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Population Genetic Structure of Three Cichlids in Ilorin, North-Central Nigeria

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A B S T R A C T

Cichlids are among the economically important which serve as a source of food for people around the world. A deep understanding of the population structure and genetic diversity of cichlids are vital for initiation of conservation policies and sustainable aquaculture. There is paucity of information on the patterns of genetic variations among and within cichlids in North-central Nigeria. This study, therefore, investigated population genetic structure of *Coptodon zillii*, *Oreochromis niloticus*, and *Hemichromis fasciatus* collected from different freshwater bodies in North-central Nigeria. Genomic DNA was extracted, and five highly polymorphic RAPD primers were used for RAPD-PCR amplification and genotyping of the fish. Genetic polymorphism within and between the three tilapia species were examined. Percentages of polymorphism loci, pairwise population matrix, analysis of molecular variance (AMOVA), and genetic distances of cichlid populations were determined using standard methods, and dendrograms were constructed using an un-weighted pair group method of arithmetic mean (UPGMA). Overall, percentages of estimated molecular variance within and among *C. zillii*, *H. fasciatus* and *O. niloticus* populations were 5% and 95%; 4% and 96% and 13% and 87%, respectively. Our results suggest that the three cichlids have close evolutionary relationship and there were no distinct genetic differences on the basis on sampling locations. *C. zillii* and *H. fasciatus* are more genetically closer than *O. niloticus*. This study concludes that RAPD is useful in studying the population genetic structure of cichlids. This study therefore recommends conservation of genetic pool of cichlid species through proper maintenance and restoration of polluted habitat to guarantee sustainable fishery production. However, markers such as microsatellite DNA can be assayed in further studies for better results.

Keywords: tilapia, random amplified polymorphic DNA markers, genetic diversity, PCR, Northcentral, freshwater

INTRODUCTION

Fishing has served as a source of livelihood and protein in many countries of the world (1). The freshwater ecosystem of Nigeria has fish diversity resources of more than 250 species making it lucrative target for fishers (2-5). Kwara state, Nigeria, has several riverine communities with intense fishing activities,

especially around Oyun, Asa, Moro, and Apodu (6-10). Cichlidae are widely cultured and widely distributed in Nigeria water bodies. Previous studies showed that cichlids are economically important fish species because they serve as a source of food especially proteins, essential amino acids, essential vitamins, and minerals, and contribute to development by providing direct and indirect employment for large and small-scale fisheries and aquaculture. They dominate the catches in the wild (6, 9, 10). They are indigenous to tropical and sub-tropical fresh waters of Africa, Mediterranean, and Middle East (11). Cichlids in African lakes are associated with long-lasting lacustrine habitats, particularly for their rapid speciation and extensive adaptive radiation (12). It has been reported that cichlids can exhibit adaptive radiation and interestingly diversifies into many ecologically varied species in a short period of time (13).

The study of genetic diversity gives information on the degree of variation in a local reproductive unit, which is essential for environmental conservation (14). The importance of this approach lies in its potential for delimiting priority areas for species conservation and sustainable use (15). Fish species' richness and diversity in morphology, color, and behavior have made them model organisms suitable for study of speciation and adaptive evolution (16). All over the world, diversity and conservation of freshwater fishes have gained the attention of many researchers in recent time (17-21). Yet, there are only a few documented reports on fish species population composition and genetic diversity in North-central Nigerian freshwater ecosystem (1). There are few available molecular studies on intra- and inter-specific fish population diversity in North-central Nigeria. This kind of study is required to provide data that can appropriately guide policy-makers on the development of management and conservation strategies toward the utilization of fish genetic resources (22). There have been few reports on the use of molecular methods to investigate genetic diversity among freshwater fish species in Nigeria (20-25).

