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MICROSATELLITE VARIABILITY OF TWO POPULATIONS OF *CLARIAS GARIEPINUS* (SILURIFORMES, CLARIIDAE) IN NIGERIA

M. O. Awodiran[1*], F. O. Adeniran[2], R. O. Akinwale[3], A. A. Akinwande[2]

[1]*Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria*
[2]*Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife, Nigeria*
[3]*Department of Aquaculture and Fisheries, University of Ilorin, Ilorin, Nigeria*
E-mail: michfemi@yahoo.com
[*]*Corresponding author*

Microsatellite Variability of Two Populations of *Clarias gariepinus* (Siluriformes, Clariidae) in Nigeria. Awodiran, M. O., Adeniran, F. O., Akinwale, R. O., Akinwande, A. A. — The study evaluated the genetic signatures of the fishes from the two populations and compared the pattern of differentiation of the two populations with a view to separating the species from the different populations into possible sub-species. Forty (40) specimens were collected from River Niger (Lokoja) and Asejire Resevoir. The DNA of the twenty (20) specimens from each population extracted from the muscle tissue using phenol-chloroform extraction (PCE) method was subjected to microsatellite DNA analysis. Seven (7) microsatellite markers (Cga01, Cga02, Cga03, Cga05, Cga06, Cga09 and Cga10) were used in the analysis. Microsatellite DNA analysis of the two populations revealed significant differentiation between the two populations as shown by the high values of heterozygosity, low level of inbreeding and non-conformance to Hardy-Weinberg's equilibrium. It is concluded from the study that microsatellite analysis showed a high potentiality for separation of the populations.
Key words: differentiation, diversity, heterozygosity, microsatellite DNA analysis, populations.

Introduction

C. gariepinus (Burchell, 1822) which belongs to the family Clariidae is an economically important freshwater fish species that contributes immensely to the annual freshwater fish production in Nigeria. It is readily acceptable among Nigerian fish farmers and consumers; hence, it commands high commercial values, and is an important source of animal protein. It also serves as a model organism in research (Volckaert et al., 1994). It is cultured intensively and extensively in Africa, Europe and Asia. The economic benefits of this fish species are due to its hardiness, fast growth, large size attainable and ability to withstand and tolerate harsh environmental conditions (Welcomme, 1988; Hecht et al., 1988).

The highest diversity of the family Clariidae is found in Africa with 14 genera and 92 species (Teugels, 1986 a), while only 2 genera with some 17 species are presently known from Asia (Teugels, 1996). The genus *Clarias* is the most common and popular of the family Clariidae containing 32 species in Africa (Teugels, 1986 b).

In spite of its economic and scientific importance, little information is available on the phylogenetic relationship among the few catfish populations in Nigeria. Microsatellite markers constitute one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms (Bartish et al., 2000; Garg et al., 2009). Therefore, the primary objective of this study was to identify genetic similarity and diversity within and between the two populations of *C. gariepinus* from two different vegetation zones of Nigeria using microsatellite markers. Scientifically sound management of fish resources relies on basic knowledge of the biology of the species, including information on the population structure. Such information may influence the development of management strategies coupled with strategies for conserving its biodiversity. Therefore, there is need to understand the genetic composition and diversity of natural population of *C. gariepinus* in order to evaluate the latent genetic differentiation across the vegetation zones of Nigeria using molecular methods, hence this study.

Material and methods

Study sites and sampling collection

Specimens of *C. gariepinus* were collected from Lokoja and Asejire with the help of local fishermen. Eighty (80) specimens of *C. gariepinus* (40 individuals from each location) were transported live to the laboratory before being sacrificed.

Asejire Lake (fig. 1) is located at about 30 km East of Ibadan, Oyo State, Nigeria. Asejire Lake is a man-made lake constructed over River Osun; the construction project was completed in 1972. It is located between latitude 7°21′45″ N and 4°08′00″ E and longitude 7.36° N and 4.13° E. The lake is Y-shaped with two unequal arms of the Y while the catchment area above the dam is 7,800 km^2 and the impounded area is 23.42 km^2. The lake has gross storage capacity of 7,403 million litres of water at an altitude of 137m above sea level (Yem et al., 2011). Asejire lies within the tropical zone and in the rainforest region of Nigeria with an average rainfall of 1,348 mm.

The second area of study is Ganaja River side in Lokoja (part of River Niger). Lokoja is located between latitude of 7°49′00″ N and 6°45′00″ E and longitude 7.817° N and 6.750° E. 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 (Alabi, 2009; Suleiman et al., 2014). Lokoja has vegetation characterized by mixed leguminous (Guinea) woodland to forest savanna.

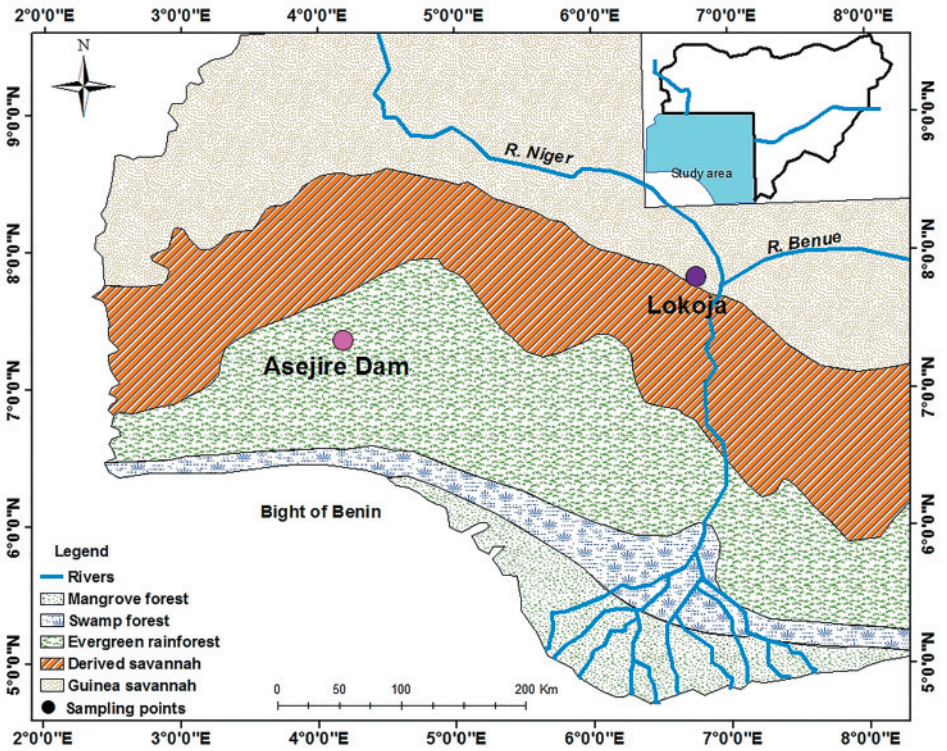

Fig. 1. The study area showing the map of sites of collection of *C. gariepinus*.

