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MOLECULAR CHARACTERIZATION OF *LATES NILOTICUS* (PERCIFORMES, LATIDAE) POPULATIONS FROM THREE NIGERIAN WATERBODIES USING RANDOM AMPLIFIED POLYMORPHIC DNA AND MICROSATELLITE MARKERS

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Molecular Characterization of *Lates niloticus* (Perciformes, Latidae) Populations from Three Nigerian Waterbodies using Random Amplified Polymorphic DNA and Microsatellite Markers. Ogbuebunu, K. E., Awodiran, M. O. — Thirty *Lates niloticus* (Linnaeus, 1758) from three Nigerian waterbodies were genotyped on six RAPD primers and five microsatellites loci. RAPD revealed that effective number of alleles (A_E) at population level per locus was within the range of 1.641 ± 0.066 to 1.645 ± 0.041 while the mean number of alleles (A_N) across populations equals 2.000. Characterization on five microsatellites loci revealed genetic diversity within and among studied populations. Observed heterozygousity (H_O) was within the range of 0.317 ± 0.335 to 0.523 ± 0.315 while expected heterozygousity (H_E) was within the range of 0.414 ± 0.306 to 0.715 ± 0.097. Proportion of differentiation (F_{ST}) within populations was 0.236. Overall gene flow (N_m) among populations equals 0.806. This study established the successful use of RAPD and microsatellite as tools for studying population structure of fish species, especially *L. niloticus*. Thus, it can be concluded that *L. niloticus* in the three (3) sampled Nigerian waterbodies is undergoing evolution.
Key words: molecular characterization, *Lates niloticus* RAPD and Microsatellites.

Introduction

Lates niloticus (Linnaeus, 1758) is one of the largest freshwater fish reaching a maximum length of nearly two meters and weighing up to 200 kg (Greenwood et al., 1966). They are commonly known as the Nile or Niger Perch and belong to the family Centropomidae and genus *Lates*. *L. niloticus* is carnivorous and predacious in nature and widely distributed throughout the Afro-tropic eco-zone (Kaufman, 1992; Kaufman and Ochumba, 1993; Kahwa, 2013). They are distributed across major river basins and lakes of tropical Africa (North, East and West Africa) and endemic to northern Nigeria (Kahwa, 2013; Reed et al., 1967).

RAPD`s are DNA fragments from polymerase chain reaction application of random segments of genomic DNA with single primer of arbitrary nucleotide sequence. RAPD serves as both forward and reverse primers, and are usually able to amplify fragments from 1–10 genomic sites simultaneously. They are quick and easy to assay, require a low quantity of DNA with no sequence data for primer construction (Welsh and McCelland, 1990) and possess high genomic abundance (Williams et al., 1990). Also, Microsatellites are DNA sequences repeated in tandem and are widely distributed in the genome. They are codominant markers that are highly polymorphic and locus specific (Ellegren, 2004; Jentzsch et al., 2008).

Molecular characterization has become more relevant in genetic diversity studies and has assisted in assessing the genetic constitution of species through quantitative and qualitative measures of diversity, using DNA markers which analyze and provide useful information on origin, relationship or relatedness, uniqueness, abundance of several genes in a gene pool of a particular fish species in a population (Groeneveld et al., 2010). This DNA markers' data can be applied in various ways such as the isolation of genes of economic and scientific value, conservation of species and isolation of information on the history of a species or genus. Today, genetic diversity studied at molecular level has become an active area of research. This includes the use of genetic markers to estimate the functional variations within and across species of fish and other animals or plants.

Methods

Study areas

Kainji Lake is located at Borgu local government area of Niger State (9°50′–1°55′ N and 4°23′–4°45′ E). River Benue, Makurdi is located in Benue state (7°44′ N and 88°31′ E). Ikere-gorge reservoir is located at Iseyin local government area of Osun State. It is one of the nine (9) dams owned and managed by the Ogun/Osun River Basin Development Authority (O-ORBDA) (3°401′ N and 3°501′ N and 8°101′ E and 8°201′ E). Fig. 1 is the map of Nigeria showing the study areas.

