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# Preliminary Study of Genetic Variation of *Heterobranchus bidorsalis* from Rivers Benue and Niger in Nigeria using Microsatellite Markers

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## Abstract

This study was carried out to compare the genetic variations of *Heterobranchus bidorsalis* from River Niger and River Benue in Nigeria using Microsatellite marker CN13. A total of 20 samples of *Heterobranchus bidorsalis* was obtained from the wild in Kebbi state and Adamawa state. The DNA was extracted using Zymo Research Extraction Kit and the Polymerase Chain reaction (PCR) was done with a PCR thermocycler Mastercycler Personal Eppendorf 22331 Hamburg Machine. The DNA band was run on 1% Agarose Gel Electrophoresis. Individual amplification success was 65%. 92% of the total alleles observed for the locus was homozygous and 8% was heterozygous. The genetic distance for the population ranged from 0.000 to 113.484 and had Cophenetic Correlation Coefficient (CP) value of 0.999. It was concluded that slight variation exists between the two populations of *H. bidorsalis*. This could be due to differences in ecological zones where the samples were collected though they belong to the same ancestor. It is therefore recommended that the two population can be crossed to produce hybrids with better vigour.

**Keywords**: *Heterobranchus bidorsalis*; Ecological zones; Microsatellite Markers and DNA.

**Introduction**:

The fish species *Heterobranchus* is very common and widely distributed throughout Africa. They go by different names in various localities and in Nigeria are collectively referred to as the Mud catfishes (Madu *et al*., 1999). The hybrid mudfish is the crossbreed between the *Heterobranchus* and *Clarias* species (Madu *et al*., 1999).

According to technical principles, there are three classes of molecular markers: nucleic acid hybridization based on complementary bases, e.g., restriction fragment length polymorphisms (RFLPs); Polymerase Chain Reaction (PCR) based on DNA amplification, e.g., random amplification of polymorphic DNAs (RAPD), amplified fragment length polymorphisms (AFLP), microsatellites or simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) (Hoshino *et al*., 2012). Microsatellites were detected in eukaryote genomes almost thirty years ago and they are the most promising PCR-based markers (Jarne and Lagoda, 1996). Microsatellites are tandemly repeated motifs of variable lengths that are distributed throughout the eukaryotic nuclear genome in both coding and non-coding regions (Jarne and Lagoda, 1996). They also appear in prokaryotic and eukaryotic organellar genomes, e.g., chloroplast (Powell *et al*., 1995) and mitochondria (Soranzo *et al*., 1999).

In addition to their co-dominant feature, i.e., the identification of all alleles of a given locus, microsatellites can also be amplified using polymerase chain reaction (PCR) in stringent conditions that usually only permit the amplification of single loci, thus facilitating data integration (Bravo *et al*., 2006). Furthermore, microsatellites are widely distributed throughout the genome, highly polymorphic and transferable between species. These features provide the foundation for their successful application in a wide range of fundamental and applicable fields (Chistiakov *et al*., 2006).

Genomic conservation of microsatellite loci has also been compared among channel catfish, blue catfish, white catfish (*Ameiurus catus*) and flathead catfish

(*Pylodictus olivaris*) (Liu *et al*., 1999; Tan *et al*., 1999), all in the family Ictaluridae. The microsatellite loci were highly conserved in all genera tested from Ictaluridae. All channel catfish primers tested successfully amplified genomic DNA from flathead catfish, and 86% of the channel catfish primers successfully amplified the genomic DNA from white catfish (Liu *et al*., 1999a). Microsatellite have also been used in the study of *Chrysichthys nigrodigitatus* (Nwafili 2014), *Pseudo-nitzschia pungens* (Nicolaus 2006).

Therefore, this study is aimed at comparing the genetic variations among strains of *Heterobranchus bidorsalis* obtained from the two major rivers in Nigeria, as a tool in fish breeding and genetics.

## Materials and Methods

### *Sample Collection*

The study was carried out on 10 wild samples each of *Heterobranchus bidorsalis* obtained from Kebbi State and Adamawa Sate (Figure 1). Fish were transported in 50 liters jerry cans to the Modibbo Adamawa University of Technology (MAUTech) Yola. They were conditioned and kept for onward tissue collection. Fin tissues (25 mg) were collected and transferred immediately into a 1.5 ml microcentrifuge tube for DNA extraction.

