AES BIOFLUX

Advances in Environmental Sciences - International Journal of the Bioflux Society

Diversity and distribution of fishes of Gaji River, Bauchi State, Nigeria

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Abstract. Fish sampling was conducted along the Gaji River with a view to assessing the Diversity and Distribution of the fishes. The river which is the major source of water for the diverse wild fauna in and around the Yankari National Park, is located on Latitude 9° 45′ 16″ and Longitude 10° 30′ 37″ and covers an area of about 2,244 km² (870 mi²) and is home to several natural warm water springs, as well as a wide variety of flora and fauna (Wikipedia 2011). A total of twenty sampling locations were established on the river and throughout the period of the work, different fishing gears which included cast nets, gill nets, entrance traps, hooks and line and in some cases seines were used. Furthermore, Mitochondrial DNA cytochrome b sequencing was employed to study the genetic diversity of *Clarias gariepinus* sampled from the study area due to its economic importance. Amplification of the cytochrome b (cyt b) gene was done using the polymerase chain reaction (PCR). A portion of mtDNA containing the gene was amplified using primers L15267, 5′-AAT GAC TTG AAG AAC CAC CGT-3′ and H15891, 5′-GTT TGA TCC CGT TTC GTG TA-3′ described by Briolay et al (1998). A total of 13 species were identified from the 4,218 individuals collected, representing 12 families and 13 genera. Their percentage distribution showed that *Oreochromis niloticus* had the highest percentage occurrence of about 31% followed by *Parachanna obscura* with 27%. This was followed by *Clarias gariepinus* which had about 20% distribution of occurrence. On the distribution of the identified species in the various sampling locations, Ruwan Bangiya had the highest occurrence of the different species with 11 out of the total 13 species found in this location. Ruwan Dinya, Ruwan Sarki and Ruwan Gajin Gwaza also had relatively high occurrence of the identified fish species. The lowest occurrence was found in Ruwan Dalamiri, Ruwan Kunkuru and Ruwan Kankwana. Ruwan Gada and Ruwan Kakkida had moderate distribution of the various species. Three haplotypes were identified and the result further showed that population distribution of *C. gariepinus* is homogenous throughout the study area during the study period. The mean genetic distance between all populations is low indicating that frequent gene flow occurs between all populations.
Key words: Nigeria, haplotype, PCR, fish diversity.

Introduction. In Africa fish is an important source of animal protein, constituting 23% of human daily animal protein intake as reported by (Konstapel & Noort 1995; FAO 1991). It is an important food for over 400 million Africans, contributing essential proteins, minerals and micronutrients to their diets. The continent is projected to need an additional 1.6 million tons of fish a year by 2015 just to maintain current consumption (TWC 2009). The rapid increases in fish supply required over the next decades will only be possible, therefore, if these fisheries are sustained and improved, while simultaneously developing aquaculture. Fish form an integral part of aquatic ecosystem and play many different roles in the food web. According to the World Conservation Monitoring Centre (1992), over 50% of all vertebrate species living on earth are fish and some 40% of these fish are freshwater species.

The study of diversity and variation has been widely carried out in different populations and species and form the basis of many biological investigations such as systematics (Yahia & Sissom 1996), evolution (Cesaroni et al 1994), animal conservation and management (Sherley 1996; Britten et al 1997) using different approaches which include the study of morphological characters (Vicario et al 1988) and DNA markers

(Carvalho & Pritcher 1995). One of the most important criteria for any efficient conservation and management programme is the taxonomic clarification of species complexes as well as the assessment of genetic biodiversity within and among populations. According to Paul-Michael (2004), species are the currency of biology. Genetic variation study is important for stock management in fisheries. The concept of 'stock' is essential for both fisheries and endangered species management (Begg & Waldman 1999). According to Begg et al (1999) that stocks are random groups of fish large enough to self-reproducing, with members of each group having similar life history characteristics. Genetic variation study is useful to understand the stock structure of a species and design an appropriate management guideline in fisheries where multiple stocks are exploited differentially (Ricker 1981). The process of defining fish stock is essential for effective management. Fisheries management generally aims to achieve maximum sustainable production from fish stocks (Begg & Waldman 1999). DNA tests and sequencing are the most popular methods for species identification and conservation studies of animal specimens at present (Tsai et al 2007; Gorgan 2009).