RAPD technique has been used for identification of different fish species because of its cost effectiveness and that it does not require prior knowledge of the DNA sequences of the studied organisms (26). RAPD is a versatile tool for the study of genetic relatedness and has been used extensively in fish to understand genetic differences that exist among populations for long-term fisheries management. Studies such as genetic variability among Scaridae fishes in Egypt (25), genetic characterization of four fish species of Synodontis (27), and genetic diversity of *Clarias gariepinus* in Nigeria using RAPD have been reported (28). Lind and his colleagues investigated genetic diversity of Nile tilapia (*Oreochromis niloticus*) throughout West Africa and reported the presence of genetically distinct and unique populations (29). Zhou et al. carried out a comparative genetic analysis between wild and cultured fish populations and reported that levels of polymorphism in cultured populations were generally low in the wild populations (30).

Apodu reservoir, Asa, Moro, and Oyun rivers are very rich in freshwater fish resources and considered as one of the hot spots of fish biodiversity in Kwara state, North-central Nigeria (2, 5, 9, 10, 31). There is paucity of information on the population genetic structure and genetic variations among cichlids in Nigerian freshwater bodies. This study, therefore, investigates genetic diversity and population structure of three common cichlids species from the four different water bodies in Kwara state, North-central Nigeria using RAPD-PCR assay. The report of this study will provide a baseline information on the population genetic structure of cichlids in Nigeria and assist in fish biodiversity knowledge.

MATERIALS AND METHODS

Sample Collection Sites

Forty-five cichlid samples representing three species *Coptodon zillii* (*C. zillii*), *Oreochromis niloticus* (*O. niloticus*), and *Hemichromis fasciatus* (*H. fasciatus*) were collected with the help of fishermen using gill net, hook and line, and traps from four different study sites in Kwara state, Nigeria. All the collected samples were used for the study. These sites comprised Apodu reservoir, Asa, Moro, and Oyun rivers, Apodu (Longitude 8˚45'25.9' N-8˚45'27.7'N), (Latitude 4˚27'35.5' E - 4˚27'41.4' E); Asa (Longitude 8˚25' N - 8˚27'N), (Latitude 4˚32' E-4˚34' E); Oyun (Longitude 4˚40'52'' N - 4˚41'52'' N), (Latitude 4˚27'41.4' E - 4˚27'35.5' E) and Moro (Longitude 4˚45'56'' N and 4˚27'⁴41.48' N). Sampling sites were selected based on regular fishing activities in these areas. The collected samples were deposited at the Fisheries and Hydrobiology unit, Department of Zoology, University of Ilorin, Nigeria. The samples were then sorted and identified according to the identification keys of Nigerian freshwater fish species (2, 5). Fin clips were obtained from adult fishes and immediately preserved in 100% ethanol to maintain the integrity of the genomic DNA for molecular analysis. The map of collection sites of the cichlids for this study is shown in Figure 1.

Genomic DNA Extraction

The DNA extraction from the fish fin tissues of samples was performed using a method previously described with some minor modifications (21). The fin clips of approximately 20 mg each were cut into minute pieces using surgical blades, and then suspended in 500 µL STE (0.1 M NaCl, 0.05 M Tris, and 0.01 M EDTA, pH 8). After adding 10% 30 µL sodium dodecyl sulfate (SDS) and 30 µL proteinase K (10 mg/mL), the mixture was incubated at 55°C for 2 h and vortexed intermittently every 30 min. The DNA was purified by successive extraction with phenol,

