogi State. *J. Res. in Forestry, Wildlife and Env.,* 2(1): 1-6.

Adeyemi SO 2013. Fish production estimates for Gbedikere Lake, Bassa, Kogi State, Nigeria. *Croatian J. Fisheries,* 71: 141-146.

Adeyemi SO & Toluhi OO 2014. Helminth parasites of Mochokid *Synodontis resupinatus* in a freshwater ecosystem in the lower Niger River, Kogi State, Nigeria. *Global J. Biol., Agric. and Health Sci.,* 3(2): 141-143.

Adeyemi SO, Bankole NO & Adikwu IA 2009. Fish gear survey of Gbedikere Lake, Bassa, Kogi State, Nigeria. *Int. J. Lake and Rivers,* 2(1): 53-56.

Alege GO 2019. Characterization of Nigerian sesame (*Sesamum indicum* L.) using random polymorphic DNA (RAPD) marker. *J. Biotech. Res.,* 5 (9): 77-84.

Ali BA, Ahmed MMM & El-Zaeem SY 2004. Technical note: application of RAPD markers in fish: Part II-Among and within families; Cichlidae (Freshwater), Mugilidae (Catadromous), Sparidae and Serranidae (Marine). *Int. J. Biotech.,* 6(4): 393–401.

Almeida FS, Lopes CM, Orsi ML, Siro RN & Sodr ELMK 2013. Genetic monitoring by RAPD markers for repopulation programs of *Salminus brasiliensis* (Pisces, Characiformes). *Acta Scientiarum,* 35 (2): 119-126.

Asagbra MC, Adebayo AS, Ugwumba AO, Ugwumba AAA & Anumudu CI 2014. Genetic characterization of fin fish species from the Warri River at Ubeji, Niger Delta, Nigeria. *Afr. J. Biotech.*, 13(27): 2689-2695.

Asensio L, Gonzalez I, Fernandez A, Rodriguez MA, Lobo E, Hernandez, PE, Garcia T & Martin R 2002. Application of random polymorphic amplified DNA (RAPD) analysis for identification of grouper (*Epinephelus guaza*), wreck fish (*Polyprion americanus*), and Nile perch (*Lates niloticus*) fillets. *J. Food Protection*, 65(2): 432-435.

Ausubel FA, Brent R, Kingston RE, Moore DD, Scidman JG, Smith JA & Struhl K 2001. Current CTAB Protocols in Molecular Biology. Green Publishing and Wiley – Interscience, New York, 202p.

Iyaji FO & Eyo JE 2009. Parasites and their freshwater fish host. *Bio-Research*, 6(1): 328 – 338.

Iyaji FO and Yaro CA 2016. Endoparasitic helminths of *Synodontis schall* (Bloch and schneider, 1801, siluriformes, mochokidae) at the confluence of Niger and Benue Rivers, Lokoja, Nigeria. *Int. J. Fisheries and Aqua. Stud.*, 4(5): 30-35.

Iyiola OA, Nneji LM, Mustapha MK, Nzeh CG, Oladipo SO, Nneji, IC, Okeyoyin AO, Nwani CD, Ugwumba OA, Ugwumba AAA, Faturoti EO, Wang Y, Chen J, Wang W & Adeola AC 2017. Barcoding of economically important freshwater fish species from North-Central Nigeria uncovers cryptic diversity. *Ecol. and Evol*., 8: 6932–6951.

Megbowon I 2019. Genetic evaluation of some tilapiine fishes using varying RAPD markers. *Int. J. Fisheries and Aqu. Stu*., 7(4): 275-279.

Nadeem MA, Nawaz MA, Shahid MQ, Dogan Y, Comertpay G, Yildiz M, Hatipoglu R, Ahmad F, Alsaleh A, Labhane N, Ozkan H, Chung G & Baloch FS 2018. DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotech. and Biotechnol. Equip.*, 32(2): 261-285.

Neekhra B, Mansoori AA, Verma S, Koiri RK & Jain SK 2014. RAPD-PCR based biomarker study in fish species (Family: *Cyprinidae*) of Madhya Pradesh, India. *Austin J. Mol. and Cell. Biol.*, 1(1): 1-6.

Nei M & Li WH 1979. Mathematical model for studying genetic variations in terms of restriction endonucleases. *Nat. Acad. Sci.*, 76: 5269-5273.

Ogbuebunu KE & Awodiran MO 2017. Molecular characterization of *Lates niloticus* (Perciformes, Latidae) populations from the three water bodies using random amplified polymorphic DNA and Microsatellite Markers. *Vestnik Zoologii*, 51(1): 31–36.

Okomoda VT, Solomon SG & Ataguba GA 2013. Physicochemical parameters, microbiological water quality and fish abundance in flooded areas of Lokoja, Nigeria. *Asian Fisheries Sci.*, 26: 101-114.

Solomon SG, Okomoda VT & Aladi SL 2012. Fish Fauna in Lower River Niger at Idah Kogi State. *J. Aqua. Eng. and Fisheries Res.*, 4: 34-40.

Suleiman B, Maruff L & Oniye SJ 2015. Radiographic studies on morphological anomalies in artificially spawned *Heterobranchus longifilis* Valenciennes, 1840 F1 generation. *Sokoto J. Vet. Sci.*, 13(1): 9-16.