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Genetic characterization of fin fish species from the Warri River at Ubeji, Niger Delta, Nigeria

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A study to evaluate the genetic similarities and differences among 11 specimens of cichlids and four specimens of mudcatfishes obtained from Warri River was carried out through DNA fingerprinting analysis using random amplified polymorphic DNA (RAPD)-PCR amplification with seven decamer primers and dendrograms through unweighted pair-group method with average (UPGMA) cluster analysis. The total number of bands generated by the seven RAPD primers, ranged between 2 to 33 for the cichlids and 8 to 28 for the catfish family, with band size between 100 to 800 bp. The primers produced 228 bands in total 119 for the cichlids and 109 for the catfishes, with 24% polymorphism. Considerable genetic variation was observed within species (especially within *Tilapia zilli, T. guineensis* and *Clarias gariepinus*), between species in the same genera (*T. zilli and T. guineensis)* and among cichlids and catfishes. The most consistent of the RAPD primers generated 87 bands among the cichlids with 23 bands (26%) polymorphic and 74% conserved. Among the catfishes, the primers produced 69 bands with 16 (23%) polymorphic. The data show that the RAPD technique was useful and sensitive in differentiating various fish genera and species.

Key words: Random amplified polymorphic DNA (RAPD), Niger Delta, aquatic diversity, phylogeny

INTRODUCTION

There is an increase in research interest in the maintenance and preservation of genetic diversity of fish as an essential ecological resource and life support system (Ekelemu and Zelibe, 2006). Fishes constitute a large percentage of the biological diversity of organisms that inhabit rivers and other water bodies and majority of the population of Niger Delta depends on catch from the wild as source of animal protein. The mudcatfishes and the tilapias are some of the groups of fishes that are frequently caught (Idodo-Umeh, 2003; Akpan, 2013).

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Therefore, scientific efforts to determine the current level of diversity of common fishes in these localities are applicable in ecology, fishery management, aquaculture and stock conservation and in resolving perennial identification problems among cichlids and mudcatfish species.

Traditionally, morphological characterization of fish has been useful in determining fish species, sex and larval stages, but it does not offer a definite or reliable method for species identification. Hence, genetic or DNA-based techniques are being used for such purposes (Teletchea, 2009; Wong, 2011). Species identification has been shown to be possible using molecular markers in Random Amplified Polymorphic DNA (RAPD) (Soufy et al., 2009). The RAPD technique is a polymerase chain reaction (PCR) technique that relies on the generation of amplification products for a given nucleic acid using an amplification-based scanning technique driven by arbitrary priming oligonucleotides (Williams et al., 1990). The amplified region consists mostly of non-coding and unstructured sequences that are of different length from one species to another (Williams et al., 1990). In this technique, polymorphism is revealed by the presence or absence of amplification products when two strains or individuals are compared (Soufy et al., 2009). High levels of DNA polymorphisms could be detected using this technique; it could produce fine genetic markers, and a large number of loci sampled without prior DNA sequence information (Ali et al., 2004; Ahmed et al., 2004; Theodorakis and Bickham, 2004).

The RAPD technique has been used in the evaluation and identification of the genetic diversities of species and subspecies of tilapia (Bardakci and Skibinski, 1994; Dinesh et al., 1996; Ahmed et al., 2004; Ali et al., 2004; Sherif et al., 2009; Soufy et al., 2009); carps (El-Zaeem et al., 2006); *Clarias gariepinus* (Saad, et al., 2009); *Prochilodus marggravii* (Hatanaka and Galetti, 2003); *Pimelodus maculatus, P. lineatus, Salminus brasiliensis and Steindachneridion scripta*, (Ramella et al., 2006). Similarly, Hassanien et al. (2004) found genetic diversity among different populations of *Oreochromis niloticus* in Egypt using this technique.

In our study, we report the usefulness of the RAPD technique in the assessment of genetic variations among species of cichlids and mud catfish from the Warri River, Niger Delta, Nigeria.

MATERIALS AND METHODS

Description of study area

The Warri River is a major navigable channel of the Niger Delta, southern Nigeria. It takes its origin from around Utagba Uno and flows through zones of freshwater swamps, mangrove swamps, dominated by *Rhizophora* species, and coastal sand ridges. It is a relatively large water body which stretches within latitudes 5°21' to 6°00' N and longitude 5°24' to 6°21'E, covering a surface area of about 255 sq km with a length of about 150 km. It drains various tributaries and empties into the brackish Forcados River that in turn empties into the Atlantic Ocean at the Bight of Benin (Figure 1).

