E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2021; 9(3): 91-97

www.fisheriesjournal.com
Received: 03-02-2021
Accepted: 07-04-2021

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Genetic diagnosis on strains of the African Catfish, *Clarias gariepinus* (Burchell 1822) in River Benue and a Tributary in North East Nigeria

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Abstract
Molecular diagnosis on strains of African catfish, *Clarias gariepinus* is fundamental step to study genetic diversity, this study was aim to reveal and compare the genetic structure of *C. gariepinus* from Benue and Donga River population. Thirty (30) matured *C. gariepinus*; 15 specimens each of Benue and Donga River were utilised for the molecular analytic study, DNA specimens were prepared for sequencing through PCR technique, partial genome sequences of 30 specimens covered 0.998% region of the *Clarias gariepinus* mitochondrion, total score compared to the whole genome ranged 0.99 - 1.00%. The Tajima's neutrality test, revealed that the 561 positions in the 29 specimen sequences has 2 segregating sites (S), the nucleotide diversity (π) was 0.000869, and the Tajima test statistic *D* was -0.087728 while the tree diagram showed 3 clusters/haplotypes within 2 divergent clades. The Tajima's relative rate test showed that 557 identical sites out of the 558 positions in all the three haplotypes. Only one unique nucleotide difference occurred in haplotype 1. Alignment of the nucleotides showed a T - C point mutation/single nucleotide polymorphism at Locus 430 indicating that the genetic distance between the species is distinct. Analysis of the genetic diversity of the population revealed clearly different strains and their respective population structures.

Keywords: *Clarias gariepinus*, clade, cluster, genetic, haplotypes and nucleotide

1. Introduction
Genetic enhancement of *Clarias gariepinus* is needed not only to meet the demands of fish production, but also to ensure profitability and the conservation of natural resources [1]. The need to monitor the levels of genetic diversity is profound because genetic diversity is closely linked to the evolutionary potential and the survival of the species. To effectively manage brood stocks for optimum productivity, it is important to compare the genetic composition of the wild population because significant loss of genetic variation, attributable to low effective number of parents, domestication selection or the mating design, among other factors, have been reported in hatcheries. Moreover, the feral introgression of the cultured into the wild as a result of escapee fishes has a great tendency of eroding the genetic diversity of the wild, especially if the genetic composition of the cultured population had not been properly managed [2].

Genetic diversity at a level below the species leads to formation of groups referred to as stocks, which are fundamental units of evolution. They are used by fishery biologists as a basis to manage commercially important organisms. Patterns of genetic diversities between stocks provide clues to the histories of the populations and also reveal the degree of evolutionary isolation [3, 4] reported that genetic monitoring is very imperative for an effective management strategy as fish population can suffer severe genetic erosion such as genetic drift, bottleneck, inbreeding, founder effect, etc., without being detected by the traditional demographic monitoring approach. Genetic variation is the raw material in a species and populations, which enables them to adapt to changes in their environment. New genetic variation arises in a population from either spontaneous mutation of a gene or by immigration from a population of genetically different individuals. The number and relative abundance of alleles in a population is a measure of genetic variation. The amount of genetic variation within and between populations can be determined by the frequency of genes and the forces that affect their frequencies, such as migration, mutation, selection and genetic drift [5].

Genetics and fishery management can interact in several ways. When the genetic population structure of a species is known, the distribution of subpopulations in mixed fisheries can be estimated [6]. Regulation of harvest to protect weaker populations can be made based on these distributions. It is important to identify and regulate for genetic changes within a population because of differential harvests of the drastic and long-term effects they may have on a population [5].

Therefore, understanding genetic variation within catfish population is a main requirement for maximizing the selective breeding of this species. The study becomes pertinent owing to the direct need of *C. gariepinus* farmers to stock superior genotypes on one hand and the need to conserve and domesticate *C. gariepinus* species, especially in Nigeria where it is the fastest growing aspect of aquaculture. Unfortunately, there is paucity of information on the genetic diversity of *C. gariepinus*.

This study was aim to assess the genetic structure of *C. gariepinus* from Benue and Donga River population, which is an important tool in fish breeding and genetics.