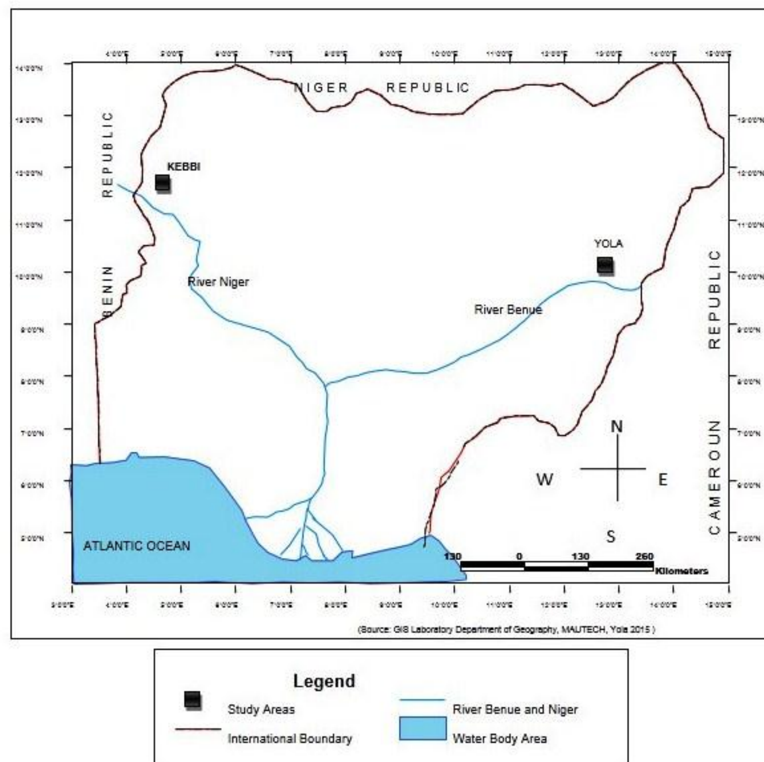


**Figure 1:** Map showing the Collection points

### *DNA extraction*

This was done in the Molecular Biology Laboratory of Chevron Biotechnology Center at the Modibbo Adama University of Technology (MAUTECH), Yola. The Genomic DNA was extracted using ZR Genomic DNA™ and manufacturer's protocol was followed. Briefly, to a 25mg of tissue sample in a micro centrifuge tube, 95µl of Nuclease free water, 95µl of 2x Digestion buffer and 10 µl Proteinase K were added. These were mixed thoroughly by votexing and later incubated in a water bath at 55°C for 3 hours. Three micro liters (3µl) of RNase was added to the tube and incubated at room temperature for 5mins. 700µl of Genomic Lysis buffer was added to the tube and mixed by votexing. It was then centrifuged at 10,000xg for one minute. The supernatant was transferred to a Zymo-spin™ IIC column placed in a collection tube and was centrifuged at 10,000xg for 1 minute. 200µl of DNA pre-wash buffer was added to the spin-column in a new collection tube. It was centrifuged at 10,000xg for 1 minute. 400µl of g-DNA wash buffer was added to the spin column and centrifuged at 10,000xg for 1 minute. The spin-column was transferred to a clean micro centrifuge tube. 100µl of Elution buffer was added to the spin column and

incubated for 2-5 minutes at room temperature. It was then centrifuged at top speed for 30 seconds to elute the DNA. The eluted DNA was stored at -20°C for further use

***Polymerase Chain Reaction (PCR) amplification***

This was done at the National Veterinary Research Institute (NVRI) Vom Plateau State. One microsatellite loci CN13 was PCR amplified in a 50µl total reaction volume containing 39.7µl of Distilled water, 1x PCR buffer, 2mM dNTP mix, 0.03U of Taq Polymerase, <100ng Template DNA, and 0.2µM each of the forward and reverse primer sets. Thermal cycling conditions were set as follows: Initial Denaturation at 94°C for 4 seconds, 35 cycles of Denaturation at 94°C for 30 seconds, annealing at 60°C for 1 minutes and extension at 68°C for 1 minutes. A final extension of 68°C for 10 minutes.