Sampling stations were established along the river at Ubeji, a remote village located behind a refinery (Figure 1). The river is known to supply drinking water for domestic use and irrigation of farm lands, recreational activities and means of transportation. Fishing activities also takes place in the river and the people depend to a large extent on their fish catches as one of the major sources of protein.

Collection of samples

Fifteen (15) fish specimens were collected from the Warri River at Ubeji for this study in June 2010 from local fishermen. The fish samples were chilled in the field, frozen and transported to the Curator, Department of Zoology University of Ibadan, where eleven were identified as cichlids (five specimens of *Tilapia zilli*, three of *T. guineensis*, one *Sarotherodon galilaeus* and two *Hemichromis fasciatus*) and four as catfishes (*C. gariepinus*). They were then stored frozen in the laboratory prior to RAPD analysis. Three individuals of *C. gariepinus* bought from the market in Ibadan served as control.

DNA extraction

DNA was extracted from the muscle tissue following the method described by Lopera-Barrero et al. (2008) with some modification. Approximately, 0.3 g of each muscle tissue was crushed with a mortar and pestle in 500 µL extraction buffer (100 mM Tris, 8.5 mM EDTA, 500 mM NaCl) and transferred to a polypropylene microfuge tube. 20% SDS was added; the mixture was vortexed briefly and incubated in a gentle shaking water bath at 65°C for 10 min. 160 µL of 5 M potassium acetate was added after bringing the sample to room temperature, then vortexed and centrifuged at 10 000 *g* for 10 min. Supernatant was removed (about 500 µL) into another Eppendorf tube and 200 µL of cold isopropanol was added and mixed after which samples were kept on at -4°C for 15 min before centrifuging at 10 000 *g* for 10 min to sediment the DNA. Supernatant was decanted after centrifuging and pellets were washed with 500 µL of 70% ethanol and centrifuged at 10 000 *g* for 10 min. The ethanol was decanted and the DNA was air-dried at room temperature (37°C) and re-suspended in 50 µL TE (10mM Tris and 1mM EDTA) buffer and stored in the freezer prior to RAPD PCR analysis. The integrity of the DNA was verified by electrophoresis on a 1% agarose gel.

RAPD PCR Analysis

The PCR consisted of a 25 µL reaction volume of 4x Go Taq (Integrated Technologies U.S.A) colourless master mix, 1 µL of 7 pmol of primer (10 bp) and 2 µL of genomic DNA (100 to 200 ng). The sequences of primers used are shown in Tables 1 and 2. The final reaction mixture was placed in a DNA thermal cycle (Apicon ThermoEX 500). The PCR programme included an initial denaturation step at 94° C for 2 min followed by 45 cycles of 94°C for 30 s for DNA denaturation annealing, extension at 72°C for 30 s and final extension at 72°C for 10 min was carried out. The samples were cooled at 4°C and the amplified DNA fragments were separated on 1.2% agarose gel and stained with ethidium bromide. The amplified DNA pattern was visualized on a UV transilluminator and photographed.