2. Materials and Methods

2.1 Experimental Sites

The sample were collected from River Benue and Donga, River Benue which lies between latitude 8º10'58.3″N and longitude 9º44'42.32″E in DMS (Degrees Minutes Seconds) or 8.18122 and 9.74431 (in decimal degrees) while Donga River which lies between latitude 7°43′00″N and longitude 10°03′00″E. It has an area of 3,121 km². The River exists year-round, the water volume fluctuates with seasons. The river overflows its bank during the rainy season (May-October) but decreases drastically in volume leaving tiny island in the middle of the River during the dry season (November-April) figure 1. The river contains several species of fish which are of economic importance to the people of Taraba State and Nigeria at large.

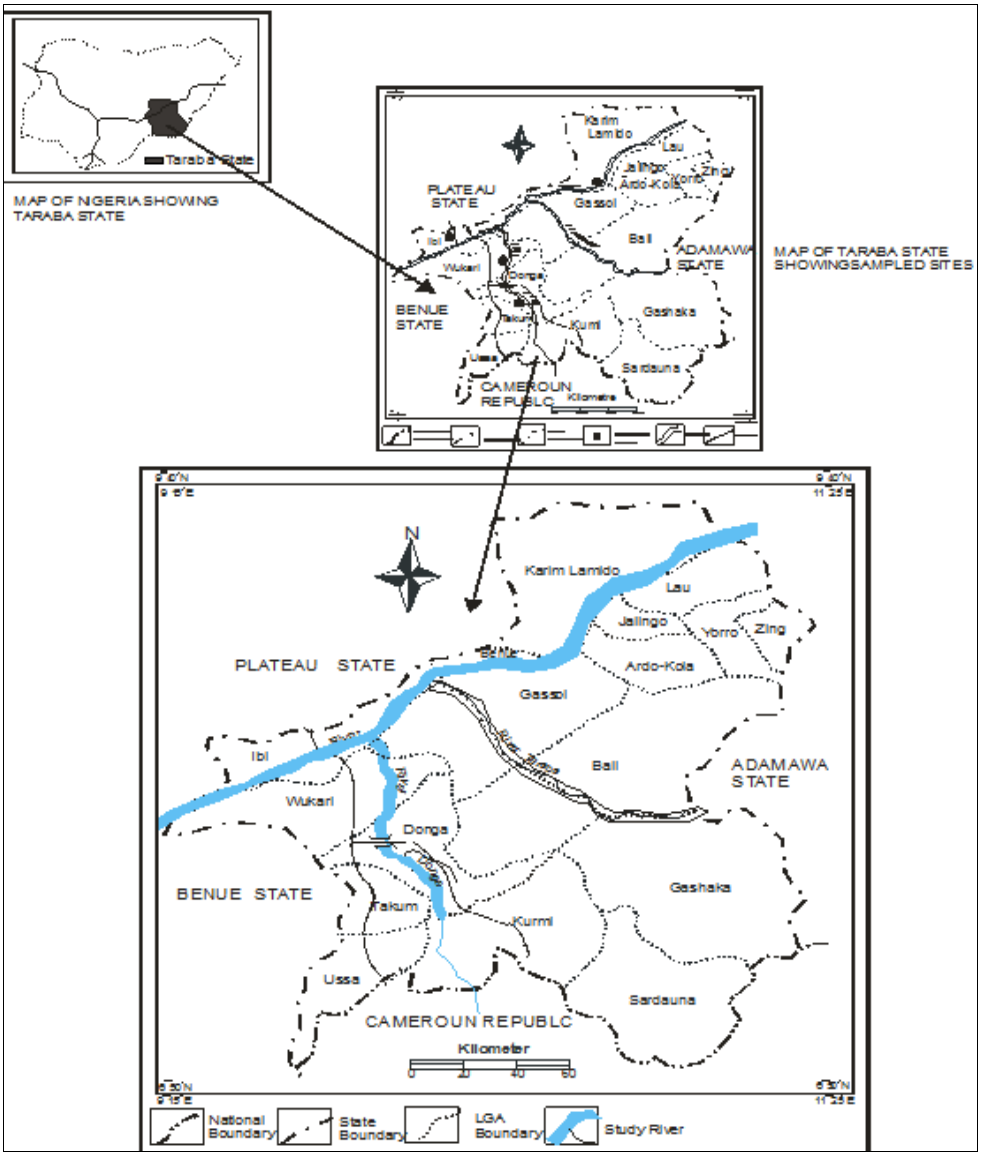

Fig 1: Map showing locations of sampling site and Rivers

2.2 Experimental Procedures

Molecular diagnosis was inferred through the 16S rRNA, a conserved mitochondrial gene region. The mitochondrial 16S rRNA gene has been widely used to explore the Phylogenetic relationships of fishes at various taxonomic levels. Therefore, the 16S rRNA gene has great potential for the inference of divergence among the fish lineages and resolution of relationships within and also ribosomal RNA gene has the ability to conserved secondary structures that are moderately well conserved among distantly related taxa [7].