***Statistical analysis***

Gel image was scored manually with binary numbers using the MS power point. All binary variables were entered into the Excel spreadsheet and data were analyzed using the Unweighted Pair Group Method of Analysis (UPGMA). The Genetic distance, similarity matrix, Cophenetic Correlation Coefficient (CP) and input data for the phylogenetic tree was computed using the online version of Unweighted Pair Group Method of analysis (UPGMA) at www.genomes.urv.cat/cgibin/UPGMAboot.

**Results**

PCR amplification was successful for sixty five percent (65%; n = 13) of the samples. 92% were homozygous while 8% of the populations were heterozygous. The result of the Number of Alleles and allelic sizes for each sample is shown in Table 1

**Table 1:** Allele size by locus for each sample

| **Samples** | **CN13 (bp)** | | **Homozygote (Ho) / Heterozygote (He)** |
|---|---|---|---|
| Yola *H. bidorsalis* sample 1 | 230 | | Homozygote |
| Yola *H. bidorsalis* sample 2 | 230 | | Homozygote |
| *Yola *H. bidorsalis* sample 3 | | | |
| Yola *H. bidorsalis* sample 4 | 200 | | Homozygote |
| *Yola *H. bidorsalis* sample 5 | | | |
| *Yola *H. bidorsalis* sample 6 | | | |
| Yola *H. bidorsalis* sample 7 | 230 | | Homozygote |
| Yola *H. bidorsalis* sample 8 | 200 | | Homozygote |
| Yola *H. bidorsalis* sample 9 | 230 | | Homozygote |
| Yola *H. bidorsalis* sample 10 | 200 | | Homozygote |
| Kebbi *H. bidorsalis* sample 1 | 220 | | Homozygote |
| *Kebbi *H. bidorsalis* sample 2 | | | |
| *Kebbi *H. bidorsalis* sample 3 | | | |
| Kebbi *H. bidorsalis* sample 4 | 250 | | Homozygote |
| Kebbi *H. bidorsalis* sample 5 | 250 | | Homozygote |
| Kebbi *H. bidorsalis* sample 6 | 250 | | Homozygote |
| *Kebbi *H. bidorsalis* sample 7 | | | |
| *Kebbi *H. bidorsalis* sample 8 | | | |
| Kebbi *H. bidorsalis* sample 9 | 200 | 150 | Heterozygote |
| Kebbi *H. bidorsalis* sample 10 | 200 | | Homozygote |

Areas marked * had no amplicons.

Similarity matrix Table 2 was computed using online version of UPGMA using binary code generated from the gel electrophoresis result. The amplified products were used to generate the binary code. Regions with amplifications were assigned a code of 1, those without amplification were assigned a code 0. Samples that did not amplify completely were excluded from analysis. Samples with 1.000 means that they are the same without variation. From the similarity table, the following samples y1, y2, y7 y8, and y10 are grouped similar, also y9 and y4, are similar. Likewise, n1, y4, y8, n6, n4, n5 and n9 are similar. The value 0.674 denotes 67.4% of

similarity. It shows that 32.6% differences has not occurred by chance.

**Table 2:** Similarity Matrix computed with Pearson coefficient

| y1 | y2 | y4 | y7 | y8 | y9 | y10 | n1 | n4 | n5 | n6 | n9 | n10 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| y1 | 1 | 1.000 | 0.091 | 1.000 | 0.091 | 1.000 | 0.091 | 0.091 | 0.091 | 0.091 | 0.091 | 0.135 | -0.091 |
| y2 | | 1 | 0.091 | 1.000 | 0.091 | 1.000 | 0.091 | 0.091 | 0.091 | 0.091 | 0.091 | 0.135 | -0.091 |
| y4 | | | 1 | 0.091 | 1.000 | 0.091 | 1.000 | 0.091 | 0.091 | 0.091 | 0.091 | 0.674 | 1.000 |
| y7 | | | | 1 | 0.091 | 1.000 | 0.091 | 0.091 | 0.091 | 0.091 | 0.091 | 0.135 | -0.091 |
| y8 | | | | | 1 | 0.091 | 1.000 | 0.091 | 0.091 | 0.091 | 0.091 | 0.674 | 1.000 |
| y9 | | | | | | 1 | 0.091 | 0.091 | 0.091 | 0.091 | 0.091 | 0.135 | -0.091 |
| y10 | | | | | | | 1 | 0.091 | 0.091 | 0.091 | 0.091 | 0.674 | 1.000 |
| n1 | | | | | | | | 1 | 0.091 | 0.091 | 0.091 | 0.135 | -0.091 |
| n4 | | | | | | | | | 1 | 0.091 | 0.091 | 0.135 | -0.091 |
| n5 | | | | | | | | | | 1 | 1.000 | 0.135 | -0.091 |
| n6 | | | | | | | | | | | 1 | 0.135 | -0.091 |
| n9 | | | | | | | | | | | | 1 | 0.674 |
| n10 | | | | | | | | | | | | | 1 |