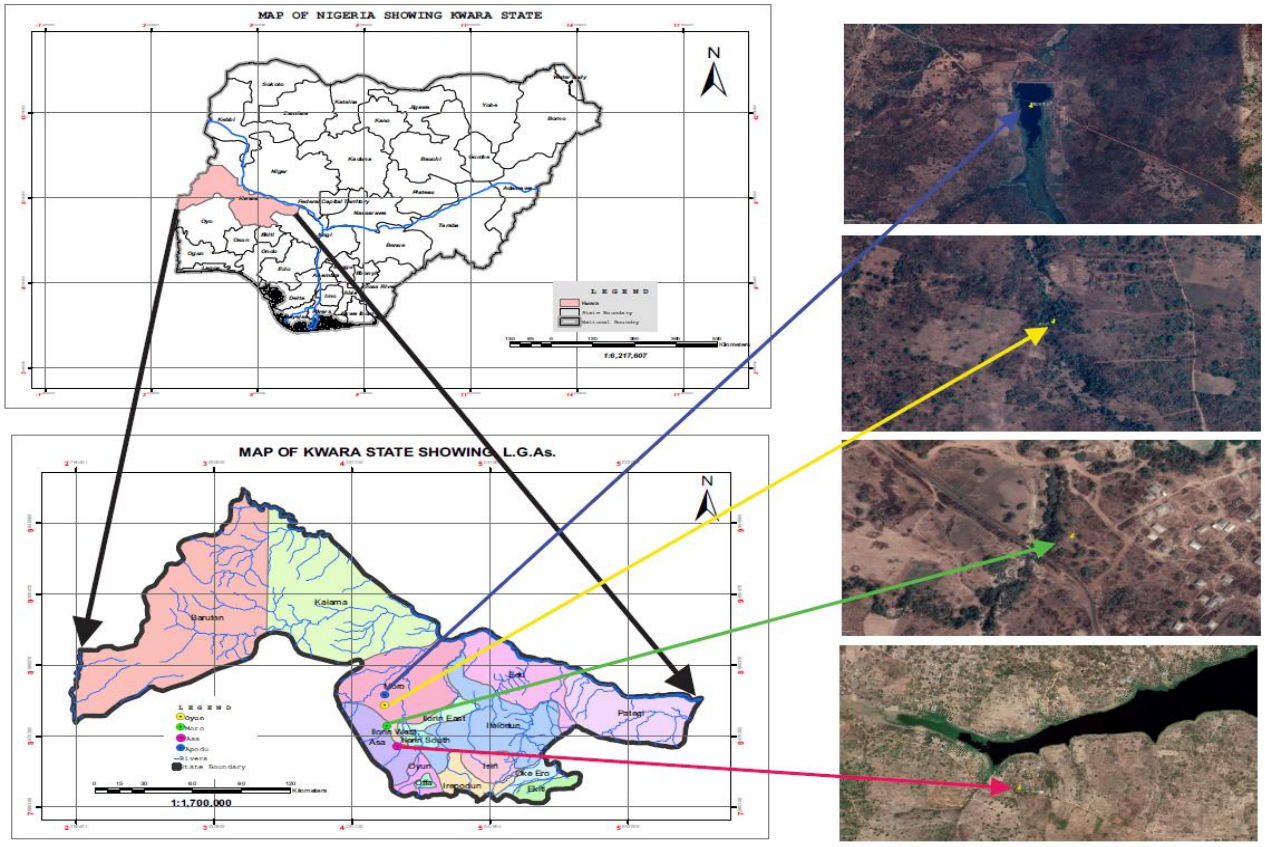

Figure 1. Map of the sample collection sites surveyed in the different study areas

phenol: chloroform: isoamyl alcohol (25:24:1) and chloroform: isoamyl alcohol (24:1), respectively. Then, the DNA was precipitated with ice-cold absolute ethanol and washed with 70% ethanol. The pellet was dried and resuspended in 50 µL TE buffer (10 mM Tris-HCl, 1 mM $Na_2EDTA.H20$, pH 7.2).

PCR Amplification and Genotyping

Five OPA series of RAPD primers, shown in Table 1, were used for PCR amplification. The PCR was carried out in 12.5 µL reactions containing 4 µL of 5x Firepol® Master Mix from Solis BioDyne, 1 µL of RAPD primer, 2 µL of template DNA (200-600 ng), and 5.5 µL of nuclease-free water. The PCR cycling conditions were as follows: initial denaturation at 94°C for 3 min, 45 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 2 min. The PCR amplicons were mixed with a 5x Blue DNA loading buffer (Bioline, UK) in the ratio of 4 µL of the sample/amplicon to 1 µL of loading buffer and were run on 2% agarose gel electrophoresis. The electrophoresis was run at 75 volts for 45 min. Solis Biodyne 100 bp DNA ladder was run alongside the DNA samples. The DNA agarose gels were stained, visualized under blue-light transilluminator, and the pictures of the viewed gel were then taken and documented for further analysis.