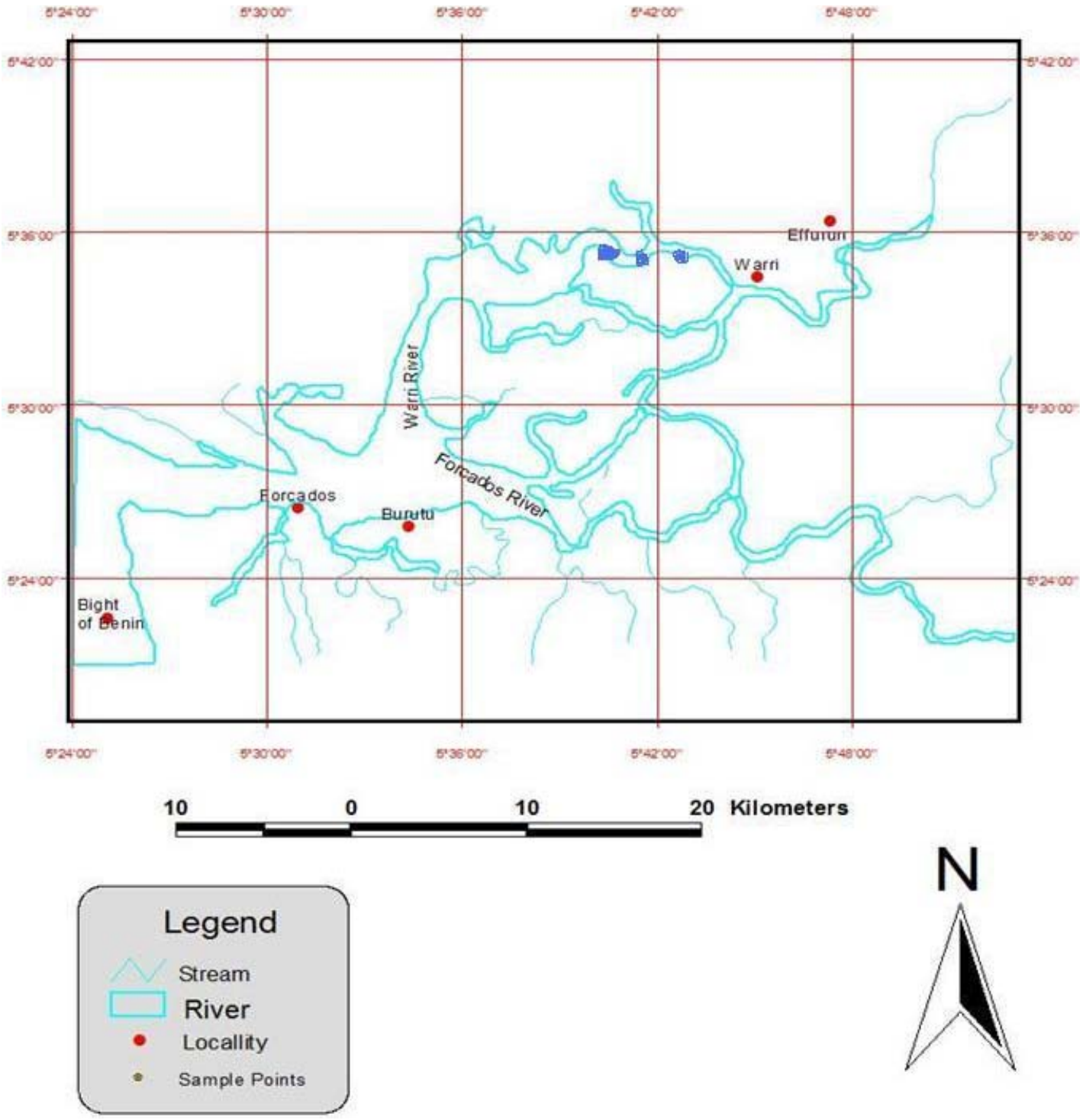

Figure 1. Sampling sites (painted in deep blue) at Ubeji in Warri River, Niger Delta.

Gel scoring and data analysis

Identified DNA bands were scored as 1 or 0 for their presence and absence respectively in each sample. Unambiguous and reproducible bands were used in analysis of the gels, hence, primers that produced no amplifications and bands were not used in analysis. These data were used to calculate genetic distance using Jaccard coefficient similarity matrices. Phylip (Felsenstein, 2005), a tool assessed through ExPASy, a web based bioinformatics resource of the Swiss Institute of Bioinformatics, was used to

Table 1. The sequences, GC% and annealing temperature of the RAPD primers used.

Primer	Sequence 5´- 3´	Annealing Tm (°C/S)
1	GAAACGGGTG	40/120
2	AATCGGGCTG	40/120
3	GGGTAACGCC	40/120
4	CAGCACCCAC	40/120
5	GTGATCGCAG	40/120
6	CCGGGAATCG	40/120
7	AGTCAGCCAC	40/120

Table 2. The sequences, GC% and annealing temperature of the specific primers used.

Primers sequences 5´ - 3´ (forward)	Sequences 3´ - 5' (Reversed)	Annealing Tm (°C/S)
GTGTGAATGTGCTTGTGTATGC	GTGCGGGGCAGTTATTGATGG	59.1/120
TGCATTTTTGTAGTGATGC	GGGCTCCTGGAGATTGC	59.1/120

Table 3. Summary of the number and characteristics of amplification products obtained from a survey of seven primers of random sequence among 11 individuals of the cichlid family.