Sampling

Thirty (30) *L. niloticus* specimens were obtained from Kainji Lake, River Benue at Makurdi and Ikere Gorge reservoir over a period of three (3) months (from September–November, 2015). Pectoral fin clips of *L niloticus* were collected with the aid of a dissecting set. The collected fin clips was preserved in 80 % ethanol in transparent sample bottles and kept at 4 °C in a thermocool freezer for subsequent genomic deoxyribonucleic acid (DNA) extraction.

DNA extraction

Genomic DNA of *L. niloticus* fins were isolated using CTAB method. 0.3 g of the fin tissue was homogenised in an Eppendorf tube, 600 µl of 2 X CTAB buffer was added after which the sample was incubated at 65 °C for 30 minutes. It was removed from the incubator and allowed to cool to room temperature, chloroform was added and the sample was mixed by gently inverting the tube several times. Thereafter, the sample was spun at 14,000 rpm for 15 minutes, the supernatant was transferred into a new eppendorf tube and equal volume of cold Isopropanol was added to precipitate the DNA. The sample was kept in the freezer for 1 hour and later spun at 14,000 rpm for 10 minutes, the supernatant was discarded and the pellet was washed with 70 % ethanol and air dried for 30 minutes on the bench. The pellet was re-suspended in 100 µl of sterile distilled water. The DNA concentration of the samples was further measured on spectrophotometer at 260 nm and 280 nm after which the genomic purity was determined (Edward and Thompson, 1991).

PCR Reaction mix for RAPD

The reaction mix was carried out in 20 µl final volume containing 60–80 ng genomic DNA, 0.1 µM of the primers, 2 mM $MgCl_2$, 125 µM of each dNTP and 1 unit of Taq DNA polymerase. The thermocycler profiles used has initial denaturation temperature of 3 minutes at 94 °C, followed by 45 cycles of denaturation temperature at 94 °C for 20 seconds, annealing temperature of 37 °C for 40 seconds and primer extension temperature of 72 °C for 40 seconds, followed by final extension temperature at 72 °C for 5 minutes was added (Mullis et al., 1986).

PCR Reaction mix for microsatellites

The reaction mix was carried out in 20µl final volume containing 60 ng–80 ng genomic DNA, 0.1 µM of forward and reverse primers, 2 mM $MgCl_2$, 125 µM of each dNTP and 1 unit of Taq DNA polymerase. The thermocycler profiles used has initial denaturation temperature of 3 minutes at 94 °C, followed by 35 cycles of denaturation temperature at 94 °C for 30 seconds, annealing temperature of 60 °C for 45 seconds and primer extension temperature of 72 °C for 60 seconds, followed by final extension temperature at 72 °C for 10 minutes was added (Mullis et al., 1986).

Gel Electrophoresis

PCR amplicon electrophoresis was carried out by size fractionation on 1.4 % agarose gels. Agarose gels were prepared by dissolving and boiling 2.8 g agarose in 200 ml 0.5 X TBE buffer solution. The gels were allowed to cool down to about 50 °C and 10µl of 5mg/ml Ethidium Bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, the PCR amplicons were loaded in the well created. Electrophoresis was done at 100 V for 2 hours. The DNA was visualized and photographed on UV light source (Smithies, 1955).

Band scoring and data analysis

The banding pattern from the gel was scored for both RAPD and microsatellites while RAPD banding profile was transformed into numerical values, where the presence of a band is scored as 1 and absence of a band was scored as 0. Genetic diversity parameters were calculated using GenAlex 6.1 Software (Peakall and Smouse, 2006).