KEY:
n = Kebbi Samples of *H. bidorsalis*
y= Yola Samples of *H. bidorsalis*

Table 3 showed the distance matrix between samples. The matrix was constructed using online version of UPGMA. Samples with distance matrix of 0.000 are the same. The higher the value of the distance matrix, the further apart they are from each other. The highest distance matrix can be seen on the sample n10 column (113.484). The distance matrix value corresponds to samples on the vertical axis on the left.

**Table 3:** Distance matrix based on Pearson coefficient

| Y1 | Y2 | Y4 | Y7 | Y8 | Y9 | Y10 | N1 | N4 | N5 | N6 | N9 | N10 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Y1 | 0 | 0.000 | 109.091 | 0.000 | 109.091 | 0.000 | 109.091 | 109.091 | 109.091 | 109.091 | 109.091 | 113.484 | 109.091 |
| Y2 | | 0 | 109.091 | 0.000 | 109.091 | 0.000 | 109.091 | 109.091 | 109.091 | 109.091 | 109.091 | 113.484 | 109.091 |
| Y4 | | | 0 | 109.091 | 0.000 | 109.091 | 0.000 | 109.091 | 109.091 | 109.091 | 109.091 | 32.580 | 0.000 |
| Y7 | | | | 0 | 109.091 | 0.000 | 109.091 | 109.091 | 109.091 | 109.091 | 109.091 | 113.484 | 109.091 |
| Y8 | | | | | 0 | 109.091 | 0.000 | 109.091 | 109.091 | 109.091 | 109.091 | 32.580 | 0.000 |
| Y9 | | | | | | 0 | 109.091 | 109.091 | 109.091 | 109.091 | 109.091 | 113.484 | 109.091 |
| y10 | | | | | | | 0 | 109.091 | 109.091 | 109.091 | 109.091 | 32.580 | 0.000 |
| N1 | | | | | | | | 0 | 109.091 | 109.091 | 109.091 | 113.484 | 109.091 |
| N4 | | | | | | | | | 0 | 0.000 | 0.000 | 113.484 | 109.091 |
| N5 | | | | | | | | | | 0 | 0.000 | 113.484 | 109.091 |
| N6 | | | | | | | | | | | 0 | 113.484 | 109.091 |
| N9 | | | | | | | | | | | | 0 | 32.580 |
| N10 | | | | | | | | | | | | | 0 |