Primer	NBF1	NBF2	NBF3	NBF4	NBF5	NBF6	NBF7	NBF8	NBF9	NBF10	NBF11
RAPD 1	1	2	0	0	0	0	0	0	1	0	1
RAPD 2	0	0	0	0	0	0	0	0	1	1	0
RAPD 3	5	2	2	1	1	5	0	4	5	2	3
RAPD 4	5	7	0	0	0	0	0	0	1	5	4
RAPD 5	4	4	2	4	1	1	4	1	3	4	5
RAPD 6	4	4	3	3	0	5	2	0	0	0	3
RAPD 7	0	0	0	0	0	0	0	0	2	1	0

NBF1-11, Number of bands in fish 1-11.

construct dendrogram using the Unweighted Pair-Group Method with Average linkages (UPGMA) method, with visualizations provided by PhyloWidget tool (Jordan and Piel, 2008).

RESULTS AND DISCUSSION

RAPD fingerprints for 15 individual fish specimens (coded F1-11 for cichlids and F12-15 for catfishes) using seven different RAPD primers (Table 1) were obtained. No residual DNA or artifacts were observed. The seven RAPD primers used produced fragments with varying bands and primers were individual specific in most of the species examined (Tables 3 and 4). The total number of bands generated by the seven RAPD primers (RAPD1-7) respectively was: 5, 2, 30, 22, 33, 24, 3 for the cichlids and 11, 8, 28, 8, 27 and 14 for the catfish family. The size of fragment amplified by these primers as shown in Tables 5 and 6 varied between 100 to 800 bp.

Of the total number of bands generated among cichlids (119), only 24% were polymorphic. Of 109 bands generated among catfishes, only 22% were polymorphic. An average of four polymorphic bands per primer was recorded. All primers yielded at least one polymorphic band except primer 2 among the cichlids. Also, primers 1, 2, 4 and 7 produced no amplification in several samples (Table 3) and thus were excluded in final analysis. The absence of amplified bands in significant number (for example two or more) of primers makes interpretation of polymorphism in such primers difficult, as the absence does not connote genetic similarity or otherwise (Hatanaka and Galetti, 2003). This underlines the importance of primer selection in using RAPD to assess genetic diversity. Primers 3, 5 and 6 (Table 1) had very intense and reproducible bands in all samples. The three primers generated 87 bands with 23 bands (26%) polymorphic and 74% conserved among cichlids. Among

Table 4. Summary of the number and characteristics of amplification products obtained from a survey of seven primers of random sequence for *Clarias gariepinus* species.

Primer	NF12	NF13	NF14	NF15
RAPD 1	0	4	4	3
RAPD 2	2	4	2	0
RAPD 3	8	9	7	4
RAPD 4	0	4	4	0
RAPD 5	6	7	7	7
RAPD 6	2	4	3	5
RAPD 7	0	2	2	2

NBF (12-15) Number of bands in fish 12 to 15.

Table 5. Summary of the number of polymorphism and size range of amplification products obtained from a survey of seven primers of random sequence among three *Tilapia* species from the Warri River.

Primer number	Total number of bands	Number of polymorphic band pair	Size range of base pair
1	5	1	400- 120
2	2	0	400
3	30	8	800- 100
4	22	4	800- 120
5	33	8	800-120
6	24	7	800- 100
7	3	1	600- 500

Table 6. Summary of the number and size range of amplification products obtained from survey of seven primers of random sequence for *Clarias gariepinus species* obtained from Warri and Ibadan.

Primer number	Total number of bands	Number of Polymorphic band	Size range of base pair
1	11	3	800-120
2	15	4	700-300
3	28	4	600- 110
4	8	2	400- 300
5	27	4	550- < 100
6	14	4	600- 110
7	6	3	600- 500

the catfishes, the three intense primers produced 69 bands with 16 (23%) polymorphic. All controls using specific primers were amplified.

A large number of DNA bands, 228 in all, were generated by the RAPD method used, with 24% being polymorphic loci. In particular, primers 3, 5, 6 and 7 produced a high level of polymorphism with unique sequences which were useful in distinguishing the cichlid and catfish families. This may be useful to distinguish evolutionary relationships and related species in higher taxonomic level (Soufy et al., 2009). In addition, primers 2 and 7 proved to be population specific for *Hemichromis fasciatus* (Table 3) and may serve as genetic markers.