Results

L. niloticus genotyped on six RAPD primers (table 1) produced fifty-four bands; expected heterozygousity (H_E) across the populations was 0.387 ± 0.015, 0.377 ± 0.027 and 0.374 ± 0.027 (table 3) for Kainji, Makurdi and Ikere-gorge respectively. The mean number of alleles (A_N) across the three populations was 2.00 ± 0.00, effective number of alleles (A_E) was 1.645 ± 0.041, 1.641 ± 0.066 and 1.643 ± 0.065 for Kainji, Makurdi and Ikere-gorge respectively, while Shannon information index (I) was within the range of 0.556 ± 0.032 to 0.574 ± 0.017 (table 3). Analysis of molecular variance (AMOVA) showed that 100 % of the total molecular variance was within the population and 0 % was among the population (table 6). The Dendrogram separates the populations of *L. niloticus* into three (3) major clusters (table 2).

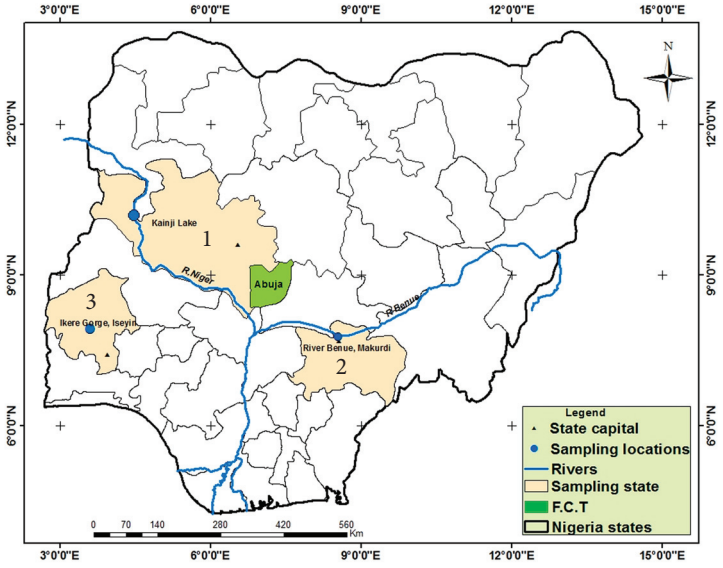

Fig. 1. Map showing the sample locations of *L. niloticus* (Linnaeus, 1758). Population 1 — Kainji lake, Population 2 — River Benue, Makurdi and Population 3 — Ikere-Gorge reservoir, Iseyin, Oyo state.

In the SSR analysis, thirty-seven alleles were scored. The mean number of alleles (A_N) per population was within the range of 11.400 ± 0.678 in Kainji Lake to 12.400 ± 0.510 in Ikere-gorge reservoir while the effective number of alleles (A_E) contributing to the population was within the range of 9.00 ± 0.599 in Kainji Lake to 10.252 ± 0.605 in Ikere-gorge reservoir (table 4). The Shannon information index (I) was within the range of 2.307 ± 0.059 in Kainji Lake to 2.413 ± 0.052 in Ikere-gorge reservoir while observed heterozygousity (H_O) per population was within the range of 0.176 ± 0.301 to 0.523 ± 0.315 in Ikere-gorge reservoir and Kainji Lake respectively (table 4). The expected heterozygousity (H_E) per population was within the range of 0.414 ± 0.306 to 0.715 ± 0.097 for Ikere-gorge reservoir and Kainji

Table 1. RAPD primer sequences

Primer names	Primer Sequences
OPH-05	AGTCGTCCCC
OPT-06	CAAGGGCAGA
OPT-20	GACCAATGCC
OPB-08	GTCCACACGG
OPB-12	CCTTGACGCA
OPB-20	GGACCCTTAC

Table 2. Microsatellites sequence

Primer Names	Sequences	Tm
UNH 934 F	ACTGCAATGAAATGCTGCTT	56
UNH 934 R	CCATTCCTCAGAGCACAACA	60
GM 166 F	TGTGAGGCTCTTCTTTTGCT	59
GM 166 R	AAGAGCGGTGGGTGGAC	62
GM 385 F	GGTGGGCAGTGTGTGTTTTT	60
GM 385 R	TTTTCATCCAGGCCTCACTT	58
UNH 919 F	TGACAGCCTGGCATAATGAG	60
UNH 919 R	CACTGAGACTGGAAGGCACA	62
GM 634 F	CTGAAACATGACTGCAGGAG	60
GM 634 R	CCCGACATTAAACTTTCAGCA	59