Table 1. RAPD primers used in this study

RAPD Primer Name	Primer sequence
OPA-03	5' AGTCAGCCAC 3'
OPA-05	5' ACGCAGGCAC 3'
OPA-09	5' GGGTAACGCC 3'
OPA-11	5' CAATCGCCGT 3'
OPAB-11	5' GTAGACCCGT 3'

Statistical Analysis

Sizes of the amplified DNA bands on the gels were scored, calculated, and compared against the DNA marker using the PyElph 1.3 software. The RAPD markers were scored by recording the presence (1) or absence (0) of these bands for each fish and each primer on Excel spreadsheets. The RAPD-DNA bands that were clear and highly reproducible in a size ranging between 200 and 1500 bp were selected for analysis. RAPD analysis was based on the assumptions that the fish populations were in Hardy-Weinberg equilibrium and that RAPD alleles exhibited the Mendelian law of segregation. In addition, it was assumed that marker alleles from different loci did not co-migrate to the same position on a gel and monomorphic fragments were homologous (co-migrate). To investigate the effect of sampling sites on genetic structuring among and within cichlids, Analysis of Molecular Variance (AMOVA) was carried out using ARLEQUIN, and percentage of

polymorphism loci were determined. Pairwise population matrix was calculated according to Nei's (1972) method among *C. zillii, H. fasciatus*, and *O. niloticus* samples from the various locations in Kwara state, Nigeria.

RESULTS

Genomic DNAs extracted from the cichlid samples was subjected to genotyping by using RAPD-PCR with five RAPD primers. Number of fragments per primer varied from 1-10 and ranged from 200-2000bp size with an average of 5 bands per primer. The PCR amplified DNA fragments differentiated populations of *C. zillii*, *H. fasciatus* and *O. niloticus* collected from Asa, Moro River, Oyun and Apodu waterbodies in Ilorin, Kwara state, Nigeria (Figure 2).

The OPA-05 primer had the highest number of polymorphic loci among all of the other primers used for the study, and the lowest was observed in OPA-03. The highest number of DNA polymorphic bands was observed using OPAB11 primer with a total of 111 bands. The total number of bands recorded for OPA3 were 43 bands. Percentages of polymorphic loci among *C. zillii, H. fasciatus* and *O. niloticus* samples from the different locations in Kwara state are shown in Table 2. Among *C. zillii* population, the highest percentage of genetic polymorphism was recorded in Oyun river with the lowest recorded in Apodu. Polymorphic loci amongst *H. fasciatus* and *O. niloticus* samples showed that the highest percentage was recorded in Apodu and Oyun, respectively, and the lowest in Asa dam cichlid population (Table2). For *O. niloticus* populations, the percentages of polymorphic loci were recorded in an increasing order of Oyun river > Moro river > Asa Dam (Table 2).

