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Genetic Differentiation of Clariid Populations using Microsatellite Markers in Kano State Rivers

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Authors' contribution

This work was carried out in collaboration among all authors. Author IOS designed the study and wrote the first draft of the manuscript. Author ROO managed the literature searches. Authors JMM and AUU performed the statistical analysis. Authors OMA and DSBU wrote the protocol. Author WOE managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

This study aimed to investigate the genetic characterization of strains of Clariid fish species in some river bodies in Kano State using microsatellite markers.One hundred and seventy seven Clariid fish samples (*Clariasgariepinus* and *Heterobranchuslongifilis*) were collected from six rivers (Thomas, Ghari, Tiga dam, Duddurun Gaya, Karaye and Bagwai) in Kano state. Blood sample was taken from each fish sample by severing the caudal peduncle and drained into FTA cards for DNA extraction, Polymerase Chain Reaction and electrophoresis to determine genetic variation between the Clariid fish populations.Genealex 6.4 software package was used to analyse the resolve bands from DNA extraction to determine their base pair and genetic variation. Results showed that the Fst values ranged from 0.00 to 0.66, Fit ranged from -0.04 to 0.12, Fis ranged from -0.35 to -0.26. It indicated a

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large number of gene flow (exchange) among the populations with a range of 0.46 to 0.87. There was an established magnitude of genetic divergence (91.86%) among the populations as shown by the result of the percentage polymorphism which depends on the number of alleles detected per locus and their frequencies. It can be concluded that since there was no inbreeding as shown in the study, none of the population exhibited genetic uniqueness. The populations had a high genetic differentiation between populations but moderate differentiation within populations. The populations were outbred populations; an indication that relatives avoided mating in the population.

Keywords: Allelic variation; genetic diversity; heterozygosity; inbreeding coefficients; outbreeding.

1. INTRODUCTION

Variability is the fundamental and basic characteristics of life. Every level of organization of life displays variation insome parameters, in space or time, within and between cells, tissues, organisms, populations and communities. The existence of variations in natural populations of organisms is a necessary condition for evolution [1]. Genetic variation is one key factor in the survival of species. Natural populations are perhaps the best gene banks which are critical resources for genetic variation for current and future application in improvement of farmed species of fish [2]. Among populations, genetic diversity can also be gained when populations that are not normally in contact with another hybridize that is when isolated population experienced migration, gene flow and genetic drift. This can occur when physical barriers are removed such as when fishes are introduced to an area or escape, or when migration patterns changes due to environmental condition.To this end, genetic studies of fish populations play an important role in the sustenance of genetic diversity [3].

Genetic markers can provide valuable information about geographic structuring, gene flow and demographic history of populations that can be highly relevant for conservation and management purposes [4].Genetic differentiation of quantitative traits among populations has been shown in many species, yet the genetic changes that can accompany divergence have received less attention.Within species, genetic diversity is partitioned among and within populations. Long before species declines into extinction, it will suffer a reduction in the level of genetic diversity within and among its populations. Often, range contraction and fragmentation of former distribution occur [5]. Fragmentation causes the formation of small isolated populations which are more vulnerable to genetic degradation. While documented extinctions of marine species are rare, the extinction of population (extirpation) is more common [5]. Genetic variation is important for the long-term survival of species; it ensures the fitness of species or populations by giving the species or populations the ability to adapt to changing environment; and lack of genetic variation or too much of homozygosity are detrimental to survival and fitness as a result of inbreeding depression [2].

Reduction in the genetic resources of natural fish populations is an important management problem. Not only has the genetic diversity of many fish populations been altered, but many populations and species have been extirpated by pollution, overfishing, destruction of habitat, blockage of migration routes and other human developments [6]. Loss of genetic diversity and locally adapted populations (and species) can compromise stability and recovery potential of marine ecosystems as well as impair their ability to adapt to changing environmental condition. There is generally limited information on genetic variation amongand within Clarias and Heterobranchus species and this greatly hampers an efficient and sustainable exploitation of these resources [7]. The African catfish *Clariasgariepinus* and *Heterobranchuslongifilis* are economically important species, but little is known about the genetic background of the natural populations of these species. Also, genetic study is needed for proper identification of the two species and determination of the genetic connection between them. Hence, the study was conceived to unravel the genetic differentiation of Clariid species in the water bodies of the study area.