The primary aim of this work is to contribute to the discovery and understanding of ecological, economic and conservation functions of the fish species in the study area. Hence, the main objectives of the work are to determine the population structure of the fishes in the study area, evaluate the differentiation and systematics of the fish species and determine the genetic diversity of *Clarias gariepinus* as a very important food fish species using cytochrome b gene molecular marker. This will enable new research and applications addressing problems of productivity and conservation of important genes in fish species of the area.

Materials and Methods

The study area. Gaji river complex is the major source of water for the diverse wild fauna in and around the Yankari National Park. Yankari National Park is a large wildlife park located in the south-central part of Bauchi State, in northeastern Nigeria. It is located on Latitude 9° 45′ 16″ and Longitude 10° 30′ 37″ and covers an area of about 2,244 km^2 (870 mi^2) and is home to several natural warm water springs, as well as a wide variety of flora and fauna. Its location in the heartland of the West African savanna makes it a unique way for tourists and holidaymakers to watch wildlife in its natural habitat. Gaji river is of great biological and economic importance as its tributaries and main channels provides an important source of cash income to the villagers around through fishing activities. Being part of a protected area, the river complex serves as home to variety of aquatic fauna, including fish, thereby conserving the genetic resources of the aquatic fauna found in the protected portion.

Fish sampling. An intensive survey of the Gaji river complex was carried out through reconnaissance visits to identify locations that are easily accessible and have potentials of retaining water for long periods. A total of twenty sampling locations were established on the Gaji river. The river, being not perennial, has some parts of it drying up in some parts of the year. The sampling locations were established based on availability of water in the river during the period of the research (Figure 1, Table 1). Throughout the period of the work different fishing gears were used to ensure total coverage of the diverse fish community in the study locations. The different fishing gears used included casting nets, gill nets, entrance traps, hooks and line and in some cases seines. Fish was sampled monthly for a period of nine months. Fish samples collected were preserved in either 10% formalin or 45% absolute alcohol depending on the fish types, for further analysis. Identification of fish species was carried out using fish the identification keys developed by Reed et al (1976); Holden & Reed (1978); Fish Base (1998); and Olaosebikan & Raji (1998).

Determination fish species diversity indices. Measures of diversity are frequently seen as indicators of the well being of ecological systems and it can be measured by recording the number of species, by describing their relative abundances or by using a measure which combines the two components (Magurran 1988). In this work, Biological Diversity indices were calculated to understand the relationships between and within the

fish species encountered in the work. The indices calculated were the Simpson's Diversity index, and Margalef's richness index.

Determination of genetic diversity of Clarias gariepinus.

DNA extraction, quantification and polymerase chain reaction. In order to preserve the tissue for DNA extraction, the muscle tissue of Clarias gariepinus was first preserved in 95% Ethanol and later Tris-NaC1-EDTA-SDS (TNES-Urea). Extraction of genomic DNA was done using AquaGenomic Extraction Kit following the manufacturer's instruction. After extraction the DNA solution was stored at 4^0C at 20^0C for long term storage as recommended by (Nguyen et al 2006). The purity of genomic mtDNA was measured by using two approaches which is by gel electrophoresis and spectrophotometer methods, the purity of genomic DNA was observed by a single band pattern that appears on a 0.8% (w/v) agarose gel after staining in ethidium bromide and visualized under ultraviolet (UV) light (Nguyen et al 2006). The quantity and quality of the extracted DNA was assessed by using Hitachi U, 1900 UV/VIS Spectrophotometer 200V. The optical Density (OD) of each diluted DNA samples were taken at two different values; OD_{260} and OD_{280} for the assessment of quality and quantity of DNA extracted. A 0.8% and 1.7 agarose gel (1st Base) in 0.5X Tris-Borate EDTA (TBE) buffer solution was prepared for assessment of DNA extraction and PCR products respectively.