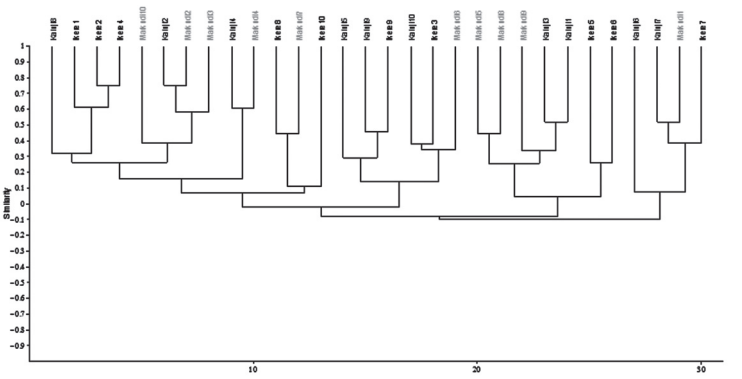

Fig. 2. Dendrogram using RAPD.

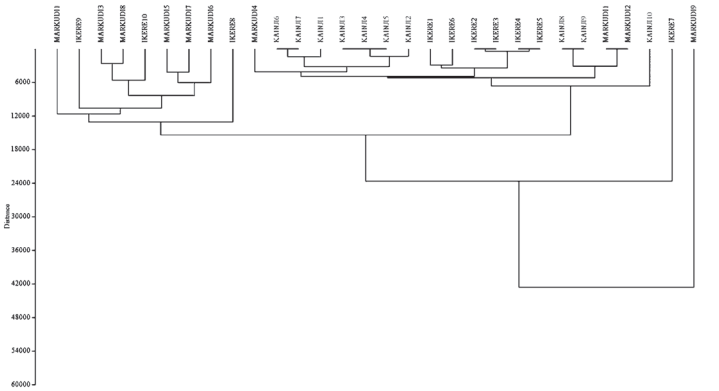

Fig. 3. Dendrogram using microsatellites.

Table 3. RAPD results

Sampling areas	MNA	ENA	Shannon index	H_E
Kainji	2.000 ± 0.000	1.645 ± 0.041	0.574 ± 0.017	0.387 ± 0.015
Makurdi	2.000 ± 0.000	1.641 ± 0.066	0.559 ± 0.031	0.377 ± 0.027
Ikere-gorge	2.000 ± 0.000	1.634 ± 0.065	0.556 ± 0.032	0.374 ± 0.027

Note. MNA — mean number of alleles; ENA — effective number of alleles; H_E — expected heterozygousity.

Table 4. Microsatellites results

Sampling areas	MNA	ENA	Shannon index	H_O	H_E
Kainji	11.400 ± 0.678	9.000 ± 0.678	2.307 ± 0.059	0.523 ± 0.315	0.715 ± 0.097
Makurdi	11.600 ± 0.600	9.568 ± 0.568	2.344 ± 0.054	0.317 ± 0.335	0.588 ± 0.167
Ikere-gorge	12.400 ± 0.510	10.252 ± 0.605	2.413 ± 0.052	0.414 ± 0.306	0.414 ± 0.306

Note. MNA — mean number of alleles; ENA — effective number of alleles; H_E — expected heterozygousity; H_O — observed heterozygousity.

Table 5. Population structure of *L. niloticus* populations using microsatellites

F_{IS}	F_{IT}	F_{ST}	N_m
0.407	0.547	0.236	0.806

Note. F_{IS} — inbreeding coefficient at population level; F_{IT} — inbreeding coefficient at total sample level; F_{ST} — proportion of differentiation; N_m — Gene flow.