2. MATERIALS AND METHODS

2.1 Study Location

The study was conducted in six selected water bodies in Kano State. These are Rivers Thomas and Ghari to the North, River Duddurun Gaya to the east, River Kano (Tiga dam) to the South, Rivers Karaye and Bagwai to the western part of the state. The first three locations were selected

due to their independent flow and their disconnected tributaries. The remaining three locations have the same origin as the Kano River and two flow towards the western part of the State while Tiga dam flows toward the Southern part (Fig. 1).Kano State is located in the semi-arid area of North-western Nigeria. It has a population of 9,383,682 comprising of 4,844,128 males and 4,539,534 females [8]. Kano State is the commercial nerve centre of Northern Nigeria. It is located between latitude $10^{o}33'$ and $12^{o}27'$ North of the equator and longitude $7^{o}34'$ and $9^{o}29'$ East of the Greenwich meridian and as such it is part of Sudano-Sahelian vegetation zone of Nigeria.

2.2 Sample Collection

The fish samples were identified using an exposition for identification by [9] and confirmed using local names provided by the fishermen. Live fish samples of *Clariasgariepinus* (143) and *Heterobranchuslongifilis* (34) from the six locations described above were purchased from commercial catches of the fishermen. *Clariasgariepinus*were collected from all locations except Tiga dam. Likewise, *Heterobranchuslongifilis*were collected in all locations except Rivers Duddurun Gaya and Thomas. The samples were adults and were transported in large bowls to the Laboratory of the Department of Animal Science, Bayero University Kano. Thirty-five blood samples (25 – *Clariasgariepinus* and 10 – *Heterobranchuslongifilis*) were transferred to FTA® Classic Cards (Whatman Bioscience, Maidestone, UK) and left to air dry and subsequently used for DNA extraction. The caudal peduncle of each fish sample was severed to drain out blood into the FTA cards. The samples were taken to a commercial laboratory (DNA Lab) in AngwaSeriki, Kaduna Metropolis, Kaduna State for analysis.

Genomic DNA was extracted individually following the procedure of [10] as follows:

1. Five 1.2mm disc of FTA® Classic Cards were punched into 1.5ml Eppendorf tubes.
2. 1000µl of 100mM Tris-base and 0.1% SDS buffer was added. Tubes were vortexed gently for 30minutes.
3. The supernatant was decanted, thereafter, 500µl of 5M guanidine thiocyanate was added and vortexed for 10 minutes.
4. The supernatant was decanted again; thereafter, 500µl nuclease-free water was added and vortexed for 10 minutes and then the supernatant was decanted.
5. The same volume of nuclease-free water was added and left to stand for 10 minutes.
6. The supernatant was again decanted and finally, 50µlnuclease-free water was added to the discs.
7. The tubes were heated at $90^{o}C$ for 10minutes in a thermocycler to complete extraction.
8. The DNA concentration was further diluted with nuclease free water in 1:20 ratio to remove impurities and prevent smearing.
9. DNA concentration was determined by measuring the intensity of absorbance of the solution at the 600 nm with a spectrophotometer and comparing to a standard curve of known DNA concentrations.
10. Measuring the intensity of absorbance of the DNA solution at wavelengths 260 nm and 280 nm was used as a measure of DNA purity. DNA absorbed UV light at 260 and 280 nanometres, and aromatic proteins absorbed UV light at 280 nm; a pure sample of DNA had a ratio of 1.8 at 260/280 and was relatively free from protein contamination. DNA was quantified by cutting the DNA with a restriction enzyme, running it on an agarose gel, staining with ethidium bromide and comparing the intensity of the DNA with a DNA marker.
11. DNA samples were stored at $-20^{o}C$ in Tris EDTA buffer pH7.8/8.0.