DNA Extraction (DNAe) from Fish Tissue

Specimens of *C. gariepinus* were kept alive for about 24 hours in the Zoology Laboratory to minimize stress, while twenty (20) specimens from each population were used for molecular studies. Approximately 1 cm × 1 cm of muscle tissue were cut freshly from fishes for molecular studies, preserved in 80 % ethanol and kept at 4 °C for subsequent DNAe of which standard method was used.

Genomic DNA for genotyping was prepared using PCE while random labeling with ^{35}S was used to visualize the PCR products (Weber and May, 1989). The final concentration of the reagents in the volume of 12.5 μL is as follow: 10–100 ng of genomic DNA, 1×PCR — buffer (75 mM Tris-HCL pH 9.0, 20 mM $(NH_4)_2SO_4$, 0.01 % Tween 20), 1 mM $MgCl_2$, and 15 mol of each primer.

For the isolation of total genomic DNA, a short procedure was applied according to a modified protocol reported by Wu et al. (1995). Muscle tissues (200–500 mg) were placed in a 1.5ml micro-centrifuge tube and homogenized by using Eppendorf micro pestle. The homogenized tissue, 0.5 ml of lysis buffer (4 mM NaCl, 0.5 mM EDTA, 0.1 % SDS and 0.02 NP 40) and 0.01 % proteinase K were added, mixed gently and incubated at 55 °C on dry bath (Genei, Model-SLM-DB-120) for 45–60 minutes for complete lyses of cells. After incubation, 250 μl chloroform and 250 μl phenol were added, mixed gently, and centrifuged at 10,000 rpm at room temperature (High Speed Brushless Centrifuge, MPW-350R) for 5 minutes and the supernatant was then transferred to a new micro centrifuge tube. Chloroform (250 μl) and 250 μl of 7.5 M ammonium acetate were added, mixed well and centrifuged at 10,000 rpm for 5 minutes at room temperature.

The DNA was precipitated from supernatant with two volumes of 99 % ethanol (ice-cold) and the DNA pellet were washed with 70 % ethanol, dried and dissolved in a Tris-EDTA buffer (10 mM Tris HCl, 1 mM EDTA, pH 7.6). UV-VIS Spectrophotometer (ND-1000, USA) was used to check quality as well as the quantity. DNA and the concentration of extracted DNA were adjusted to 50 ng/μl for PCR amplification.

C. gariepinus tissue was collected and stored at –20 °C for about 2 days, put into a small tube and centrifuged for about 30s. About 5 μl of proteinase K was added to the tubes containing the centrifuged samples, then 1.2 μl of RNase was added and was centrifuged for about 30s, the tubes were slightly vortexed for 30 s and then centrifuged for another 30s. The tubes containing the specimens were put into hot water bath and incubated overnight at 55 °C. 300 ml of TE buffer was added to the tubes after it was taken out from hot water bath, 6 μl of phenol was then added to the tubes, then (25 : 24 : 1) phenol, chloromethane and iso-amyl alcohol was prepared in the ratio specified and then carefully added into the tubes which were then centrifuged for 10 minutes.

The supernatant filtrate was collected from the upper part of the tube to new tubes while the solution prepared was added to the filtrate in new tubes and covered. The tubes were centrifuged and the resultant filtrate was collected from the upper part of the centrifuged tubes and put in another new tubes, solution prepared was added to the new tubes containing the new filtrate and centrifuged.

The resultant filtrate was collected from the centrifuged tubes into another tube, and then ethyl alcohol absolute was added to the collected filtrate and allowed to settle for 12 hours, then centrifuged while the upper filtrate was poured away with care so as to prevent the settled DNA from being poured away. 70 % alcohol was added to the DNA that settled down in the tubes, covered, shaken and was centrifuged, the upper filtrate was poured away, then, the tubes was put into test tube racks wrapped carefully with tissue paper and kept in the tubes containing the resultant DNA samples which were arranged into micro test-tube racks, wrapped with tissue paper and air-dried for 10 minutes for it to re-dissolve. The air-dried DNA pellets were dissolved in 300 μl 1 x TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH = 8.0) to prevent protein and other contaminants that may inhibit PCR reactions. The covered tubes were vortex and returned to the oven. Little of the content of the tubes was taken and diluted with sterile water to determine the purity and concentration of the DNA samples in the spectrophotometer which were kept in the refrigerator and then centrifuged.

Samples were centrifuged before DNA isolation protocol commenced, the procedure was in accordance with saturated salt procedure described by Sambrook et al. (1989) and Barlett et al. (1996) which involved tissue thawing and thorough homogenizations, centrifugation (which was observed regularly to avoid clogging), DNA wash, DNA precipitation and solubilization was done.

Total genomic DNA isolated from the tissue, beginning with cell lyses and exactly 200 μl of whole tissue was pipetted into 1.5 ml micro centrifuge tube and suspended in 300 μl lysing buffer (44 mM NH_4Cl, 10 mM NH_4HCO_3) with centrifugation at 1000 rev for 10 minutes to pellet cells; RNA-free genomic DNA was required and 20 μl of RNase of stock solution was added to the samples the resultant supernatant was removed and replaced with 1ml SET buffer (10 mM Tris-HCl, PH = 8.0, 200 mM of common salt (NaCl), 0.1M EDTA and 0.5 % SDS) and the samples digested with 0.5 μg/μl Proteinase K stored dried at 4 °C to ensure efficient lyses and yield of homogeneous solution, samples and buffer was mixed and vortex for 15s and the resultant mixtures was placed in hot water bath, incubated overnight at 55 °C. After overnight incubation, high molecular weight DNA was extracted twice with phenol-chloroform-isoamyl (25 : 24 : 1v/v)–(phenol chloromethane and isoamyl alcohol).

In each case, phenol-chloroform-isoamyl added to the resultant filtrate collected into new tubes after repeated centrifugations and the DNA samples precipitated by 70 % ethyl alcohol absolute. The resultant tubes of DNA samples was arranged into micro test tube racks, wrapped with tissue paper and was air-dried for 10 minutes to dissolved. The air-dried DNA pellets was dissolve in 300 μl 1 x TE buffer (10 mM Tris–HCl,

1 mM EDTA, pH = 8.0) to prevent protein and other contaminants that may inhibit PCR reactions and vortex. The content of each micro centrifuge tube of the DNA was divided into two, one for further use and be stored at –20 °C and the other tube diluted with sterilized water in other to determine the purity and concentration of each DNA sample.