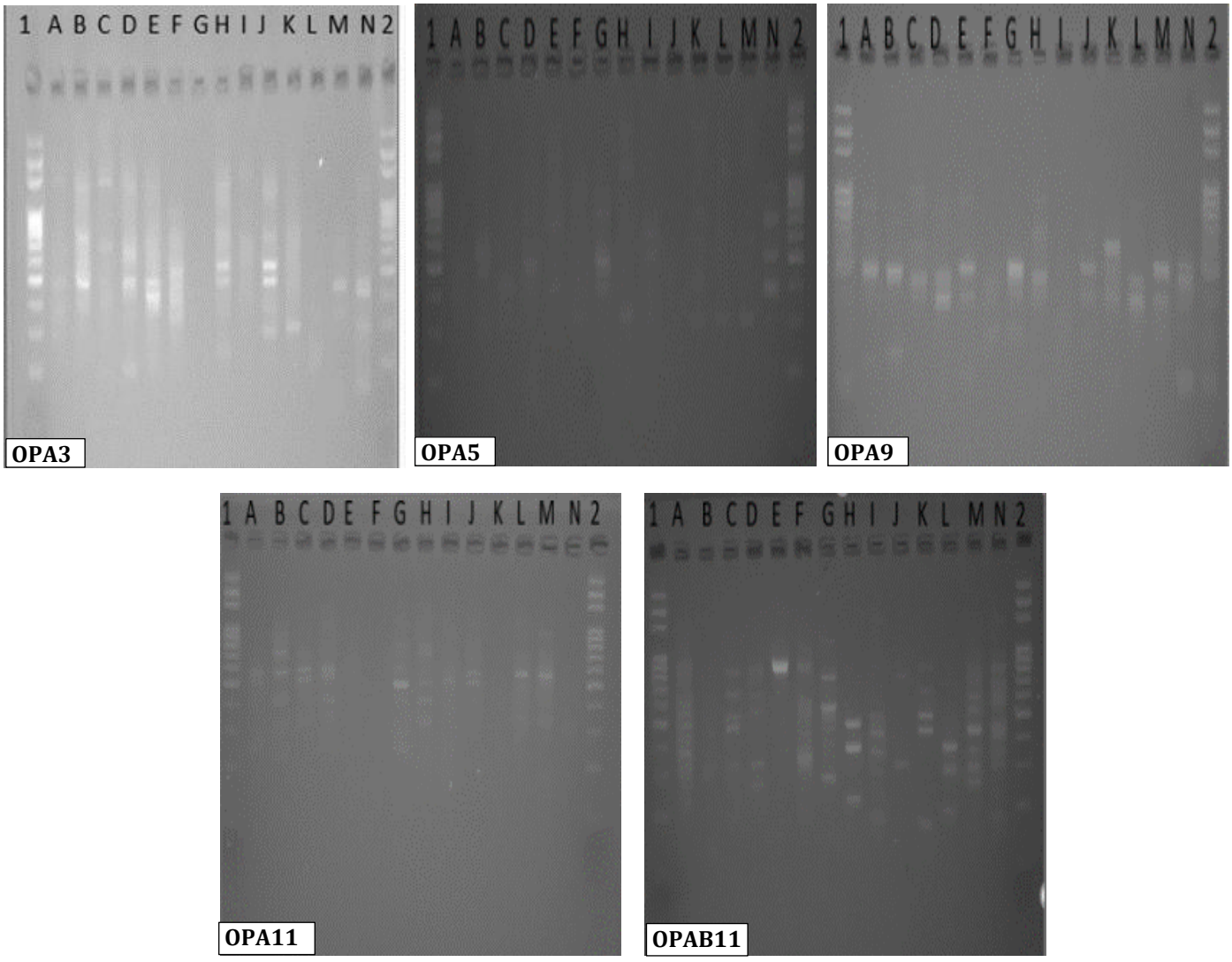

Figure 2. The amplification patterns of some cichlid samples using RAPD OPA-03, 05, 09, 011 and OPAB 11 primers. There are several polymorphic bands ranging from 200 bp to about 2000 bp

Table 2. Percentages of polymorphic loci among Cichlid samples

	Locations (water bodies)	Polymorphic loci (%)	Mean±SE
Coptodon zillii	Asa	66.67	62.22±6.79
	Oyun	71.11	
	Apodu	48.89	
Hemichromis fasciatus	Asa	14.29	35.71±13.53
	Apodu	74.29	
	Oyun	34.29	
	Moro	20.00	
Oreochromis niloticus	Asa	23.53	43.14±9.95
	Oyun	55.88	
	Moro	50.00	

Genetic similarities among *C zillii* samples from these different locations are also shown in Table 3. For *H. fasciatus,* the percentage of polymorphic loci was reduced in Asa and high in Apodu reservoir. For *C. zillii,* the percentage of polymorphic loci was less in Asa and high in Oyun river. C. *zillii* population, the highest values were recorded in Oyun river reservoir with the lowest recorded in Asa dam. Genetic similarities among *C. zillii* samples from these different locations are also shown in Table 3. Polymorphic loci amongst *H. fasciatus* and *O. niloticus* samples showed that the highest percentage was recorded in Apodu and Oyun respectively and lowest in cichlid population collected from Asa dam (Table 2). Result of analysis of molecular variance among the cichlid samples collected from four different sampling sites in Kwara state (Figure 3A-C).