Mitochondrial DNA sequencing of cytochrome b. The PCR was conducted using G-STORM Dual compartment 48 wells and Effendorf Master Cycler 77 wells Thermocyclers. PCR products were stored at -20^0C to avoid denaturing. A portion of mtDNA containing the gene was amplified using primers L15267, $5'$-AAT GAC TTG AAG AAC CAC CGT-$3'$ and H15891, $5'$-GTT TGA TCC CGT TTC GTG TA-$3'$, previously described by Briolay et al (1998). Purification of PCR products were done by using Promega wizard SV Purification Kit by the Centrifugation method following the Promega procedure (Anon 2006).

Purified PCR products were sequenced in the forward direction. The sequence results were analysed using Chromas Lite Version 2.01. and BioEdit Sequence Alignment Editor (Tamura et al 2007). Haplotypes were identified by creating a new alignment in Alignment Explorer/CLUSTAL. The sequences were again edited manually to check any nucleotides gaps that were not in the position. This was done to find best alignment of all sequences by referring to chromatogram (using Cromas Lite Version 2.01) of each haplotypes.

Results and Discussion. A total of 13 species were identified from the 4,218 individuals collected, representing 12 families and 13 genera. Table 1 shows the species distribution and the families of all the species identified. Their percentage distribution showed that *Oreochromis niloticus* had the highest percentage occurrence of about 31% followed by *Parachanna obscura* with 27%. This was followed by *Clarias gariepinus* which had about 20% distribution of occurrence.

On the distribution of the identified species in the various sampling locations, Ruwan Bangiya had the highest occurrence of the different species with 11 out of the total 13 species found in this location. Ruwan Dinya, Ruwan Sarki and Ruwan Gajin Gwaza also had relatively high occurrence of the identified fish species (Table 3). The lowest occurrence was found in Ruwan Dalamiri, Ruwan Kunkuru and Ruwan Kankwana. Ruwan Gada and Ruwan Kakkida had moderate distribution of the various species. Depending on the duration of any diversity studies and the purpose or design of the study, the number of species to be recorded can vary. More species could have been encountered in this work if the study was carried out for longer duration. This result compares with Muchlisin & Azizah (2009) who worked on fish diversity in a region in Indonesia. The results also showed that Ruwan Bangiya, Ruwan Bugudu and Gajin Gwaza had the highest Diversity indices. Ruwan Boda also had high evenness value despite relatively low Diversity index. This agrees with the findings of Muchlisin & Azizah (2009). It is also in line with the results obtained by Bhat (2003). Moses (2000) also worked on fisheries of southern Nigeria. According to him, the productivity of mangrove wetlands is very high (Mercer & Hamilton, 1984); because of this, and coupled with the fact that such areas offer some measure of protection and shelter, mangrove ecosystems attract

and support high densities of larval and juvenile stages of many commercially important species of both pelagic and demersal fishes; example of such fishes include bonga.

Fish Species Diversity. The Simpson's diversity index and Margalef's richness index of the fish calculated based on the locations indicate that Ruwan Bugudu had the highest index of diversity of 4.65 followed by Ruwan Bangiya with 4.55. Ruwan Kakkida, Ruwan Gajin Makeri, Ruwan Bakin Ruwa, Ruwan Binga and Ruwan Dinya had high values of diversity index ranging between 3.60 to 3.95 (Table 2). The value of the index increases with increasing diversity in a location. The least diversity index value of 1.16 was recorded in Ruwan Ganga. Interms of species richness in the sites, The Margalef's species richness index of 1.73 was recorded in Ruwn Bangiya eventhough it had a relatively lower Diversity index value compared to Ruwan Bugudu. The lowest richness index value of 0.50 was recorded in Ruwan maciyar Maje. Ruwan Dalamiri, Ruwan Kunkuru, Ruwan Kankwana had relatively same richness interms of their species composition.

Table 1

Fish occurrence in sampling sites of Gaji river, Bauchi State, Nigeria

Fish species	Sampling site																			
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
Heterotis niloticus	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+	-
Clarias gariepinus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrocynus lineatus	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-
Parachanna obscura	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oreochromis niloticus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Protopterus annectens	+	+	+	+	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-
Gymnarchus niloticus	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Tilapia zilli	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Schilbe mystus	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Ctenopoma kingsleyae	+	+	+	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
Synodontis ocellifer	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gnathonemus abadii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Polypterus senegalus	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+