DNA Yield and Purity

The concentration (purity) of total DNA from each *C. gariepinus* was determined. The absorbance at 260 nm and 280 nm was measured and the optical density (OD) or appropriately called purity of DNA values was calculated as the ratio of absorbance at 260 nm to absorbance at 280 nm and for pure DNA, an A260/280 ratio expected to be 1.7–1.9 (QI Amp Blood kit, 1996). Isolated DNA samples values that fall within this range were selected for use. Following the determination of the OD 260/280 value of the samples, the concentration of the total DNA was adjusted to 100 ng/µl and exactly 1 µl of the DNA samples was used as template PCR and the quality and concentration of fewer number of samples were assessed by agarose gel electrophoresis in initial trial during which reaction conditions were optimized and reaction programme developed.

PCR

In this study, seven commercially available oligonucleotide primers (ten to twenty bases long) were used to initiate PCR amplifications. Primers were randomly selected on the basis of $(GT)_n$ content and annealing temperature for Microsatellite PCR amplifications. The primers used included Cga01, Cga02, Cga03, Cga05, Cga06, Cga09 and Cga10. The primers were chosen since they were already used in the literature for clariid fish's population studies (Galbusera et al., 1996).

The composition of the PCR reaction mixture with the final volume of 25 µl in each micro-PCR tube, contained 1 µl template DNA, 2.5 µl of 10 x Buffer, 1 µl of 25 mM dNTPs, 2 µl (that is, 1 µl labeled forward plus 1 µl unlabeled reverse form) of each pair of primer, 0.2 µl of (5 U/µl) *Taq* DNA polymerase, 2.2 µl of 25 mm/mol Mg2+ and 16.1µl sterilized distilled water.

The thermo cycling PCR reaction was carried out in the thermal cycler at (94 °C, 300 s) of initial denaturation, 35 cycles of denaturation at (94 °C, 60 s), annealing temperatures of the primers were adjusted through optimization using several temperature values, and extension at 72 °C, 60 s. This was followed by final extension at 72 °C, 60 s.

After PCR, the PCR products (samples) were heat-denatured at 94 °C for 600 s in the PCR system and were transferred to ice-box (chilled at 0 °C) before loading into the gel containing 12 % polyacrylamide solution (PAGE), which contained 210 g of 6 M urea, 38 g acrylamide and 2 g N,N'-methylene bisacrylamide (19 : 1), added together with TBE-buffer made up of 5.4 g Tris, 2.75 g boric acid and 2 ml of EDTA and all these were standardized with sterilized water. Exactly 25 µl of 30 % TEMED ($C_6H_{16}N_2$) and 250–300 µl of 10 % ammonium persulphate (AP) were added to each 60 ml of PAGE solution to serve as cross-links, then they were mixed carefully and poured gently into the gel cassettes made up of 20 x 20 cm double-glass plates clamped together by iron-clips.

Following this, two plastic combs, each with 26 flat-teeth wells were gently inserted into the upper part of the glass, a wedge gel of 0.4 mm thick was used. Before loading the amplified products, 1µl of blue load was placed on a tray and added to 10 µl of each of the amplified product, mixed gently and loaded to each lane in each of the glass trough. The fourteenth lane in each of the glass trough contained only the commercial internal size standard (4 µl loading dye mixed with 1 µl of microsatellite marker- either pBR322 DNA/MspI or ROX to determine the size of the amplified products. Buffer 1 x TBE was placed in the glass trough to run the gel. PCR products were separated on poly acrylamide gel electrophoresis which lasted for 2 hours at 100V, 10 mA, using a drop of ethidium bromide (etBr) as staining agent before visualization of the products under UV trans-illuminator. The photographs were taken while Genotyper (version 2.0) DNA fragment analysis software (EASTMAN KODAK) was used for all analyses.

Electrophoresis was conducted on 2 % Agarose gel and scored by comparison to 8-bp standard DNA ladder (Jena Bioscience, LÖbstedter, Germany) with the following values 75, 154 and 220.

Data analyses

Data obtained from the seven polymorphic loci were induced in the analyses, allele frequencies, mean number of alleles per locus, observed and expected heterozygosity were obtained from Microsatellite Analyzer (MSA) Version 4.05 by Dieringer and Schlötterer (2003) computer package.

Based on microsatellites and allele frequencies that were produced, the proportion of homozygosity was obtained by using the method of Nei (1972), the gene diversity (heterozygosity) of each locus per population called (hi) was also determined. The gene diversity among loci in each population (i. e. H), was expressed according to Nei (1987) and Zhang et al. (2010), the genetic variability parameter that considered the alleles including rare alleles called polymorphism information content (PIC) was obtained, and the mean of this parameter (PIC) was also calculated using the method of Botstein et al. (1980). The cumulative power of discrimination across all populations (CPD) was determined using the method of Fan et al. (2002). Allele frequencies were also generated. Input allele frequency data was carried out using Microsoft excels worksheet and analyzed with DISPAN package. This was done to obtain the unbiased gene diversity (H_S), total heterozygosity (H_T) and

the genetic differentiation or the fixation coefficient of subpopulations within total populations. F-statistics which included observed heterozygosity (H_O), total inbreeding estimates (F_{IT}) and inbreeding coefficient of individuals within the subpopulations (F_{IS}) for the loci were generated with F_{STAT} version 2.9.3.2 computer software programme (Goudet, 2002). Wright's F_{ST} values among pairs of populations were generated using F_{STAT} software, Shannon information index (P), the identity similarity matrices (I), standard genetic distance (Nei, 1972) were determined.

Results

Molecular results

Table 1 shows the characterization of the seven loci which includes the different primer sets used, repeat arrays, primer sequences, gene bank accession numbers, specific annealing temperature, amplicons size and observed number of alleles. All the primers were amplified successfully and resulted in clearly scorable bands.

The design of primers for microsatellite loci

The microsatellite primers which were isolated through the production and screening of a library of short fragments of genomic DNA of *C. gariepinus*, PCR conditions for the seven loci were optimized to yield clear bands, whose sizes were in accordance with the sizes predicted by the sequence information, the sizes of the PCR products and number of alleles are shown in table 2. All of these primer sets produced PCR bands that were inherited in Mendelian fashion. The number of alleles per locus ranged from 3 to 7. Variation of allele sizes is quite low for Cga03 locus which indicates that the individuals in the population did not differ greatly at the locus specified by this marker. The seven markers were used to analyze specimens collected from Lokoja and Asejire which revealed fairly high amount of allelic polymorphism and heterozygosity from 8–70 % (mean 53 %) for 40 specimens (table 2).