2.3 Experimental Fish
The experimental fish utilised for the molecular analytic study were 30 matured *C. gariepinus* specimens; 15 specimens from each of River Benue and Donga. The specimens were collected from fisherfolks at the main landing sites of the River Benue and Donga over a period of six (6) months February - July 2020. These were identified to species level, following the descriptions of [8], then conditioned inside individual hapa inside a mobile fish pond of 4m x 3m for eight (8) weeks.

2.4 DNA Extraction
DNA extraction followed [9] Procedure. Fin clip of each of the selected specimens were aseptically obtained using scissors and kept in separate sample bottle. These were individually macerated with sterile pestle inside sterile mortal, 1ml Dellaporta DNA Extraction Buffer (DEB) added. Specimens of extract of each fish tissue were transferred into separate 1.5ml Eppendorf tube, 50µl of 20% Sodium Dodecyl Sulphate (SDS) added, and incubated in a water bath at 65ºC for 30minutes, then cooled to room temperature. 160µl of 5M Potassium Acetate was added, mix briefly and centrifuged at 13000rpm for 10minutes. The supernatants were transferred into autoclave tubes and 2/3 volumes of cold Isopropanol / Isopropyl alcohol added. Tubes were inverted 3-5 times gently and incubated at -20ºC for 1 hour, centrifuged at 13000rpm for 10minutes and the supernatant discarded. To purify, 500µl of 70% ethanol were added to the residue DNA pellet, then centrifuged for 5minutes at 13000rpm. The DNA pellets were dried at 37ºC for 10-15 minutes, resuspend in 50µl of Tris-EDTA (TE) buffer and stored at -20ºC for further lab analysis.

2.5 Polymerase Chain Reaction
In preparation for sequencing, DNA specimens were prepared for sequencing through PCR technique. The PCR preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM $MgCl_2$, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol 16Sar-F CGCCTGTTTATCAAAAACAT 16Sbr-R CCGGTCTGAACTCAGATCACGT primers polymerase (Promega, USA) made up to 42 µl with sterile distilled water then 8µl (100ng/µl) DNA template. The PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA). The PCR profile followed an initial denaturation at 94°C for 5 min; 35 cycles, of 94°C for 45 seconds annealing, 58°C for 45seconds and 72°C for 1-minute extension and a final termination at 72°C for 10 minutes. The PCR templates were then stored at 4ºC [10, 11].

2.6 PCR Integrity Test
The integrity of the amplified about 1.5Mb gene fragment of each specimen were confirmed on a 1. % Agarose gel ran to amplification. The buffer (1XTAE buffer) was prepared and subsequently utilised to prepare 1.5% agarose gel. The suspension of these was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2l) of 10X blue gel loading dye was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well. The gel loading dye gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel. The gel was electrophoresed at 120V for 45 minutes, visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside all the experimental samples in the gel. The samples were ran in batches of 20 and 10 specimens, each having 100bp ladder. Presence of electrophoresis bands indicate amplification of the DNA template.

2.7 Purification of the Amplified Products in Preparation for Sequencing
After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each of about 40µl PCR amplified product in a new sterile 1.5 µl tube Eppendorf, mix thoroughly by vertexing and kept at -20°C for at least 30 min. These were centrifuged for 10 min at 13000 g and 4°C. Supernatants were removal (invert tube on trash once), then pellet was washed by adding 150 µl of 70% ethanol, mixed, then centrifuged for 15 min at 7500 g and 4°C.

Again, all supernatants were removed by inverting the tube on tray inverting the tube on paper tissue, and let it dry in the fume hood at room temperature for 10-15 min. These were resuspended with 20 µl of sterile distilled water and kept in -20ºC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 from thermo scientific (USA).

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual. The sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit.