Table 3. Pairwise population matrix of Nei unbiased genetic identity among cichlids

Asa Dam	Oyun River	Apodu Reservoir	Location
1			Asa Dam
0.968	1		Oyun River
0.914	0.977	1	Apodu

Asa Dam	Moro River	Oyun River	Apodu	Location
1				Asa dam
0.765	1			Moro river
0.726	0.73	1		Oyun river
0.762	0.754	0.865	1	Apodu reservoir

Asa Dam	Moro River	Oyun River	Location
1			Asa Dam
0.89	1		Moro River
0.848	0.758	1	Oyun River

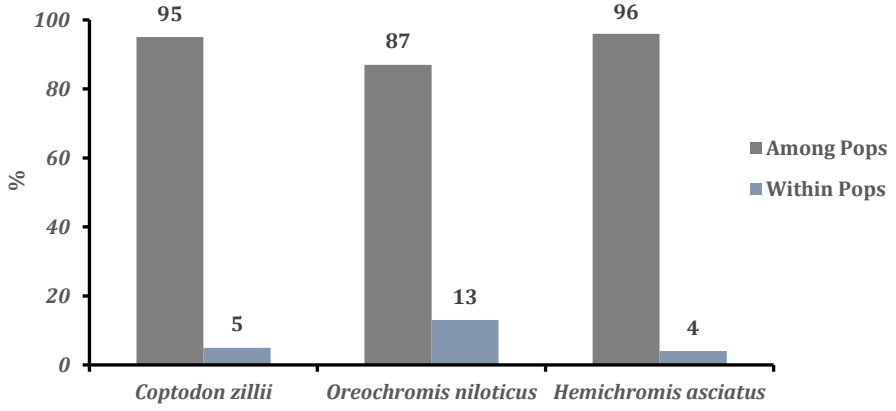

Figure 3. Percentages of molecular variance within and among Cichlid populations

In summary, percentages of estimated molecular variance among and within *C. zillii*, *H. fasciatus* and *O. niloticus* populations were 5% and 95%; 4% and 96% and 13% and 87%, respectively (Figure 3). Data from Figure 3 suggests that the cichlid populations are not genetically differentiated based on locations probably because these

populations might share the same alleles at similar frequencies. This explains the reason why genetic relatedness was high. Percentages of polymorphic loci among *C. zillii, H. fasciatus*, and *O. niloticus* samples from the different locations in Kwara state are shown in Table 3. Among *C. zillii* population, the highest percentage was recorded in Oyun river with the lowest recorded in Apodu. Polymorphic loci amongst *H. fasciatus* and *O. niloticus* showed that the highest percentages were recorded in Apodu and Oyun respectively, with the lowest in Asa dam cichlid population (Table 2). Genetic similarities among *C. zillii* samples from the different locations are also shown in Table 3. Genetic similarity values among the *O. niloticus* samples were high among samples collected from Asa dam and Moro river, but the similarity was low among cichlid samples from Moro and Oyun rivers (Table 3). Table 4 is a summary of the values of percentages of molecular variance within and among sampled cichlid populations.

Table 4. Summary of analysis of molecular variance (AMOVA) among and within cichlid populations

Species	Source of variation	df	Sum of squares	Estimated variance	Percentage of variation
Coptodon zillii	Among Population	2	30.016	0.538	0.05
	Within Population	19	214.575	11.293	0.95
	Total	**21**	**244.591**	**11.831**	**1**
Hemichromis fasciatus	Among Population	3	31.35	0.413	0.04
	Within Population	6	56.75	9.458	0.96
	Total	**9**	**88.1**	**9.872**	**1**
Oreochromis niloticus	Among Population	2	23.25	1.25	0.13
	Within Population	6	48.08	8.014	0.87
	Total	**8**	**71.333**	**9.264**	**1**