Gene frequencies and genetic variability

Gene frequencies of the polymorphic loci for each population are shown in table 3. Genetic variability which was based on specimen size of 20 fish per locus per population

Table 1. Characteristics of the microsatellite markers used in this study

Locus name	Repeat array	Primer sequences (5′–› 3′)	Genbank accession number	($MgCl_2$) in mm	Annealing temperature (°C)	Size of PCR product (bp)	Number of alleles
Cga01	$(GT)_{15}$	GGCTAAAAGAACCCTGTCTG	U30862	1	59	92–102	5
		TACAGCGTCGATAAGCCAGG					
Cga02	$(GT)_{10}N_2(GT)_8$	GCTAGTGTGAACGCAAGGC	U30863	1	58	102–110	5
		ACCTCTGAGATAAAACACAGC					
Cga03	$(GT)_{21}$	CACTTCTTACATTTGTGCCC	U30864	1	56	100–168	6
		ACCTGTATTGATTTCTTGCC					
Cga05	$(GT)_{11}N_2(GT)_2$	TCCACATTAAGGACAACCACCG	U30866	1.5	60	194–206	7
		TTTGCAGTTCACGACTGCCG					
Cga06	$(GT)_5N_2(GT)_9$	CAGCTCGTGTTTAATTTGGC	U30867	1.5	60	134–140	3
		TTGTACGAGAACCGTGCCAGG					
Cga09	$(GT)_3N_3(GT)_{11}N$	CGTCCACTTCCCCTAGAGCG	U30871	1	65	180–204	6
	$(GT)_6N_2(GT)_4$	CCAGCTGCATTACCATACATGG					
Cga10	$(GT)_2N_2(GT)_{15}$	GCTGTAGCAAAAATGCAGATGC	U30870	1	60	100–114	5
		TCTCCAGAGATCTAGGCTGTCC					

Table 2. Fixation indices of the two populations of *C. gariepinus* obtained from microsatellite data

	Cga01	Cga02	Cga03	Cga05	Cga06	Cga09	Cga10
Fst:	0.64	0.08	0.44	0.10	0.26	0.09	0.19
Fit:	0.69	0.73	0.73	0.60	1.00	0.52	0.24
Fis:	0.14	0.70	0.53	0.55	1.00	0.47	0.05
Gst:	0.48	0.10	0.28	0.10	0.18	0.07	0.11

Fis over all loci:	0.51
Gst over all loci:	0.19

Table 3. Indices of genetic variability

Parameter	Cga01		Cga02		Cga03		Cga05		Cga06		Cga09		Cga10	
	Lokoja	Asejire	Lokoja	Asejire	Lokoja	Asejire	Lokoja	Asejire	Lokoja	Asejire	Lokoja	Asejire	Lokoja	Asejire
Heterozygosity Observed	0.27	0.35	0.00	0.29	0.00	0.44	0.17	0.70	0.00	0.00	0.44	0.08	0.00	1.00
Heterozygosity Expected	0.35	0.38	0.35	0.70	0.64	0.48	0.81	0.76	0.51	0.53	0.53	0.58	0.66	0.53
Number of Alleles	3	2	2	5	3	4	7	4	2	2	5	3	4	2
Size of PCR product	92–96	100–102	104–106	102–110	100–146	144–168	194–206	200–206	134–138	138–140	180–202	200-204	100–114	100–102
*Ho	0.66	0.64	0.62	0.34	0.38	0.55	0.21	0.32	0.51	0.50	0.49	0.46	0.36	0.5
*He	0.34	0.36	0.38	0.66	0.62	0.45	0.79	0.69	0.49	0.50	0.51	0.54	0.64	0.5
*Ne	1.51	1.56	1.62	2.96	2.60	1.82	4.73	3.17	1.98	1.99	2.06	2.15	2.77	2.00
*PIC	0.31	0.30	0.38	0.60	0.54	0.42	0.76	0.63	0.37	0.37	0.48	0.43	0.59	0.38

*Ho — homozygosity, *He — heterozygosity, *Ne — effective number of alleles, *PIC — polymorphic information content.

and the mean effective numbers of alleles were 2.47 and 2.24 respective to Lokoja and Asejire populations. While the polymorphic Information Content across the seven loci of the two populations were 0.49 ± 0.24 in River Niger (Lokoja) population and 0.45 ± 0.26 in Asejire population. Observed heterozygosity of the two populations fall in a small range, between 0.125 (± 0.077) in Lokoja population to 0.409 (± 0.197) in Asejire population.

The proportion of homozygosity by the equation proposed by Nei (1978), the effective allele number (Ne) per locus, the gene diversity (heterozygosity) of each locus across the two populations (Ho) and the genetic variability parameter that considered all alleles including rare alleles called polymorphism information content (PIC) was determined (table 4).

The PIC measures the level of polymorphism of each locus/marker and its values ranged from 50–79 %. In other words, PIC is a measure of the worth of a marker in

Table 4. Genetic variability indices per population per locus

	Cga01	Cga02	Cga03	Cga05	Cga06	Cga09	Cga10
Ho	0.33	0.45	0.26	0.18	0.40	0.44	0.36
Ne	3.07	2.24	3.83	5.45	2.51	2.29	2.79
He	0.67	0.55	0.74	0.81	0.60	0.56	0.64
PIC	0.61	0.50	0.70	0.79	0.53	0.54	0.59

Table 5. Mean homozygosity, heterozygosity, $^{**}F_{IS}$ and standard error (SE) in parenthesis

Population	Mean homozygosity	Mean effective number of alleles	Mean heterozygosity	Mean of PIC (SE)	Mean of heterozygosity observed (SE)	Mean of heterozygosity expected (SE)	$^{**}F_{IS}$
Lokoja	0.41	2.47	0.54	0.49 (0.24)	0.125 (0.077)	0.55 (0.216)	0.741
Asejire	0.47	2.24	0.53	0.45 (0.26)	0.409 (0.197)	0.566 (0.218)	0.257

$^{**}F_{IS}$ = He– Ho/He.
F_{IS}— fixation index; He — heterozygosity and Ho — homozygosity.

assessing diversity among genotypes being study. The measures of allelic variability are presented also in table 3 while the mean effective numbers of alleles are shown in table 5, which contributes to the populations ranged from 2.24–2.47.

The gene diversity among loci in the two populations (H) according to Nei (1987) and Zhang et al. (2010) was 0.66 and the mean of this parameter (PIC) was calculated using Botstein et al. (1980) which was 0.61. Cumulative power of discrimination (CPD) according to Fan et al. (2002) across the two populations is 78 %. The identity similarity was 0.48 (1 — Genetic Distance).

Departure from Hardy-Weinberg equilibrium (HWE)

Among the fourteen tests (seven loci, two populations) for Hardy-Weinberg equilibrium (HWE) (table 6), significant departures were observed in the two populations, with Lokoja population exhibiting HWE deviations at all the loci under study, while the Asejire population showed deviation at only one locus (Cga03). Of all the loci studied, only (Cga03) showed significant non-conformance to HWE within Asejire population.