Template DNA was amplified using polymerase chain reaction (PCR). PCR was carried out in 10µl reaction volumes containing 20 to 40ng (50-120 ng/µl) genomic DNA, 2µl5x Taq mastermix of Taq DNA polymerase, 100 µM of each dNTP, 2mM $MgCl_2$, Tween-20, Nonidet P-40, red dye, gel loading buffer, stabilizers, 0.3µl each of the forward and reverse primer sets and 5.9µl of PCR grade water.Seven microsatellite markers isolated by [10] were used as primers. They were obtained from Jena Bioscience, Lobstedter, Germany. The primers are Cga01, Cga02, Cga03, Cga05, Cga06, Cga09 and Cga10. Amplifications were carried out in a Real-time thermocycler as follows: 1 cycle at $94^{o}C$ (5min) for pre-denaturation, 35 cycles at $94^{o}C$ (30sec) for denaturation, 35 cycles at $55^{o}C$ (30sec) for annealing, 35 cycles at $72^{o}C$ (30sec) for extension and 1 cycle at $72^{o}C$ (5min) for final

extension to complete amplification. PCR condition for each marker was optimized: 1cycle for 2minutes at 95^0C, 25 cycles for 15seconds at 95^0C, 15seconds at 55^0C, 45seconds at 68^0C, 1 cycle for 5minutes at 68^0C and finally, 1 cycle 2days at 4-10^0C. Electrophoresis was conducted on 2% Agarose gel containing a negative control to detect potential PCR contamination, and each gel contained a positive control using a sample that had been genotyped in order to standardize allele scoring among gels. Scoring was done by comparison to 8 bp (75, 154, 220, 298, 344, 396, 504 & 1632) standard DNA ladder to identify the approximate size of a molecule run on a gel during electrophoresis, with the aid of gel analyser (Jena Bioscience, Lobstedter, Germany)