2.8 Statistical Analysis
Bio-Edit software and MEGA X were utilized for all genetic analysis while Sequences were edited on Bio-edit prior to analysis.

3. Results
Plates 1 showed the gel electropherogram of the PCR Specimens of the 30 *Clarias gariepinus* Specimens. The plate showed that all the specimens amplified. The NCBI identity of the sequences of the nucleotides of the specimens is presented in Table 1. The sequence of the sample 28 failed; hence it was not included in the nucleotide analysis. The partial genome sequences of the remaining 29 specimens covered 0.998% region of the *C. gariepinus* mitochondrion, complete genome NCBI accession KT001082.1. The specimens total score compared to the whole genome ranged 0.99 - 1.00%. The partial genome sequences of 29 specimens shows that 19 specimen has 0.99% genome range while 10 has 1% genome range. The percentage of genome range 0.99% reveal that Benue has 11 specimens (36.67%) while Donga has 8 specimens (26.67%), in 1% genome range Benue 4 specimen (13.33%) while Donga 6 specimen (20%).

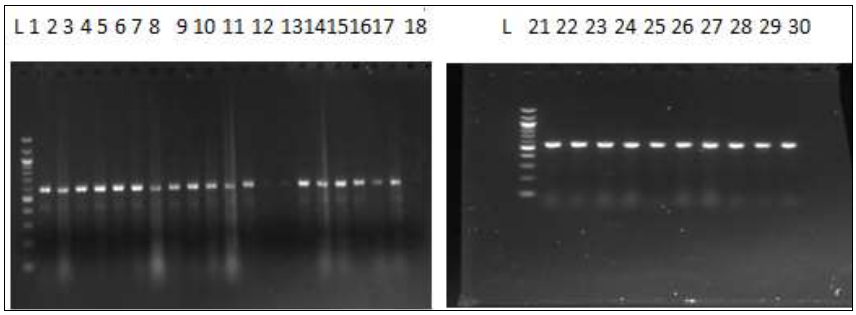

Plate 1: Gel Electropherogram Showing Amplification of the PCR Specimens of the 30 *Clarias gariepinus* Specimens *Lane L indicate ladder

Table 1: The sample ID, Location, NCBI identity and length (basepair) of nucleotide sequences of the 30 *Clarias gariepinus* specimens

S/No.	Sample ID Description	Location	Total Score	Length (Base pair)	Accession No.
1	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
2	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
3	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
4	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
5	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1026	1.00	KT001082.1
6	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
7	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
8	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1026	1.00	KT001082.1
9	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
10	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
11	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1026	1.00	KT001082.1
12	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
13	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
14	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1027	0.99	KT001082.1
15	*Clarias gariepinus* mitochondrion, partial genome	Benue River	1026	1.00	KT001082.1
16	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1027	0.99	KT001082.1
17	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1026	1.00	KT001082.1
18	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1027	0.99	KT001082.1
19	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1026	1.00	KT001082.1
20	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1026	1.00	KT001082.1
21	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1027	0.99	KT001082.1
22	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1027	0.99	KT001082.1
23	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1027	0.99	KT001082.1
24	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1027	0.99	KT001082.1
25	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1026	1.00	KT001082.1
26	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1027	0.99	KT001082.1
27	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1027	0.99	KT001082.1
29	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1026	1.00	KT001082.1
30	*Clarias gariepinus* mitochondrion, partial genome	Donga River	1026	1.00	KT001082.1

The Tajima's neutrality test [12] in Table 2, revealed that the 561 positions in the 29 specimen sequences has 2 segregating sites (S), the nucleotide diversity (π) was 0.000869, and the Tajima test statistic *D* was -0.087728.

Table 2: Results from Tajima's Neutrality Test [1]

M	S	p_s	Θ	Π	D
29	2	0.003565	0.000908	0.000869	-0.087728

Note: This analysis involved 29 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 561 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [2].