Lake respectively (table 4). Analysis of molecular variance (AMOVA) showed that 62 % and 38 % of the total molecular variance was within and among populations respectively (table 6). Changes in genetic and

Table 6. Analysis of molecular variance (AMOVA), %

	RAPD	MICROSATELLITES
Within population	100	62
Among population	0	38

Table 7. Conformity to Hardy-Weinberg equilibrium

Populations	Locus	Df	ChiSq	Probability	Significance
Kainji	UHH 934			Monomorphic	
Kainji	GM 166	1	0.311	0.577	ns
Kainji	GM 385	1	10.00	0.002	**
Kainji	UNH 919	1	10.00	0.002	**
Kainji	GM 634	1	10.00	0.002	**
Makurdi	UHH 934	3	15.566	0.001	**
Makurdi	GM 166	15	18.750	0.225	ns
Makurdi	GM 385	21	22.540	0.369	ns
Makurdi	UNH 919	6	20.278	0.002	**
Makurdi	GM 634	10	40.000	0.000	**
Ikere-gorge	UHH 934	6	13.511	0.036	*
Ikere-gorge	GM 166	6	8.776	0.187	ns
Ikere-gorge	GM 385	1	0.123	0.725	ns
Ikere-gorge	UNH 919	3	20.000	0.000	**
Ikere-gorge	GM 634	3	20.000	0.000	***

Note. ns — not significant; *P < 0.05; **P < 0.01; ***P < 0.001; H_0 (P > 0.05) — null hypothesis — population mating randomly; H_1 (P < 0.05) — alternative hypothesis — population not mating randomly.

genotypic structure which measures the extent of inbreeding, genetic drift (divergence) and conformity to Hardy-Weinberg equilibrium in fish populations was determined using hierarchical F-statistics. The inbreeding coefficient value at population level (FIS) was 0.407 (table 5); this was relatively higher than F-value of zero (0), hence, indicating divergence and inbreeding within the populations. Also, the coefficient FIT which measures the divergence and inbreeding at sample level was 0.547 (table 5), the value clearly extends from the range of zero (0) to one (1), therby indicating the presence of inbreeding. The coefficient FST (fixationa index) which is significant in assessing genetic differentiation within populations was 0.236 (table 5). This indicates that on the average, 23.6% of total genetic diversity is present within a given population of the fish. Similarly, the overall gene flow (Nm) among the fish population was 0.806 (table 5); this gave an estimate of the average number of migrants between the studied populations per generation. The F-statistics value which clearly indicates heterozygotes deficiency was compared to Hardy-Weinberg equilibrium (table 7). This indicates significant departure from Hardy-Weinberg equilibrium by showing deviations for at least one locus per population at P < 0.05, P < 0.01 and P < 0.001(except locus GM 166 in the three (3) populations and GM 385 in Makurdi and Ikere-gorge populations). Figs 2 and 3 show the UPGMA dendrograms' clusters depicting the genetic relationship among the populations of *L. niloticus* studied.

Conclusion

The characterization of *L. niloticus* from three Nigerian waterbodies using RAPD and Microsatellites markers revealed genetic diversity and comparatively, microsatellites consistently recorded higher values in all genetic diversity indices which indicate great allelic diversity and high genetic variation. With reference to previous studies on *L. niloticus* Lake Victoria populations (Basiita et al., 2015 and Mwanja et al., 2014), there is an affirmative correlation in high genetic diversity with this present study; as expected heterozygosity, which was relatively high in both studies (Basiita et al., 2015 (0.49) and Mwanja et al., 2014

(0.68) correlates with expected heterozygosity for both molecular makers (RAPD (0.387) and (microsatellites (0.715) in this study. Both markers were able to characterise *L. niloticus* populations and establish their successful use as viable tools for studying the population structure of fish species. Therefore, from all genetic diversity indices studied, we conclude that *L. niloticus* in the sampled locations are undergoing inbreeding, non-conformity to hardy Weinberg equilibrium and evolution.

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