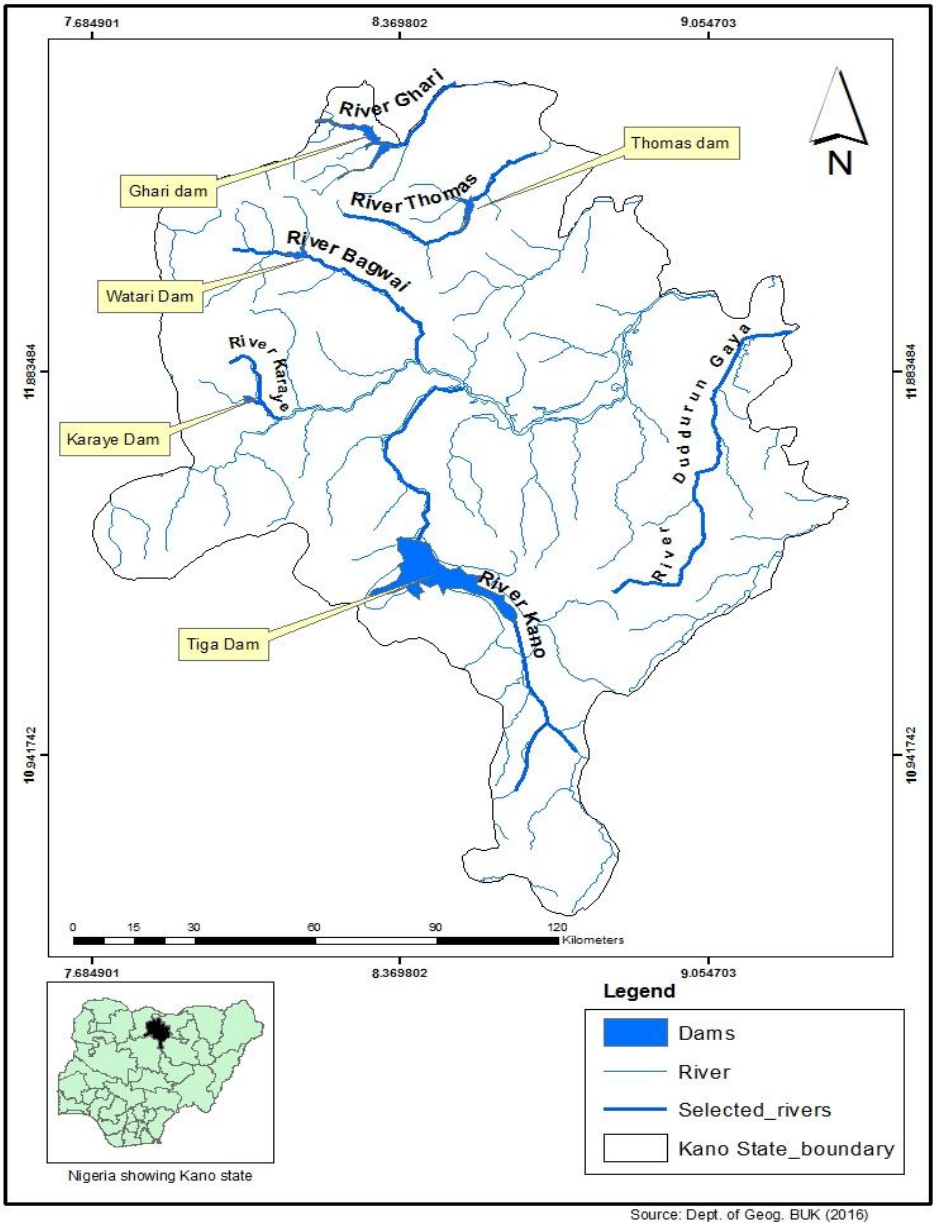

Fig. 1. Study area

2.3 Data Analysis

Allele frequencies for each locus within each sampled population were computed. This was calculated locus by locus with the expression:

$$FreqAllele\_x = \frac{2\mathrm{Nxx} + \mathrm{Nxy}}{2\mathrm{N}}$$

Where Nxx is the number of homozygotes for allele X (XX), and Nxy is the number of heterozygotes containing the allele X (Y was any other allele to be tested). N = the number of samples. These were tested for deviation of observed genotype frequencies from those under Hardy-Weinberg equilibrium using the Markov chain exact tests provided in the Genepop software [11]. Estimate of microsatellite diversity within populations such as total alleles TNA, mean number of alleles MNA, allelic richness (ar), observed and expected (H_o and H_e) heterozygote as well as nuclear pairwise F_t, values corrected for multiple testing was calculated using MS analyser 4.05 [12,13] was used to infer genetic inbreeding coefficient $F_{is.}$To quantify the extent of molecular variation, locus-by-locus analysis of molecular variance (AMOVA) was performed using Genealex 6.4 [14]. In the current study, Fst was used to determine the potential differences between the two statistics. F-statistics were obtained using AMOVA approach and population pairwise based on microsatellite loci as implemented in Genealex 6.4.

3. RESULTS

The pairwise genetic differentiation (Fst) between each pairs of populations is shown in Table 1. Populations from different genetic clusters appeared to be more differentiated from each other, corresponding well to the classification of the genetic cluster. The obtained values showed that there was free interbreeding among populations in different magnitude. The values ranged from 0.00 for complete panmixis, to 0.66 indicating that some of the populations still share some amount of genetic diversity and the populations were highly differentiated.