Genetic diversity among populations

Results of the hierarchical analysis of molecular variance (AMOVA) within and among the two populations were categorized a priori into two groups. The AMOVA analysis revealed significant, although minimal differentiation between the populations

Table 6. Conformity to Hardy-Weinberg Equilibrium

Population	Locus	DF	Chi Sq	Probability	Significance
Lokoja	Cga01	190	176.667	0.747	ns
Lokoja	Cga02	210	212.778	0.434	ns
Lokoja	Cga03	231	231.667	0.475	ns
Lokoja	Cga05	190	168.667	0.865	ns
Lokoja	Cga06	210	176.667	0.954	ns
Lokoja	Cga09	171	151.111	0.861	ns
Lokoja	Cga10	276	257.222	0.785	ns
Asejire	Cga01	171	157.867	0.756	ns
Asejire	Cga02	190	192.889	0.428	ns
Asejire	Cga03	171	203.889	0.044	*
Asejire	Cga05	190	188.889	0.509	ns
Asejire	Cga06	210	229.111	0.174	ns
Asejire	Cga09	153	170.556	0.157	ns
Asejire	Cga10	210	243.889	0.054	ns

Key: ns = not significant, *P < 0.05 (significant).

(G_{ST} = 0.19, p < 0.01) table 2. Similarly, the variation among individuals within populations was substantial albeit moderately valued at F_{IS} = 0.51, p < 0.01. Within individual variation accounted for 99 % of the total genetic variation whilst only 1 % was partitioned in the variation among the two populations.

By and large, the pair-wise measures F_{ST} employed to assess differentiation between populations were congruent and indicated low to extensive genetic differentiation among pair-wise population comparisons after FDR correction. Pair-wise F_{ST} values (table 4) for all combinations of populations demonstrated significant differentiation that ranged from 2.47 and 2.24 was not significantly differentiated, corresponding to high genetic differentiation (0.5213) between Lokoja and Asejire populations. In both instances, results from population pair-wise F_{ST} estimates showed that the largest genetic difference was uncovered between the two populations with significant values of 0.741 and 0.257 respectively, whereas the smallest differentiation was observed between the individuals of population of Asejire with the pair-wise F_{ST} values of 0.257.

Inbreeding coefficient of the subpopulation of *C. gariepinus* within the two populations per locus

The average θ value (unbiased estimated of F_{st}) across the seven loci was 0.2571 (Cga01 = 0.6378, Cga02 = 0.0805, Cga03 = 0.4358, Cga05 = 0.1046, Cga06 = 0.2586, Cga09 = 0.0949, Cga10 = 0.1933) and 95 % CI (Information Content) of its estimate using a bootstrap method were between 0.08054 and 0.63784 which suggested the genetic structure of the two populations of *C. gariepinus* from the two vegetation zones under study (table 2).

Inbreeding Coefficient of the Subpopulation of *C. gariepinus* within the two populations per Locus are shown in table 2. The average θ value (unbiased estimated of F_{ST}) across the seven loci was 0.2579 and 95 % CI of its estimate using a bootstrap method were between 0.0222 and 0.1294 suggesting genetic structure of the two populations of *Clarias* in Nigeria.

Genetic differentiation among the two populations and genetic distances between the two populations of *C. gariepinus* based on microsatellite data (Nei, 1972)

The values for genetic differentiation (Gst) as shown in table 7 were significantly different from each other while the genetic distance in table 8 revealed the value of Nei's genetic distance to be relatively high in the two populations. Mean heterozygosity values for the two populations were 0.54 and 0.53. Nei's genetic distance showed greater differences between the populations under study (0.5213).

Table 7. Genetic differentiation between two populations of *C. gariepinus*

	Cga01	Cga02	Cga03	Cga05	Cga06	Cga09	Cga10
Ht	0.67	0.55	0.74	0.82	0.60	0.56	0.64
Hs	0.35	0.50	0.53	0.74	0.50	0.52	0.57
*Gst	0.48	0.10	0.28	0.10	0.18	0.07	0.11

*Genetic differentiation (Gst) = heterozygosity total (Ht) — heterozygosity within sub population (Hs), heterozygosity total (Ht).

Table 8. Genetic distances between the two populations of *C. gariepinus* based on microsatellite data

Population	Lokoja	Asejire
Lokoja	0.0000	
Asejire	0.5213	0.0000

Phylogenetic dendrogram

Two main clusters or clades were observed from the phylogenetic Dendrogram (fig. 2) produced which showed the genetic relationship across the populations studied (fig. 1). At R-Square 0.5, the first phylogroup/clade consists majorly of individuals from Lokoja population while the second clade is made up of individuals from both Lokoja and Asejire populations. The first clade diverged at R-Square of about 60% while the second one separated earlier at about 40 %. The Dendrogram depicts homogeneity in the Lokoja population as shown in the first clade. The sub cluster of Lokoja in the second clade or cluster separates differently together from the second sub cluster which consists of Asejire. The study showed that both populations were different genetically from each other.

Discussion

Five alleles were found at Cga01 locus, while at Cga02, Cga03, Cga05, Cga06, Cga07, Cga09 and Cga10 loci, number of alleles found were 5, 6, 7, 3, 6 and 5 respectively. Galbusera et al. (1996) in their study on isolation and characterization of seven loci of *C. gariepinus* reported a range of five to fourteen numbers of alleles. Though Agbebi et al. (2013) used four loci on the preliminary characterization of genetic strains in clariid species of *C. gariepinus* and *Heterobranchus bidorsalis*; observed the same number of alleles as in this work.

The positive relation between numbers of repeats units and level of allele size variation was apparent in human (Carvalho and Hauser, 1994). The same trend of relationship was observed in Thai silver barb (Kamonrat, 1996), level of allele variation of *C. gariepinus* microsatellite loci (average 16.3 alleles per locus) is high compared to 7.7 alleles per locus (range 5–14) reported for seven microsatellite loci of *C. gariepinus* (Galbusera et al., 1996).

However, the level of allele variation for Cga loci is underestimated due to small sample size (40 individuals). Other species of *Clarias* were identified with average of 13.8 alleles per locus (Kamonrat, 1996). High microsatellite allele variation was reported in number of