Key: + = Presence, - = Absence, A= Ruwan Dinya, B= Ruwan Bugudu, C= Ruwan Bangiya, D= Ruwan Sarki, E= Ruwan Gada, F= Ruwan Boda, G= Ruwan Gajin Gwaza, H= Ruwan Ganga, I= Ruwan Kakkida, J= Ruwan Gajin Makeri, K= Ruwan Bakin Ruwa, L= Ruwan Maciyar Maje, M= Ruwan Maidabaki, N= Ruwan Lika-Lika, O= Ruwan Binga, P= Ruwan Wambai, Q= Ruwan Dalamiri, R= Ruwan Kunkuru, S= Ruwan Kankwana, T=Ruwan Salmanu

Table 2

Biological indices of fishes in Gaji river, Nigeria according to sampling sites

Location (Site)	Species richness (Margalef's Index)	Simpson's Index (D)	Reciprocal of D
Ruwan Dinya	1.51	0.25	3.95
Ruwan Bugudu	1.14	0.22	4.65
Ruwan Bangiya	1.73	0.21	4.55
Ruwan Sarki	1.51	0.31	3.28
Ruwan Gada	1.27	0.25	4.00
Ruwan Boda	1.04	0.33	2.99
Gajin Gwaza	1.65	0.24	4.11
Ruwan Ganga	1.06	0.86	1.16
Ruwan Kakkida	1.23	0.28	3.60
Ruwan Gajin Makeri	0.87	0.28	3.60
Ruwan Bakin Ruwa	1.24	0.27	3.66
Ruwan Maciyar Maje	0.50	0.27	3.66
Ruwan Maidabaki	1.08	0.38	2.61
Ruwan Lika-Lika	0.74	0.19	5.29
Ruwan Binga	0.86	0.25	3.92
Ruwan Wambai	1.11	0.34	2.91
Ruwan Dalamiri	0.72	0.37	2.71
Ruwan Kunkuru	0.73	0.28	3.57
Ruwan Kankwana	0.74	0.30	3.39
Ruwan Salmanu	1.03	0.32	3.10

Molecular diversity

DNA extraction in Clarias gariepinus. There are many methods to store and preserve tissue for molecular studies. For this study, muscle tissue of *Clarias gariepinus* was preserved in TNES-Urea. This buffer has several advantages over other preservation buffer. A total of 20 samples were extracted for further analysis in performing DNA sequencing using AqualGenomic Extraction Kit. The DNA quality and quantity value based on spectrophotometer was satisfactory ranging from 1.1 to 2.2 with a least value of DNA quantity of 10ng/ µL.

Optimization of cytochrome b PCR conditions. $MgCl_2$, concentration of 2.0 mM was chosen for DNA amplification as it resulted in visibly clear fragment. Optimization was further conducted for primer concentration to eliminate the unspecified products. Annealing temperature of 50^0C was established because it successfully amplified the DNA fragment by producing clear and high intensity band while the established PCR condition is shown in Table 3. A total of 11 samples were successfully amplified by using the optimized PCR conditions obtained. Sample with amplified Cyt b mtDNA fragment were then purified to eliminate enzymatic reactions present from the amplification reactions.

Table 3

Established PCR conditions

Reagent	Volume (µL)
10x PCR Buffer	2.5
25mM $MgCl_2$	2.8
10mM dNTP	0.5
5mM Primer L	0.8
5mM Primer H	0.8
Taq Polymerase	0.4
ddH_2O	14.7
DNA template	2.5
Total volume	25µL

Purification of PCR product. Purification of Cyt b PCR was only performed on amplified products with clear and high intensity bands. Plate 1 shows the purification results of Cyt b PCR product. Only purified Cyt b PCR products that had high bands were sent for sequencing. PCR amplification was repeated on samples which did not show high intensity bands after purification. A total of 11 samples were sent for sequencing.