Dendrogram analyses

Dendrogram was constructed (Figure 2) after data generated from the RAPD primer band analysis. The UPGMA cluster analysis of the similarity matrix based on RAPD data separated the cichlids specimens into three clusters and the catfishes one (Figure 2). The first (C) formed by two *T. zilli*, one *T. guineensis* and one *S. galilaeus*; the second (D) by one *T. zilli*, one *T.*

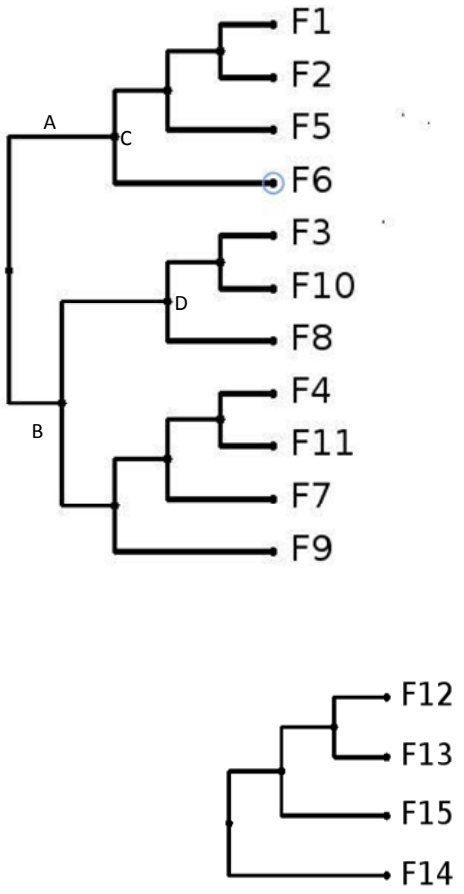

Figure 2. Dendrogram of finfish samples in Warri River at Ubeji plotted by Unweighted Pair-Group Method of Averages (UPGMA). Key for the leaves: F1-4 and 11, *Tilapia zilli*; F5, *Satherodon galileaus*; F6-8, *Tilapia guineensis*; F9-10, *Hemichromis fasciatus*; F12-15, *Clarias species.*

guineensis and one *H. fasciatus*; and the third (E) by two *T. zilli*, one *T. guineensis* and one *H. fasciatus*. The second and third clusters of the cichlids had smaller genetic distances and hence, a closer profile, forming clade B (Figure 2). Of the 11 leaves (specimens) of the cichlid dendrogram, seven were in clade B.

The Tilapia specimens showed genetic variation among themselves. Three of the *T. zilli* specimens - F3, F4 and F11 were of close distance to the *H. fasciatus* specimens F10 and F9. In particular, F3 shared a node and same branch length with F10, while F4 and F11 also clustered with F9. Also, all three specimens of *T. guineensis*- F6, F7 and F8 were in different clusters and were several nodes away from each other. The clustering of the *H. fasciatus* specimens followed a similar pattern. This rather wider variation than expected may be due to smaller number of primers that were eventually used for the dendrogram, as it reduces the primers available for complete analysis; however, the results may indicate a need for in-depth and large scale diversity study of cichlids at the study site.

In addition, F5, a *S. galilaeus* specimen had a profile close to that of F1 and F2, both *T. zilli* specimens (Figure 2). The dendrogram suggests that in each clade or cluster, there are always different taxa- no single species clustered together completely.

Several authors have demonstrated the effectiveness of the RAPD method in discriminating between species or subspecies in cases where close morphological characteristics and attributes have low resolving power (Ahmed et al., 2004; Saad et al., 2009). In this study, *T. zilli* and *T. guineensis* had different profiles from the RAPD primers, despite similar morphological features. In Figure 2, *T. guineensis* specimens F6, 7 and 8 formed a

separate branch at nodes C, D and E. Individual species possess qualities which make them appear similar morphologically but are different genetically as has been confirmed in this study.

Furthermore, there was also considerable variation in the RAPD profiles among the catfishes as shown in the dendrogram (Figure 2), with only two F12 and F13 showing very close genetic profiles by sharing a node. Overall, considerable genetic variation was observed within species (especially within *T.zilli, T. guineensis and C. gariepinus*), between species in the same genera (*T. zilli and T. guineensis)* and among cichlids and catfishes. Genetic variation observed in the study could be result of various factors such as differences in growth rate, fertility and phenotype of each genus and might also be due to individual ability to thrive in a highly contaminated environment. The Warri river site is close to a refinery and water bodies in the Niger Delta are believed to have experienced pollution (Akpan, 2013). However, the differences observed may be adduced to reasons such as hybridization among and between species in the wild, the peculiarities of the RAPD technique, or primer selection. Conclusively, we believe that the sensitivity of the RAPD technique with no prior knowledge of the genome played an important role in the detection of the observed differences in the fish samples.

Conflicts of interest

The authors declare that they have no conflict of interest.

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