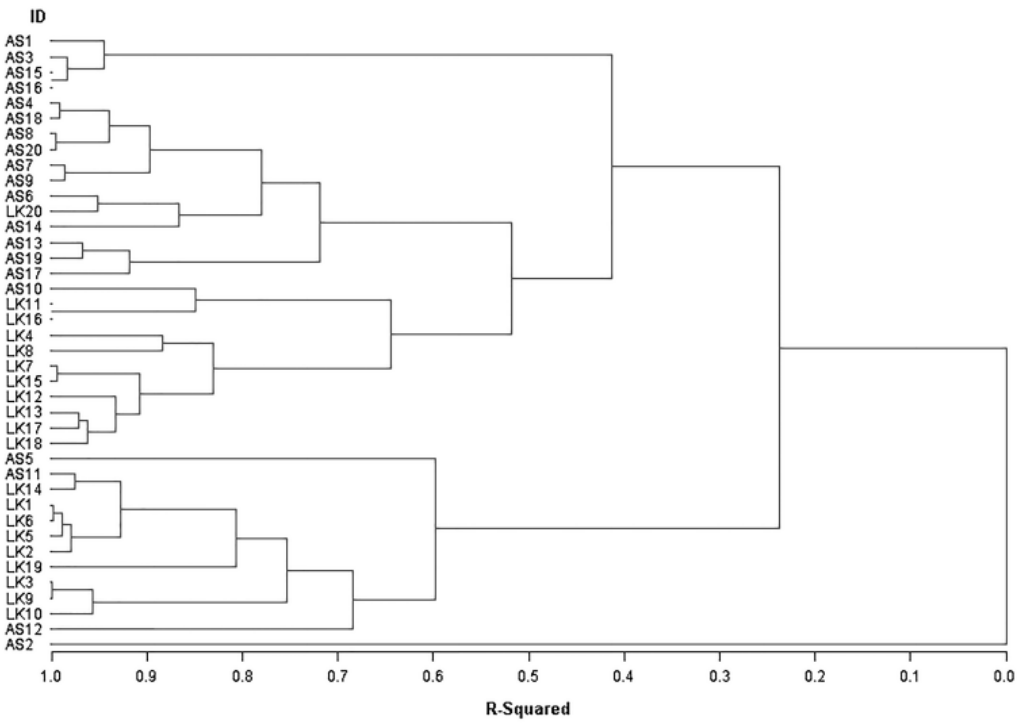

Fig. 2. Genetic dendrogram of *C. gariepinus* from two vegetation zone (Lokoja and Asejire) of Nigeria.

fishes such as Whiting (14–23 alleles/ locus, Rico, 1997), Red sea bream (16–32 alleles/ locus, Takagi et al., 1997) and Atlantic cod (8–46 alleles/ locus, Bentzen et al., 1996). Relative low variation was observed among microsatellite loci of Brown trout having 5–6 alleles/ locus (Miller and Kapuscinski, 1996) and Sea bass having 4–11 alleles/ locus (Garcia et al., 1995).

Cga03 which has only a few alleles is well-suited for population genetic studies (Galbusera et al., 1996), especially when specimen size is small. The highly variable loci like Cga05 and Cga06 are ideal for genome mapping, pedigree study (Reilly et al., 1995) and assessment of inbreeding of aquaculture stocks (Takagi et al., 1997). Due to the high variability detected by these microsatellite loci, it was reported that they were suitable for genetic characterization of *Clarias* species (Daud et al., 1989; Lawonyawut, 1995). The loci were also reported useful for taxonomic studies of this taxa as well as the identification of their natural hybridization (Kamonrat, 1996).

Most of the loci exhibited consecutive alleles differed by multiple number of repeat unit. For instance, Cga05 expressed allele difference by one base-pair which was unexpected from the dinucleotide-microsatellite sequence.

Analyses of the microsatellite loci revealed good level of genetic informativeness having a PIC mean of 0.6085 which is higher than the threshold of 0.5 which is the acceptable standard for genetic markers to be informative. The heterozygosity percentage observed for all the alleles (53 %) falls within the observed levels of reported heterogeneity in fish, which range from 24 % to 90 % (O'Connell and Wright, 1997).

The observed heterozygosity by loci for the two populations ranged between 0.08 and 0.70 with an average of (Hi = 0.267 ± 0.137). This falls below the value reported by Agbebi et al. (2013) (0.446± 0.063) but is above the range of Hi in *C. gariepinus* populations sampled across Africa (0.06 to 0.15) (Teugels et al., 1992; Agnese et al., 1997). The expected heterozygosity follows the same trend.

Microsatellite analysis of the two populations studied showed significant deviations from Hardy-Weinberg equilibrium (HWE) in the two populations studied. Ferguson et al., (1996) opined that deviation from Hardy-Weinberg equilibrium can occur due to the mixing of heterogeneous gene pools. Moreover, violations of the Hardy Weinberg equilibrium can cause deviations from expectation. Reduction in size of a population is considered to be one of the few factors that might be responsible for deviation from Hardy-Weinberg equilibrium (Nasren et al., 2009).

From the application of microsatellite loci to the two populations of *C. gariepinus*, the populations were adequately represented, which appears to have deviated from HWE. Further investigations detected recent population bottleneck along with over-representation of homozygotes in Asejire. On the other hand, although not unusual, the populations exhibited non-conformance to HWE. High values of heterozygosity-based indices suggested that a generous number of breeding individuals were accounted for, and created the present populations with the exception of Lokoja, whereby significant yet low level of inbreeding was detected.

Genetic diversity which provides information on the variability among and between the two populations is central to the development of sustainable breeding strategies for competitively commercialized culture of clariid fish species of study. Genetic variation of *C. gariepinus* in this study was characterized by mean allele diversity (2.51 alleles/ population per locus) compared to the averages for freshwater fish (A = 9.1 ± 6.6 averaged across 13 species (De Woody and Avise, 2000). However, the mean heterozygosity (0.53) observed in this microsatellite analysis were marginally the same with the unrelated *C. striata* population investigation of Kedah (0.52) (Jamaluddin et al., 2011) and markedly inflated compared to the populations in Thailand (0.039) (Hara et al., 1998).

The present result implied that the two populations of *C. gariepinus* studied may have significant potential impact to the local genetic diversity. However, since genetic variation of the populations is substantially higher, there is the need to carry out another study to

investigate sine qua non whether there is any incidence of the populations of C. *gariepinus* escaping and introgressing into the either populations. Genetic introgression from different populations of the same species has been reported to be one of the factors responsible for genetic erosion and extinction of species (Ezilrani and Christopher, 2012). A comparison between the two populations of *C. gariepinus* from farms with wild populations sampled at streams or freshwater bodies in close proximity to the farms could be carried out to assess whether there have been significant changes in the diversity of the population.

The AMOVA analysis detected significant albeit low population association among the populations in this study. In addition, 99 % of the total variation was partitioned to within individuals' components whereas only 1 % of variation was found among the populations assigned. This implies that there was little variation between the populations of which the majority of the variation exists on the individual level without any regards to its population.

The high degree of genetic variability demonstrated in the populations has led to questions on whether this level of diversity could be maintained for a sustained response from long-term selection for commercially important traits (Davis and Hetzel, 2000). This is because, major concerns of aquaculture practices do not only revolve around gathering but also maintaining as much of the naturally occurring variation as possible within the populations (Lind et al., 2009). Domestication of populations over generations, associated with the decline in genetic variation due to founder's effect, non-random mating, genetic drift, selection (based on commercial traits) and inbreeding may lead to loss of genetic diversity. Therefore, retention of the genetic diversity of the populations is the requisite to the long-term success of breeding programs.

The genetic distance observed between the two populations in this study was 0.5213. This is comparable to genetic distances reported in other African catfishes. Genetic distances vary from 0.271 to 0.916 in the genus *Chrysichthys* and it varies from 0.01 to 0.34 between populations of *Chrysichthys nigrodigitatus* (Agnese, 1989); from 0.003 to 0.112 between populations of *Chrysichthys auratus*; 0.803 between *C. gariepinus* and *C. anguillaris* as reported by Agnese et al. (1997).