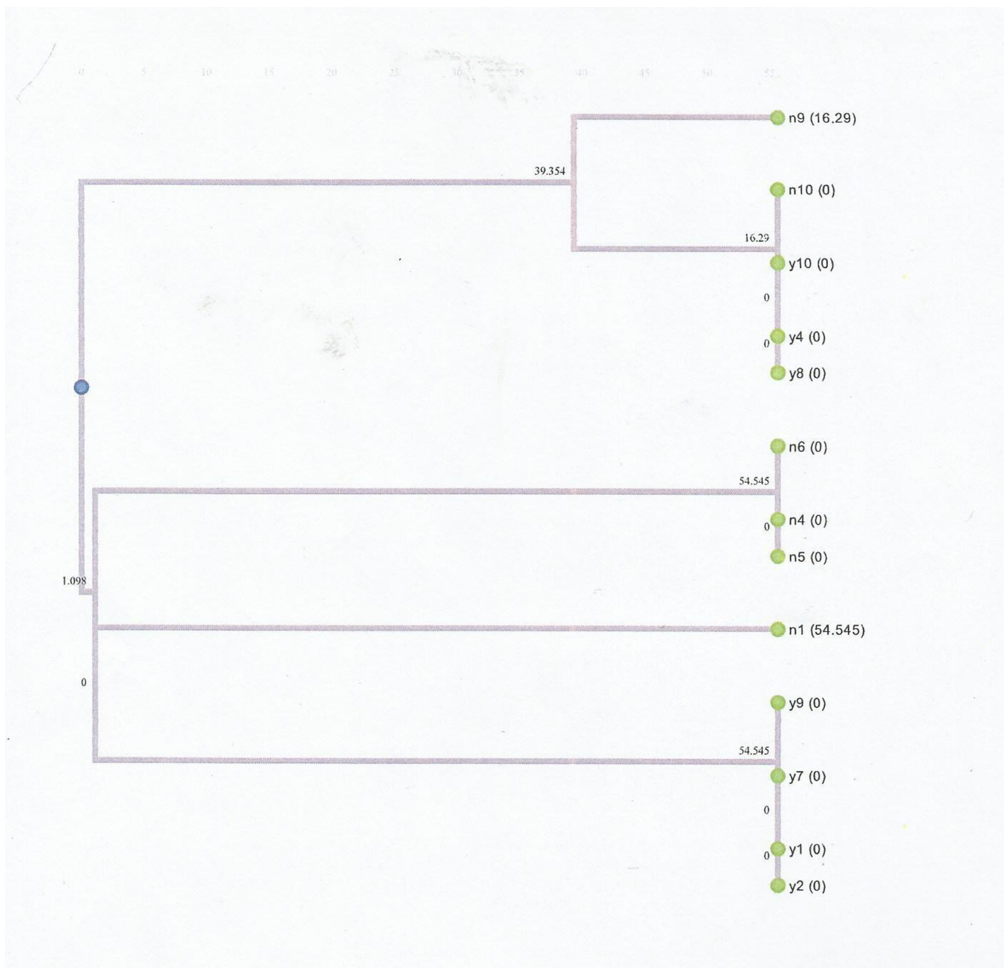


**Figure 2:** The phylogenetic tree constructed using UPGMA. The tree was constructed using clustered binary number obtained from the gel electrophoresis.

## Discussion

The individual sample amplification success was 65%. Agbebi *et al*., (2013) observed 95% amplification success using 5 Microsatellite loci. Mburu and Hanotte (2005) reported that one of the causes of non-amplification can be due to too high or low annealing temperature. If the temperature is too high, the primers will not anneal perfectly and with a high temperature, there will be no amplification at all.

The locus assayed was polymorphic for one sample with two numbers of alleles. The rest were non polymorphic. The range of alleles observed per locus one to two does not agree with the three to eight range observed by Volkaert *et al*., (1998) in the analysis of five microsatellite loci in ten samples of *Clarias gariepinus*. The number of alleles observed in *H. bidorsalis* and *C. gariepinus* ranged from four to eight as observed by Agbebi *et al*., (2013). Chiang *et al*., 2008 observed a range of two to eight alleles per locus in captive stock of endangered freshwater fish *Varicorhinus alticorpus* (*Cyprinidae*).

The genetically homogenous samples were observed not to maintain the same allele frequencies for the microsatellite loci. This can be due to variability of the genetic constitutes of the samples as they have to adapt to certain environmental factors in the different populations. This agrees with the result that environmental barriers, historical process and life histories for example, mating systems may all, to some extent shape the genetic structure of populations (Francois and Nicolas 2002).

The range of genetic distance observed in this study was 0.000 to 113.484 showing that some of the samples were closely related while some were far apart from each other. Agnese (1989) reported that genetic distance varying from 0.271 to 0.916 in the genus *Chrysichthys*, Agbebi *et al*., (2013) observed a genetic distance of 0.8364 to 0.8888. The values obtained in this study was greater than those

reported by Agnese (1991) between populations of *Chrysichthys auratus* (0.003 to 0.112).

Similarity matrix is a measure of genetic variability. A value of 1.000 indicates that two population are the same without variation. The further the value is from 1.000, the further apart the two populations are from each other. The similarity index from this study -0.091 to 1.000 indicated that although they are not very wide apart, they are not identical either.

**Conclusion**

Microsatellite markers is a reliable and authentic method for determining genetic variation as compared to other markers such as RAPD which has limited detection of polymorphism and other disadvantages. From the above study, it can be concluded that slight variation exists between the two populations of *H. bidorsalis*. This could be due to differences in ecological zones where the samples were collected though they belong to the same ancestor. It is therefore recommended that the two strains can be crossed to produce hybrids since there are slight variations in their DNA.

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