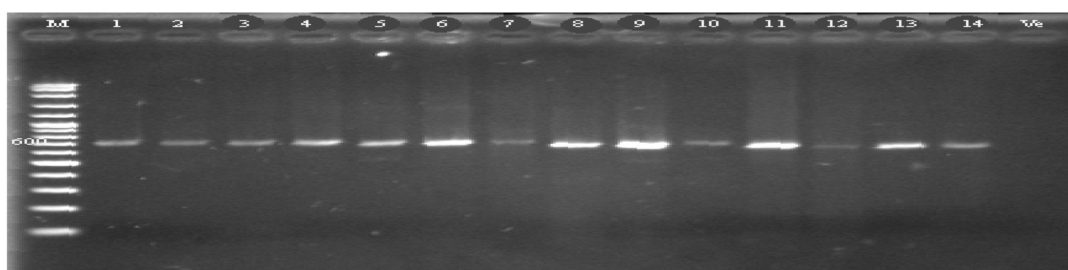

Figure 1. Purified PCR products for *Clarias gariepinus.*

Cytochrome b sequencing. Based on the sequencing results, only 11 samples were chosen for further analysis using BioEdit and Mega 4. Samples were chosen based on peaks on the chromatogram produced through sequencing. Sequencing with high peak and with little overlap indicated good satisfactory results. For most samples, the sequencing generated more than 580 base pair. Forward sequences for each sample were compared and edited by using BioEdit sequence alignment to ensure that all ambiguous sites were corrected. All sequences were BLAST in National Center for Biotechnology Information (NCBI) to confirm that the obtained sequences were Cyt b gene sequences from *Clarias gariepinus.* Accession numbers assigned to the identified haplotypes are shown in Table 4. From the 11 individuals, 3 haplotypes were identified. The sequences of the identified haplotypes, relative distribution of the nucleotide compositions and molecular diversity indices are shown in Figure 2 and Tables 5 and 6.

- hap01
CACCCATTATTCAAAATTGTCAACGACGCACTCATCGACCTTCCCGCCCCCTCTAATATC
TCCGCATGATGAAACTTCGGCTCTCTACTATTATTATGTCTTGGAGTACAAATCCTCACA
GGTCTATTCCTAGCCATACACTACACTTCTGATATCTCAACCGCATTCTCATCAGTAGTA
CACATCTGCCGAGACGTCAACTACGGATGAATTATCCGAAACCTTCACGCCAACGGAGCA
TCCTTCTTCTTCATCTGCATCTACCTTCACATTGGCCGCGGTCTTTACTACGGCTCATAC
CTATACAAAGAAACATGAAACATTGGCGTTGTACTACTCCTTTTAGTAATAATAACAGCC
TTCGTAGGATACGTACTACCATGAGGACAAATATCCTTCTGAGGTGCCACAGTAATCACA
AACCTCTTATCAGCCGTACCTTACATAGGAGATGCCCTAGTCCAATGAATCTGAGGAGGT
TTCTCCGTAGACAATGCAACACTTACACGATTTTTCGCATTCCACTTCCTCCTACCATTC
ACAATCATCGCAGCTACAATTCTACACGCTCTATTCCTACACG
- hap02
CACCCATTATTCAAAATTGTCAACGACGCACTCATCGACCTTCCCGCCCCCTCTAATATC
TCCGCATGATGAAACTTCGGCTCTCTACTATTATTATGTCTTGGAGTACAAATCCTCACA
GGTCTATTCCTAGCCATACACTACACTTCTGATATCTCAACCGCATTCTCATCAGTAGTA
CACATCTGCCGAGACGTCAACTACGGATGAATTATCCGAAACCTTCACGCCAACGGAGCA
TCCTTCTTCTTCATCTGCATCTACCTTCACATTGGCCGCGGTCTTTACTACGGCTCATAC
CTATACAAAGAAACATGAAACATTGGCGTTGTACTACTCCTTTTAGTAATAATAACAGCC
TTCGTAGGATACGTACTACCATGAGGACAAATATCCTTCTGAGGTGCCACAGTAATCACA
AACCTCTTATCAGCCGTACCTTACATAGGAGATGCCCTAGTCCAATGAATCTGAGGAGGT
TTCTCCGTAGACAATGCAACACTTACACGATTTTTCGCATTCCACTTCCTCCTACCATTC
ACAATCATCGCAGCTACAATTCTACACGCTCTGTTCCTACACG
- hap03
CACCCATTATTCAAAATTGTCAACGACGCACTCATCGACCTTCCCGCCCCCTCTAATATC
TCCGCATGATGAAACTTCGGCTCTCTACTATTATTATGTCTTGGAGTACAAATCCTCACA
GGTCTATTCCTAGCCATACACTACACTTCTGATATCTCAACCGCATTCTCATCAGTAGTA
CACATCTGCCGAGACGTCAACTACGGATGAATTATCCGAAACCTTCACGCCAACGGAGCA
TCCTTCTTCTTCATCTGCATCTACCTTCACATTGGCCGCGGTCTTTACTACGGCTCATAC
CTATACAAAGAAACATGAAACATTGGCGTTGTACTACTCCTTTTAGTAATAATAACAGCC
TTCGTAGGATACGTACTACCATGAGGACAAATATCCTTCTGAGGTGCCACAGTAATCACA
AACCTCTTATCAGCCGTACCTTACATAGGAGATGCCCTAGTCCAATGAATCTGAGGAGGT
TTCTCCGTAGACAATGCAACACTTACACGATTTTTCGCATTCCACTTCCTCCTACCATTC
ACAATCATCGCAGCTACAATTCTGCACGCTCTATTCCTACACG