Tables 2, 3 and 4 show the sample size, number of alleles, number of effective alleles, Shanon information index, observed heterozygosity, expected heterozygosity and fixation index for each of the populations studied locus by locus measuring the amount of genetic diversity among the populations studied. The highest genetic diversity occurred in Cga03 (Ne = 8.00, Ho = 1.00 and He = 0.88) for CgG (Table 2) and Cga09 (Ne = 8.00, Ho = 1.00 and He = 0.88) for CgK (Table 3). The lowest genetic diversity was observed in Cga06, Cga09 and Cga10 (Ne = 2.00, Ho = 1.00 and He = 0.500) for HIG (Table 3). Higher genetic diversity was also obtained in CgB with mean number of alleles (Na) of 5.71 with fixation index of -0.25 as depicted in Table 4.Most of the microsatellite loci were characterized with negative values of fixation index which might be linked to sampling variance within the clariid population.Mean number of alleles were 4.39 and detected across all populations for the seven microsatellites examined (Table 5). The averagenumber of effective alleles were totalled 4.05 while the total information index was 1.35. Total observed heterozygosity was 0.92 indicating that the markers were sufficiently polymorphic to determine genetic diversity in the fish populations studied. Expected and unbiased heterozygosity were 0.69 and 0.82 respectively. Mean Ho was higher than He indicating efficiency of heterozygotes at these loci. The mean fixation index for the population was -0.36 indicating that the population had more heterozygotes than expected in contrast to positive values that indicate fewer heterozygotes.

Table 6 shows the levels of intra and inter-population variation of Fis, Fst and Fit. These are inbreeding coefficients: within population (Fis), between population (Fst) and overall population (Fit) for the seven microsatellite loci investigated. Inbreeding within population (Fis) was negative ranging from -0.26 to -0.38. The inbreeding between populations (Fst) ranged from 0.22 to 0.34which showed high genetic differentiation ($p<0.05$) among the population.Inbreeding in the overall population (Fit) ranged from -0.04 to 0.12. The estimate of gene flow ranged from 0.48 to 0.87 indicating effective gene flow betweenpopulations. The degree of genetic differentiation within population was very low, that of between populations was very high while overall showed a moderate degree of genetic differentiation. The percentage polymorphism was 100% except for the sixth locus (Cga09) where it was 48.86% indicating that Cga09 had a lower genetic polymorphic loci in the populations investigation.

Table 7 shows the Analysis of molecular variance (AMOVA) of hierarchical gene diversity. The result indicated that 83% of the genetic variation was explained by within-individual, whereas 7% of the variation was explained by

Table 1. Population pairwise genetic differentiation values based on microsatellite loci (Fst) (gene differentiation)

Locus	CgD	CgG	CgB	CgT	CgK	HlG	HlB
CgD	-						
CgG	0.003	-					
CgB	0.00	0.003	-				
CgT	0.01	0.01	0.003	-			
CgK	0.01	0.02	0.003	0.02	-		
HlG	0.65	0.66	0.59	0.66	0.44	-	
HlB	0.00	0.003	0.00	0.01	0.00	0.28	-

Cg=Clariasgariepinus
Hl=Heterobranchuslongifilis
D-River Dudusingaya,
G-River Ghari,
B-River Bagwai,
T-River Thomas
K- River Karaye

Table 2. Sample size, no of alleles, no of effective alleles, information index, observed heterozygosity, expected and unbiased expected heterozygosity and fixation index for CgD, CgG and HIB

Locus	N	Na	Ne			IH_OH_euHeF		
CgD	4	4.00	4.00	1.39	1.00	0.75	0.86	-0.33
Cga01								
Cga02	4	4.00	4.00	1.39	1.00	0.75	0.86	-0.33
Cga03	4	4.00	4.00	1.39	1.00	0.75	0.86	-0.33
Cga05	4	4.00	4.00	1.39	1.00	0.75	0.86	-0.33
Cga06	4	4.00	4.00	1.39	1.00	0.75	0.86	-0.33
Cga09	4	4.00	4.00	1.39	1.00	0.75	0.86	-0.33
Cga10	4	4.00	4.00	1.39	1.00	0.75	0.86	-0.33
Mean	4.00	4.00	4.00	1.39	1.00	0.75	0.86	-0.33
SE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CgG								
Cga01	4	4.00	4.00	1.39	1.00	0.75	0.86	-0.33
Cga02	4	4.00	4.00	1.39	1.00	0.75	0.86	-0.33
Cga03	4	8.00	8.00	2.08	1.00	0.88	1.00	-0.14
Cga05	4	5.00	4.00	1.49	1.00	0.75	0.86	-0.33
Cga06	4	7.00	6.40	1.91	1.00	0.84	0.96	-0.19
Cga09	4	6.00	5.33	1.73	1.00	0.81	0.93	-0.23
Cga10	4	6.00	5.33	1.73	1.00	0.81	0.93	-0.23
Mean	4.00	5.71	5.30	1.67	1.00	0.80	0.91	-0.26
SE	0.00	0.57	0.57	0.10	0.00	0.02	0.02	0.03
HIB								
Cga01	3	4.00	4.00	3.60	1.00	0.72	0.87	-0.39
Cga02	3	3.00	4.00	2.57	1.00	0.61	0.73	-0.64
Cga03	3	4.00	8.00	3.60	1.00	0.72	0.87	-0.39
Cga05	3	5.00	4.00	4.50	1.00	0.78	0.93	-0.29
Cga06	3	5.00	6.40	4.50	1.00	0.78	0.93	-0.29
Cga09	3	5.00	5.33	4.50	1.00	0.78	0.93	-0.29
Cga10	3	4.00	5.33	3.60	1.00	0.72	0.88	-0.39
Mean	3.00	4.29	3.84	1.38	1.00	0.73	0.88	-0.38
SE	0.00	0.29	0.27	0.08	0.00	0.02	0.03	0.05