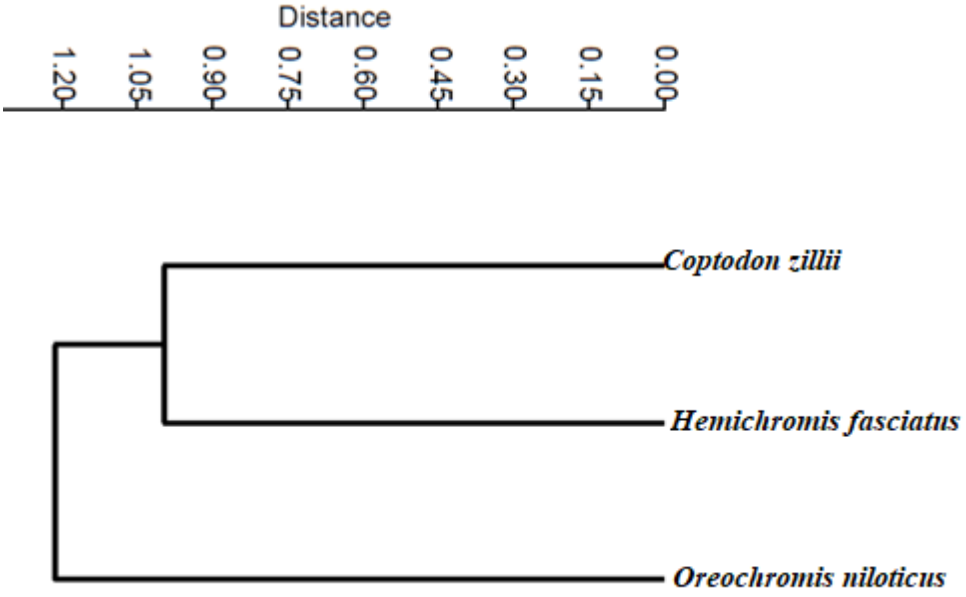

Figure 4. Dendrogram ofC. zillii, H. fasciatus and O. niloticuspopulations

DISCUSSION

Genetic distances of cichlid populations and dendrograms were constructed using an un-weighted pair group method of arithmetic mean (UPGMA) according to Nei's (32). The study of genetic diversity among the different types of fish is currently receiving growing attention all over the world (33). Cichlids are very diverse in the number of species and the phenotypes (13). This diversity allows for the study of the evolution, adaptation, and selection (34). They are also evolving new species and subspecies which are reproductively isolated without ecological separation (35). Different molecular methods such as microsatellite, restriction fragment length polymorphism and DNA barcoding have been used to characterize fishes but for this study RAPD was used. RAPD

has been used extensively for genetic analysis because for its successful genotyping, and there is no need to have a prior knowledge of the DNA sequence of the organism to be studied. It has applications in gene mapping, population genetics and plant and animal breeding (36). Population can be differentiated geographically or genetically using RAPD tool, which has been used to determine the adaptation within species to different environmental condition (37). It has also been used in determining the genetic variation of intra- and inter-population in Tilapia and *M. vittatus* that helps to differentiate the level of geographic isolation (38, 39).

RAPD markers have been used to generate new DNA markers in fish (33). Five RAPD primers were used in this study, and they exhibited varying ranges of band polymorphism. The RAPD fragments size varied from 200-2000bp size as it was reported by previous studies that RAPD markers are highly reproducible in size ranging between 200 and 1500 bp (28, 40). The five polymorphic primers in this study showed a total of 87, 72 and 51 bands in *C. zillii, H. fasciatus* and *O. niloticus,* respectively. A previous study reported a total of 202, 203 and 181 bands in populations of *O. niloticus, C. zillii* and *O. aureus* (41). Basavaraju and colleagues also observed 57.1% polymorphic bands out of 492 band recorded from 8 primers used to study diversity in common carp (*Cyprinus carpio* L.) (42). Also, it has been documented that the RAPD technique has been applied extensively to the study of phylogenetic relationship in different fish species including among cichlid species (38). Genetic variability study among individuals and populations is very important and can generate data on animals' response to environmental changes and survival (43). A recent study carried out on tilapia fishes reported high level of genetic variation among cichlids genera studied (44). Another study reported genetic differences within species (*T. zillii, T. guineensis*, and *C. gariepinus*), between species of the same genera (*T. zillii* and *T. guineensis*) and among *T. zillii, T. guineensis*, and *C. gariepinus* (45). Genetic diversity within and between two different populations of *M. vittatus* with a polymorphism of 64.98% using five RAPD primers has been studied (46). The mean percentages of genetic polymorphic loci recorded in this study is an indication that the studied cichlid populations have separate gene pools. The high intrapopulation variability and genetic homogeneity across the populations could have arisen by high levels of gene flow, which may have occurred over time. Alternatively, it may be that these populations have simply not been separated long enough to accumulate detectable genetic variations we observed in our study.