Figure 2. Sequences of identified haplotypes of *Clarias gariepinus* from Gaji river

Table 4

NCBI genbank Accession No. of identified Haplotypes of *Clarias gariepinus* in Gaji River

Haplotype	Genbank Acc. No.
C. gariepinus hap 01	GU906879
C. gariepinus hap 02	GU906880
C. gariepinus hap 03	GU906881

Table 5

Relative distribution of Nucleotide composition of *Clarias gariepinus* in Gaji River

Base	Rel. Percentage
Cytosine (C)	29.85%
Thymine (T)	28.13%
Adenine (A)	27.74%
Guanine (G)	14.29%
Total	100.00%

Table 6

Molecular diversity indices of *Clarias gariepinus* in Gaji river

Index		Value
Sample size (Good sequences)	:	11.0000
No. of haplotypes	:	3
Deletion weight	:	1.0000
Transition weight	:	1.0000
Transversion weight	:	1.0000
Allowed level of missing data	:	5.0000 %
Number of observed transitions	:	2
Number of observed transversions	:	0
Number of substitutions	:	2
Number of observed indels	:	0
Number of polymorphic sites	:	2
Number of observed sites with transitions	:	2
Number of observed sites with transversions	:	0
Number of observed sites with substitutions	:	2
Number of observed sites with indels	:	0
Number of observed nucleotide sites	:	583
Number of usable nucleotide sites	:	583

Low GC content and low number of haplotypes were recorded in this study. However, Alves et al (2001) reported higher values. Sequences of the entire cyt b gene done by Alves et al (2001) and a segment of the control region (1140 and 678 bp, respectively) from 42 specimens representing nine tributaries of the Guadiana River drainage revealed 35 `composite' haplotypes, leading to a high estimate of diversity. Maintained gene flow between two populations can also reduce genetic variation between the two groups. Gene flow will resist the differentiation of gene pool that occurs when mutation, genetic drift and natural selection favouring adaptation to local environmental condition will all lead to the genetic differentiation of local populations. Gene flow may either constrain evolution by preventing adaptation to local condition or promote evolution by spreading new genes and combinations of genes throughout a species' range (Slatkin 1987).

Conclusion. The Gaji river has high species diversity as indicated by the number of Families represented. The overall results showed that the mean genetic distance between all the sampled populations is low indicating that frequent gene flow occurs between the populations. Despite the high species diversity, there is low genetic diversity as shown by the low number of haplotypes identified in the earlier results. This can be attributed to the absence of barriers which could have caused diversity in the gene pool of members of same species.

Acknowledgements. The support of Abubakar Tafawa Balewa University for the award of study fellowship and the Education Trust Fund (ETF), Nigeria for providing grant towards sponsoring the major author to Universiti Sains Malaysia to carryout part of the work is highly acknowledged.

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Received: 12 December 2011. Accepted: 15 February 2012. Published online: 28 August 2012.
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How to cite this article:
Nayaya A. J., Siti-Azizah M. N., Adikwu I. A., Istifanus W. A., Ezra A. G., Muchlisin Z. A., 2012. Diversity and distribution of fishes of Gaji River, Bauchi State, Nigeria. AES Bioflux 4(2):50-58.