N-Sample Size, Na-No of Alleles, Ne-No of Effective Alleles, I- Information Index, Ho-Observed Heterozygosity, He-Expected, uHe-unbiased expected Heterozygosity, F-Fixation Index

Table 3. Sample Size, no of alleles, no of effective alleles, information index, observed heterozygosity, expected and unbiased expected heterozygosity and fixation index for CgT, CgK and HIG

Locus	N	Na	Ne	I	H_O	H_e	uHe	F
CgT	4	5.00	4.67	1.56	1.00	0.78	0.89	-0.28
Cga01								
Cga02	4	6.00	5.33	1.73	1.00	0.81	0.93	-0.23
Cga03	4	6.00	5.33	1.73	1.00	0.81	0.93	-0.23
Cga05	4	4.00	3.56	1.32	1.00	0.72	0.82	-0.39
Cga06	4	5.00	4.00	1.49	1.00	0.75	0.86	-0.33
Cga09	4	3.00	2.67	1.04	1.00	0.63	0.71	-0.60
Cga10	4	6.00	5.33	1.73	1.00	0.81	0.93	-0.23
Mean	4.00	5.00	4.40	1.52	1.00	0.76	0.87	-0.38
SE	0.00	0.44	0.39	0.10	0.00	0.03	0.03	0.05
CgK								
Cga01	3	5.00	4.50	1.56	1.00	0.78	0.93	-0.29
Cga02	3	4.00	3.60	1.33	1.00	0.72	0.87	-0.39
Cga03	4	6.00	5.33	1.73	1.00	0.81	0.93	-0.23
Cga05	2	2.00	2.00	0.69	1.00	0.50	0.67	-1.00
Cga06	4	6.00	5.33	1.73	1.00	0.81	0.93	-0.23
Cga09	4	8.00	8.00	2.08	1.00	0.88	1.00	-0.14
Cga10	4	5.00	4.57	1.56	1.00	0.78	0.89	-0.28
Mean	3.43	5.14	4.40	1.52	1.00	0.76	0.87	-0.38
SE	0.30	0.71	0.40	0.10	0.00	0.03	0.03	0.05
HIG								
Cga01	0	0	0	0	0	0	0	0
Cga02	0	0	0	0	0	0	0	0
Cga03	0	0	0	0	0	0	0	0
Cga05	0	0	0	0	0	0	0	0
Cga06	1	2.00	2.00	0.69	1.00	0.50	1.00	-1.00
Cga09	1	2.00	2.00	0.69	1.00	0.50	1.00	-1.00
Cga10	1	2.00	2.00	0.69	1.00	0.50	1.00	-1.00
Mean	0.43	0.86	0.86	0.30	0.43	0.21	0.43	-1.00
SE	0.30	0.40	0.40	0.14	0.20	0.10	0.20	0.00