The genetic distances calculated from microsatellite data do not agree with geographic distance, the smallest genetic distance was observed between the populations under study which were separated by distance of more than 1,000 km with disconnected river systems and other barriers. Theoretically, the result suggested certain level of gene flow between the populations under study, in fact natural interbreeding between the populations is impossible and there has been no record on transplantation of *C. gariepinus* between the two populations.

Moreover, the phylogenetic tree produced in this study does not give a definite pattern of separation into distinct sub populations. Population structuring of natural stocks of *C. gariepinus* observed in this study was fundamentally supported by the study previously done by Na-Nakorn et al. (1998). It seems reasonable to suppose that the *Clarias* populations have been subdivided into some small genetic groups from analyzing microsatellite data sets separately, and it indicates the need for genetic conservation of this species. However, there is room for further investigation of those genetic distances due to the genetic distance obtained on the microsatellite data sets which was moderately high in the two populations studied. To discuss these population structures, further studies are required in dealing with large number of populations intensively sampled from different vegetation zones of Nigeria. Moreover, large sample size and more markers may be required.

The present morphological analyses of two populations of *C. gariepinus* do not suggest any definite pattern of separation into sub populations. Application of genetic techniques may provide explanation for the lack of phenotypic differentiation between the two populations of study. This may indicate similar populations of *C. gariepinus* from two different vegetations of rainforest and guinea savanna of Nigeria.

Significant levels of genetic differentiation, ranging from low to high was observed among and between the two populations. Analysis of Molecular Variance (AMOVA) showed minimal, but significant differentiation between the populations. Differentiation estimators based on the microsatellite data revealed a positive correlation between population genetic delineation and spatial distribution (which included hydro-geographic factors) of *C. gariepinus* populations. On the contrary, physical barrier such as geographical distance has increased the genetic dissimilarity between the populations.

This study also has implications for management and conservation. There is global need to preserve genetic resources for maintaining species and their genetic diversities. The identification of steps or actions that will minimize the effects of genetic losses within natural populations of fish species is one of the basic mechanisms for maintaining variability and genetically healthy populations. This work has shown further the usefulness of microsatellite markers in managing and conserving Clariid fish species.

References

Agbebi, O. T., Ajagbe, O., Makinde, L., Afolabi, O. 2013. Production, growth and effect of varying stocking density of *Clariobranchus* fry. *International Journal of Fisheries*, **4** (4), 73–78.

Agnese, J. F. 1989. *Differenciation genetique de plusieurs especes de siluriformes O uest Africians ayantu n interet pour aquaculture*. PhD Thesis, University of Montpellier France, **14**, 59–63.

Agnese, J. F., Teugels, G. G., Galbusera, P., Guyomard, R., Volckaert, F. 1997. Morphometric and genetic characterization of sympatric populations of *Clarias gariepinus* and *Clarias anguillaris*. *Senegal Journal of Fish Biology*, **50**, 1143–1157.

Alabi, M. O. 2009. Urban sprawl, pattern and measurement in Lokoja, Nigeria. *Theoretical and Empirical Researches in Urban Management*, **13** (4), 158–154.

Barlett, J., Langdon, S., Simpson, B., Stewart, M. Katsaros, D., Simondi, P., Love, S. Scott, W. Williams, A., Lessells, A., Macleod, K., Smyth, J., Miller, W. 1996. The prognostic value of epidermal growth factor receptor mRNA expression in primary ovarian cancer. *Br J Cancer* **73**, 301–306.

Bartish, I. V., Gorkava, L. P., Rumpunen, K., Nybom, H. 2000. Phylogenetic relationship and differentiation among and within populations of *Chaenomeles* Lindl. (Rosaceae) estimated with RAPD's and isozymes. *Theoretical Applied Genetics*, **101**, 554–563.

Bentzen, P., Harris, A. S., Wright, J. M. 1991. Cloning of hypervariable minisatellite and simple sequence microsatellite repeats for DNA fingerprinting of important aquacultural species of Salmonids and Tilapia. *In*: Burke, T., Dolf, G., Jeffreys, A. J., Wolf, R., eds. *DNA fingerprinting: Approaches and Applications.* Birkhauser Yerlag, Basel, 243–262.

Bentzen, P., Taggart, C. T., Ruzzante, D. E., Cook, D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. *Canadian Journal of Fish Aquacultural Science*, **53**, 2706–2721.

Botstein, D., White, R. L., Skolnick, M., David, R. 1980. Construction of genetic linkage map in man using restriction gment length Polymorphism. *Animal Journal of Human Genetics*, **32**, 314–331.

Brooker, A. B. J., Cook, D., Bentzen, P., Wright, J. M., Doyle, R. W. 1994. Organization of microsatellite differs between mammals and cold-water teleost fishes. *Canadian Journal of Aquacultural Science*, **51**,1959–1966.

Carvalho, G. R., Hauser, L. 1994. Molecular genetics and stock concept in fisheries. *Review in Fish Biology and Fisheries*, 4, 326–350.

Daud, S. K., Patimah, I., Kijima, K. 1989. Genetic variability and relationship among four species of freshwater Catfish. *Malay Applied Biology*, **18**, 23–31.

Davis, G. P., Hetzel, D. J. S. 2000. Integrating molecular genetic technology with traditional approaches for genetic improvement in aquaculture species. *Aquacult. Res.,* **31** (1), 3–10.

De Woody, J. A., Avise, J. C. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology*, **56** (3), 461–473.

Dieringer, D., Schlötterer, C. 2003. *The software used for analysis was the Microsatellite Analyzer (MSA) Version 4.05.*

Ezilrani, P., Godwin Christopher, J. 2012. Genetic Tools and Catfish Improvement. *Asian Journal of Experimental Biological Sciences,* **3** (2), 407–414.

Fan, J., McCandliss, B. D., Sommer, T., Raz, M., Posner, M. I. 2002. Testing the efficiency and independence of attentional networks. *Journal of Cognitive Neuroscience,* **14**, 340–347.

Ferguson, A., Taggart, J. B., Prodohl, P. A., McMeel, O., Thompson, C., Stone, C., McGinnity, P., Galbusera, P., Volckaert, F. A., Hellemans, B., Ollevier, F. 1996. Isolation and characterization of microsatellite markers in the Africa catfish *Clarias gariepinus* (Burchell 1822). *Molecular Ecology*, **5**, 703–705.

Galbusera, P., Volckaert, A., Hellemans, B., Ollevier, F. 1996. Isolation and characterization of microsatellite markers in the African Catfish, *Clarias gariepinus* (Burchell 1822). *Molecular Ecology*, **5**, 703–705.