Ecological factor can bring about a population genetic differentiation. This study revealed that Asa dam cichlid samples have low level of genetic polymorphisms in all species studied compared to those collected from the water bodies of Apodu, Oyun and Moro. Molecular variance among the cichlid species studied was high in *C. zillii* followed by *H. fasciatus* and the lowest in *O. niloticus* when all samples from all locations were pooled together (Table 4). Molecular variance was higher within population than among population. The genetic variability observed might be due to aquatic pollution that have probably occurred over a long period of time in the waterbodies studied. This observation is consistent with a previous report on RAPD analysis of *O. niloticus* from various locations in Riyadh river which revealed genetic diversity in the fish population studied (47). Previous reports have suggested that Apodu, Oyun and Moro rivers may contain several types of aquatic pollutants which may be one of the reasons for genetic variability observed among fish populations in these studied waterbodies (48-50). Analysis of *O. niloticus, H. fasciatus* and *C. zillii* Cichlid populations for phylogenetic relationships with RAPD data generated showed distinct clusters according to fish species (Figure 4).

Analysis of molecular variance (AMOVA) showed that *C. zillii* and *H. fasciatus* were genetically closer, while *O. niloticus* appeared as an outgroup. In addition, the observed high level of genetic polymorphism within the studied cichlid populations and low level of genetic differentiation among populations may probably be due to extensive gene flow between natural populations of cichlids in each sampling site. This further suggests the existence of inbreeding in the studied cichlid populations that may lead to loss of genetic diversity and enhance genetic relatedness as observed in this study. Pairwise population matrix based on Nei's standard-UPGMA dendrograms showed genetic relatedness among *O. niloticus*, *H. fasciatus*, and *C. zillii* collected from different sampling sites. In summary, the dendrogram of total genetic relatedness and distance revealed that *C. zillii* and *H. fasciatus* were genetically closer, while *O. niloticus* appeared to be an outgroup (Figure 4). The level of genetic similarities in the population of species was higher in Apodu and Moro compared to Asa. This may further suggest that the Asa water bodies is slightly polluted. It has been suggested that genetic polymorphism allows fishes to develop adaptive mechanisms to survive in a constantly changing environmental conditions (44). Genetic variability in a population is important for biodiversity since it helps a population to adapt to changes in the environment. In addition, the genetic variation allows adaptation to change environmental conditions. This is consistent with a previous study (51) that reported low level of genetic variation in the fish stock collected from three different rivers in southern Kerala, India. Genetic diversity among different populations was studied and a high degree of genetic polymorphism was detected among Tilapia from Egypt (33, 52). Hence, studying fish management and conservation using RAPD has been a useful method to detect or estimate genetic diversity and variations as the pattern of bands are species-specific and may vary based on

the environmental changes (53). Selective breeding and sound management strategies are required to help improve and conserve tilapia fish stock (54).

Our findings showed that RAPD-PCR assay yields reliable and reproducible data for population genetic study of fishes.This confirms the efficacy of RAPD assay as a quick and efficient method for generating DNA markers in fish and environmental assessment. This study recommends conservation of genetic composition among the cichlids and their differentiation patterns by maintaining and restoring habitats, and careful management of fisheries. This will improve economic and nutritional qualities of fishes in Nigeria and Africa as a whole. In conclusion, it was validated in this study that RAPD markers can evaluate genetic variability within and among the wild populations of cichlids. However, other DNA markers, such as microsatellite DNA, can be used in further studies for better results.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTIONS

OAI conceived and designed the experiments; ATA, SOO, RDS, MMM and KOK assisted in the collection and preservation of the collected samples; OAI, RDS, ATA, LMN, ACA, KOK and SOO provided technical support towards the experiments; OAI, SOO and RDS, ACA wrote the first draft. All authors contributed to data interpretation and article preparation. All authors read and approved the final draft of the manuscript.

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