N-Sample Size, Na-No of Alleles, Ne-No of Effective Alleles, I- Information Index, Ho-Observed Heterozygosity, He-Expected, uHe-unbiased expected Heterozygosity, F-Fixation Index

Table 4. Sample size, no of alleles, no of effective alleles, information index, observed heterozygosity, expected and unbiased expected heterozygosity and fixation index for CgB

Locus	N	Na	Ne	I	H_O	H_e	uHe	F
CgB	3	4.00	3.60	1.33	1.00	0.72	0.87	-0.39
Cga01								
Cga02	4	6.00	5.33	1.73	1.00	0.81	0.93	-0.23
Cga03	4	5.00	4.57	1.56	1.00	0.78	0.89	-0.28
Cga05	4	7.00	6.40	1.91	1.00	0.84	0.96	-0.19
Cga06	4	5.00	4.57	1.56	1.00	0.78	0.89	-0.28
Cga09	4	7.00	6.40	1.91	1.00	0.84	0.96	-0.19
Cga10	4	6.00	5.33	1.73	1.00	0.81	0.93	-0.23
Mean	3.86	5.71	5.17	1.68	1.00	0.80	0.92	-0.25
SE	0.14	0.42	0.39	0.08	0.00	0.02	0.01	0.03

N-Sample Size, Na-No of Alleles, Ne-No of Effective Alleles, I- Information Index, Ho-Observed Heterozygosity, He-Expected, uHe-unbiased expected Heterozygosity, F-Fixation Index

among-individual and 10% of the variation was explained by among population with estimated variability ranging from 0.25 to 2.84. The analysis of molecular variation indicated that majority of

the variation were partitioned within individual in the populations.

4. DISCUSSION

The validation of microsatellites markers showed a high level of genetic diversity in these loci, in the clariid populations in Kano State rivers.In the clariid population, the observed heterozygosity (H_o) ranged from 0.000 to 1.00 and an average of 5.71 alleles per locus. These values confirm the high genetic variability when compared with related species, such as pacu *P. mesopotamicus* (H_o range from 0.068 to 0.911 and average of 8.5 alleles per locus), and tambaqui *C. macropomum* (H_o range from 0.430 to 0.880 and average of 12.8 alleles per locus) [15].In contrast to neutral markers (microsatellites in noncoding regions), gene-associated microsatellites might not be sensitive to selection pressure and, therefore, they have low values of gene diversity.Also, this was an indicating that the populations are not experiencing panmixis (free interbreeding) but they still share some amount of genetic diversity and the populations were somehow differentiated. The mean inbreeding coefficient between (Fst = 0.29) observed in this study was lower than the values reported for *Clariasbatrachus* (Fst = 0.55) and *Clariasnieuhofii* (Fst = 0.48) in Thailand [12,13]. It was also lower than the Nei's among heterozygosity observed in *Clariasgariepinus* (Fst = 0.44) [14] and slightly greater than the one reported for *Clariasanguillaris* in Africa, (Fst = 0.15) by [14]. Values of inbreeding coefficient within (Fis) in most natural populations are close to zero. The Fis value provides the non-random union of gametes in population, which is the mating among individuals in the population which is related more than average relationship. The higher the inbreeding coefficient (Fis) value, the more the degree of inbreeding. The negative values of inbreeding coefficient (Fis) point towards outbreeding and excess of heterozygotes in a population. For populations mating at random, genes are equally related-within or between individuals and in this case, value of inbreeding coefficient is zero (Fis = 0). Therefore, estimates of Fis that differ significantly indicate departures from random mating. Any avoidance of mating of relatives will cause Fis to exceed 0 and to be negative. More commonly, Fis is positive, which could be interpreted as evidence of inbreeding [15]. In this study, Fis values were negative (-0.26 to -0.38, mean = -0.34) indicating a deviation from inbreeding and show an outbred populations meaning that they are less related. These results were in harmony

Table 5. Grand mean for sample size, no. alleles, no. effective alleles, information index, observed heterozygosity, expected and unbiased expected heterozygosity and fixation index