Garcia, F. J., De Leon, Dallas, J. F., Chatam, B., Canonne, M., Versini, J. J., Bonhomme, F. 1995. Development and use of microsatellite markers in sea bass, *Dicentrachus labrax* (Linnaeus 1758) (Perciformes: Serranidae). *Molecular Biology*, *Biotechnology*, **4**, 62–68.

Garg, R. K., Sairkar, P., Silawat, N., Mehrotra, N. N. 2009. Genetic polymorphism of two populations of catfish Aorichthys Seenghala (Sykes) using RAPD fingerprinting. *International Journal of Zoology*, **3**, 130–134.

Goudet, J. 2002. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9, 3.2). Government of Kenya. Economic Recovery Strategy for Wealth and Employment Creation. *Ministry of Planning and National Development* 40.

Hara, K., Yonezawa, K., Weng, Q. P., Kozlowski, M. T., Belham, C., Avruch, J. 1998. Amino acid sufficiency and mTOR regulate kinase. *Journal of Biological Chemistry*, **14** (4), 84–94.

Hecht, T., Uys, W., Britz, P. J., eds. 1988. *Culture of sharptooth catfish, Clarias gariepinus, in southern Africa*. National Scientific Programmes Unit: CSIR, SANSP Report 153, 146.

Jamaluddin, M. F., Hayashi K., Ohta H., Kurimoto, K., Aramaki, S., Saitou, M. 2011. Polypeptide binding specification of saccharomyces cerevisae, oligosaccharyl transferase accessory proteins Ost3p and Ost6p. *Protein Science*, **20** (5), 849–855.

Kamonrat, W. 1996. Spatial Genetic Structure of Thai Silver Barb *Puntius gonionotus* (Bleeker) Populations in Thailand. PhD thesis, Dalhousie University, Dalhousie, Canadian Ecology, 11–93.

Lawonyawut, K. 1995. Hybridization and Genetic Manipulation in *Clarias* catfish. PhD thesis, Institute of Agriculture, University of Stirling, Scotland. *Protein Science*, 62–70.

Lind, C. E., Evans, B. S., Knauer, J., Taylor, J. J. U., Jerry, D. R. 2009. Decreased genetic diversity and a reduced effective population size in cultured silver-lipped pearl oysters (*Pinctada maxima*). *Aquaculture*, **286** (1–2), 12–19.

Miller, L. M., Kapuscinski, A. R. 1996. Microsatellite DNA markers reveal new levels of genetic variation in northern pike. *Transaction of Animal Fish Society*, **125**, 971–977.

Na-Nakorn, U., Sukmanomon, S., Nakajima, M., Taniguchi, N., Kamonrat, W., Poompuang, S., Nguyen, T. T. T. 2006. MtDNA diversity of critically endangered Mekong giant catfish (*Pangasianodon gigas* Chevey, 1913) and closely related species implications for conservation. *Ecology, Animal Conservation*, **9** (4), 483–494.

Na-Nakorn, U., Hara, M., Seki, S., Taniguichi, N. 1998. Isozymes variation of *Clarias macrocephalus* from four locations in Thailand. *Fishes Science*, **64**, 526–530.

Nasren, S., Islam, M. N., Khan, M. G. Q., Islam, M. S., Alam, M. S. 2009. Genetic variation and differentiation in the Stinging catfish, *Heteropneustes fossilis* (Bloch), populations assessed by heterologous microsatellite DNA markers. *Indian J. Biotechnol*., **8**, 85–90.

Nei, M. 1972. Genetic distance between populations. *Animal Nation*, **106**, 283–292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York. *Canadian Journal of Fish Aquatic Science*, 512.

O'Connell, M., Wright, J. M. 1997. Microsatellite DNA in fishes. Review in Fish Biology and Fisheries. *Canadian Journal of Fish Aquatic Science*, **7** (3), 331–363.

Rico, C., Ibrahim, K. M., Hewitt, G. M. 1997. Stock Composition in North Atlantic populations of Whiting using Microsatellite markers. *Journal of Fish Biology,* **51**, 462–475.

Sambrook, J., Fritsch, E. F., Maniatis, T. 1989. *Molecular cloning: A laboratory manual*. 3rd Edn. Cold Spring, Harbor Laboratory Press, New York, 6–45.

Suleiman, Y. B., Matazu, M. B., Davids, A. A., Mozie, M. C. 2014. *The Application of Geospatial Techniques in Flood Risk and Vulnerability Mapping for Disaster Management at Lokoja*. Kogi State State, Nigeria, **4** (5), 54–61.

Takagi, M., Taniguchi, N., Cook, D., Doyle, R. W. 1997. Isolation and characterization of microsatellite loci from red sea bream, *Pagrus major* and detection in closely related species. *Fishery Science*, **63**, 199–204.

Teugels, G. G. 1986 a. A systematic revision of the African species of the genus *Clarias* (Pisces: Claridae). *Mus. Research Africa centre*, *Belgium*, 63–72.

Teugels, G. G. 1986 b. A systematic revision of the African species of the genus *Clarias* (Pisces: Claridae). *Annales du Musée Royal de l'Afrique Centrale*, **247**, 1–199

Teugels, G. G. 1996. Taxonomy, phylogeny and biogeography of catfishes (Ostariophysi; Siluroidei) an overview. *Aquatic Living Resources*, **119**, 2–15.

Teugels, G. G., Ozouf-Costaz, C., Legendre, M., Parrent, M. 1992. Akaryological analysis of the artificial hybridization between *Clarias gariepinus* (Burchell 1822) and *Heterobranchus longifilis* (Valencinnes, 1840) (Pisces: Clariidae). *Journal of Fish Biology*, **40**, 81–86.

Volckaert, F. A., Hellemans, B. A., Galbusera, P., Ollevier, F., Sekkali, B., Belayew, A. 1994. Replication, Expression, and Fate of Foreign DNA during Embryonic and Larval Development of the African catfish. *Molecular Marine Biology and Biotechnology*, **3** (2), 57–69.

Weber, J. L., May, P. E. 1989. Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction. *American Journal of Health Genetics*, **44**, 388–396.

Welcomme, R. L. 1988 International Introduction of Inland Aquatic Species. Fish and Agricultural Organization. *Fishery Techniques*, **294**, 318.

Wu, Q., Chen, M., Buchwald, M., Phillips, R. A. 1995. A simple, rapid method for isolation of high quality genomic DNA from animal tissues. *Nucleic Acids Reserves*, **23**, 5087–5088.

Yem, I. Y., Bwala, R. L, Bankole, N. O., Olowosegun, M. O., Yaji, A. 2011. Analysis of Ichthyofaunal Diversity and Peculiarities of Some Lakes in Nigeria. *Journal of fisheries International,* 6, 26–30.

Zhang, T., Kong, J., Wang, W., Wang, Q. 2010. Genetic variability assessed by microsatellites in the breeding populations of the Shrimp *Penaeus* (*Fenneropenaeus*) *chinensis* in China. *Aquaculture*, **310** (1–2), 229–233.

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