	N	Na	Ne	I	Ho	He	uHe	F
Mean	3.25	4.39	4.05	1.35	0.92	0.69	0.82	-0.36
SE	0.18	0.28	0.25	0.07	0.04	0.03	0.04	0.03

N-Sample Size, Na-No of Alleles, Ne-No of Effective Alleles, I- Information Index, Ho-Observed Heterozygosity, He-Expected, uHe-unbiased expected Heterozygosity, F-Fixation Index

Table 6. Inbreeding coefficients and estimate of gene flow (Nm) over all populations for each locus

Locus	Fis	Fit	Fst	Nm	% Polymorphic loci
Cga01	-0.33	0.11	0.33	0.50	100%
Cga02	-0.35	0.11	0.34	0.48	100%
Cga03	-0.26	0.12	0.30	0.59	100%
Cga05	-0.38	0.11	0.36	0.45	100%
Cga06	-0.34	-0.04	0.22	0.87	100%
Cga09	-0.35	-0.05	0.23	0.86	42.86%
Cga10	-0.35	-0.04	0.23	0.86	100%
Mean	-0.34	0.05	0.23	0.66	91.86%
SE	0.01	0.03	0.22	0.07	8.16

Fis, Fit and Fst – Inbreeding coefficients
Nm – Estimate of gene flow

Table 7. Analysis of molecular variation (AMOVA) of hierarchical gene diversity

Source	df	SS	MS	Est. Var	%
Among Population	6	36.21	6.04	0.34	10
Among Individual	21	70.00	3.33	0.25	7
Within Individual	28	79.50	2.84	2.84	83
Total	55	185.72		3.42	100

df – degree of freedom,
SS – sum of square,
MS – mean square,
Est Var – estimated variance

with the reports of [16] who reported values of -0.25 to -0.59 and those of [17] who obtained values in range of -0.17 to -0.46.

The seven loci assayed were polymorphic for all the populations, with each having at least four allele per population. The range of alleles observed per locus, three to eight was in perfect agreement with the three to eight range observed by [18] in the analysis of five microsatellite loci in ten samples of *Clariasgariepinus*. However, [19] observed a range of five to 14 alleles in the characterization of seven microsatellite loci in *Clariasgariepinus*. The genetically heterogenous samples were observed to maintain the different allele frequencies for a microsatellite locus. This could be because those samples were under the different selection pressures, hence they were forced to maintain the different allele frequencies or because of intensive exchange of migrants in the populations. Allele frequencies in heterogeneous populations may be established as a result of random drift due to the long-term isolation or because the populations are subjected to different selection pressures[20]. The result of this study was in agreement with those of [20] who used four microsatellite to characterize cultured *Clariid* fish strains in Abeokuta, Ogun State, Nigeria.

The genotypic data obtained showed a good level of informativeness having a percentage polymorphism value of 100% for all loci exceptthe sixth locus, which was below the threshold for which genetic markers begin to be informative. The percentage of polymorphism value depends on the number of alleles detected per locus and their frequencies. The heterozygosity percentage observed for all the alleles (91.86%) fall within the observed levels of heterozygosity in fish, which range from 24 to 95% [20]. The observed average heterozygosity (Ho = 0.92 ± 0.04) was higher than the values in *Clariasgariepinus* populations collected across Africa (0.06 to 0.15)by [20]. The expected heterozygosity observed in this study (He = 0.69 ± 0.03) was also higher than the values observed from a previous study by [21,22] (He = 0.05 to 0.15).However, the Ho and He values were within the range given by [23,24] (Ho = 0.48 to 1.00, He = 0.70 to 0.95).

5. CONCLUSION

The analysis of these polymorphic markers in clariid populations revealed that clariid population in river Bangyi had the higher genetic diversity. AMOVA demonstrated that the highest proportion of variation was presented within the populations. The F_{ST} values showed the occurrence of genetic structure among the clariid population in Kano river.Our results are fundamental to beginning a breeding programme, since the genetic structure should be taken into consideration when composing an initial base population for genetic improvement programmes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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