Abbreviations: *m* = number of sequences, *n* = total number of sites, *S* = Number of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, π = nucleotide diversity, and *D* is the Tajima test statistic

3.1 Evolutionary relationships of taxa

The tree diagram (Figure 3) showed 3 clusters/haplotypes within 2 divergent clades. Clade 1 contained 4 out of the 15 (26.67%) Benue River specimens (Benue 5,8, 11,15) and 6 out of 14 (42.86%) Donga River specimens (Donga 17, 19, 20, 25, 29, 30). Clade 2 cluster 1 contain 2 out of 15 (13.33%) Benue specimen (Benue 1,2) while Clade 2 cluster 2 contained 9 out of 15 (60.0%) Benue (Benue 3, 4, 6, 7, 9, 10, 12, 13, 14) and 8 out of the 14 (57.14%) Donga specimens (16, 18, 21, 22, 23, 24, 26, 27). The tree in Figure 2 showed the haplotype in clade 1 rooted to *C. gariepinus* NCBI voucher MF683196.1, while clade 2 rooted to the *C. gariepinus* NCBI voucher KT001082.1.

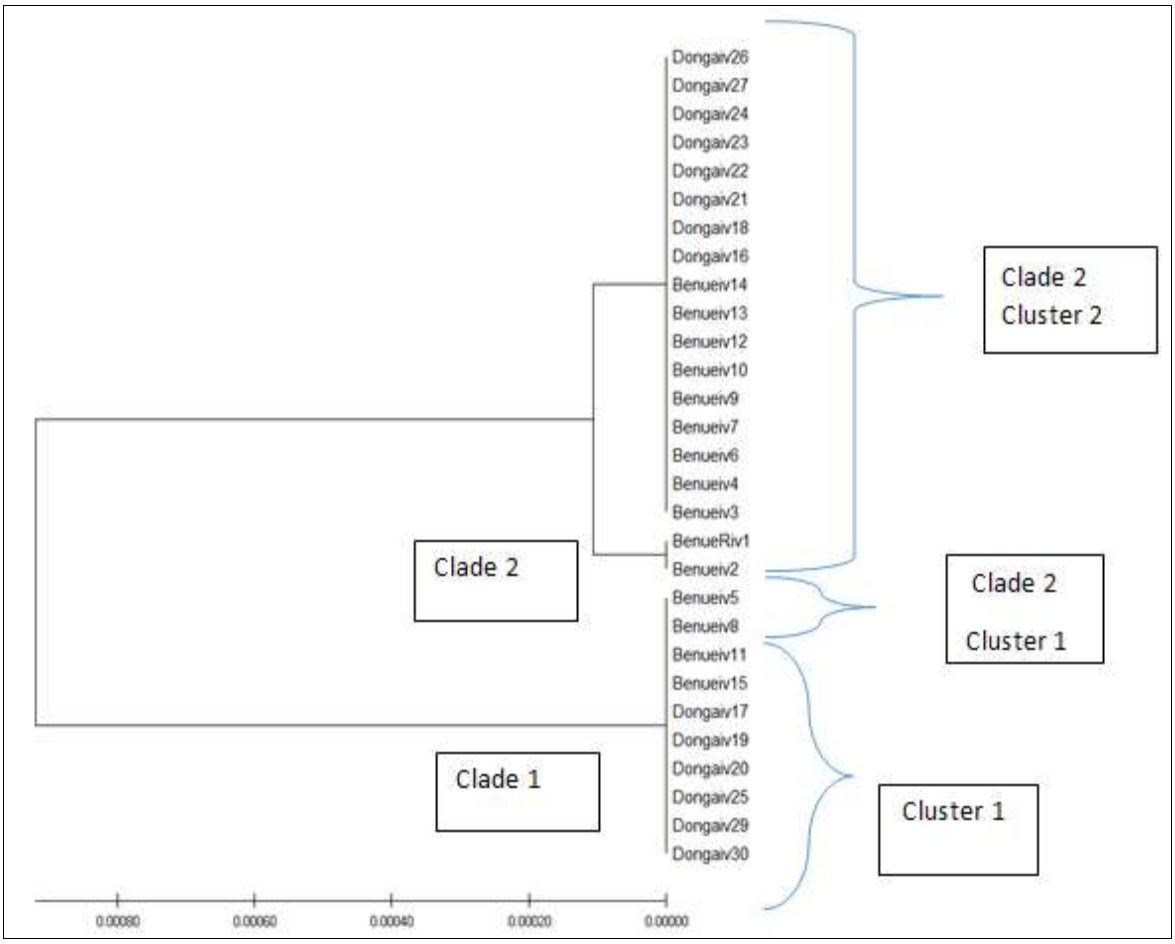

Fig 2: Tree of Life Showing the Evolutionary Relationships of the taxa of the Specimens

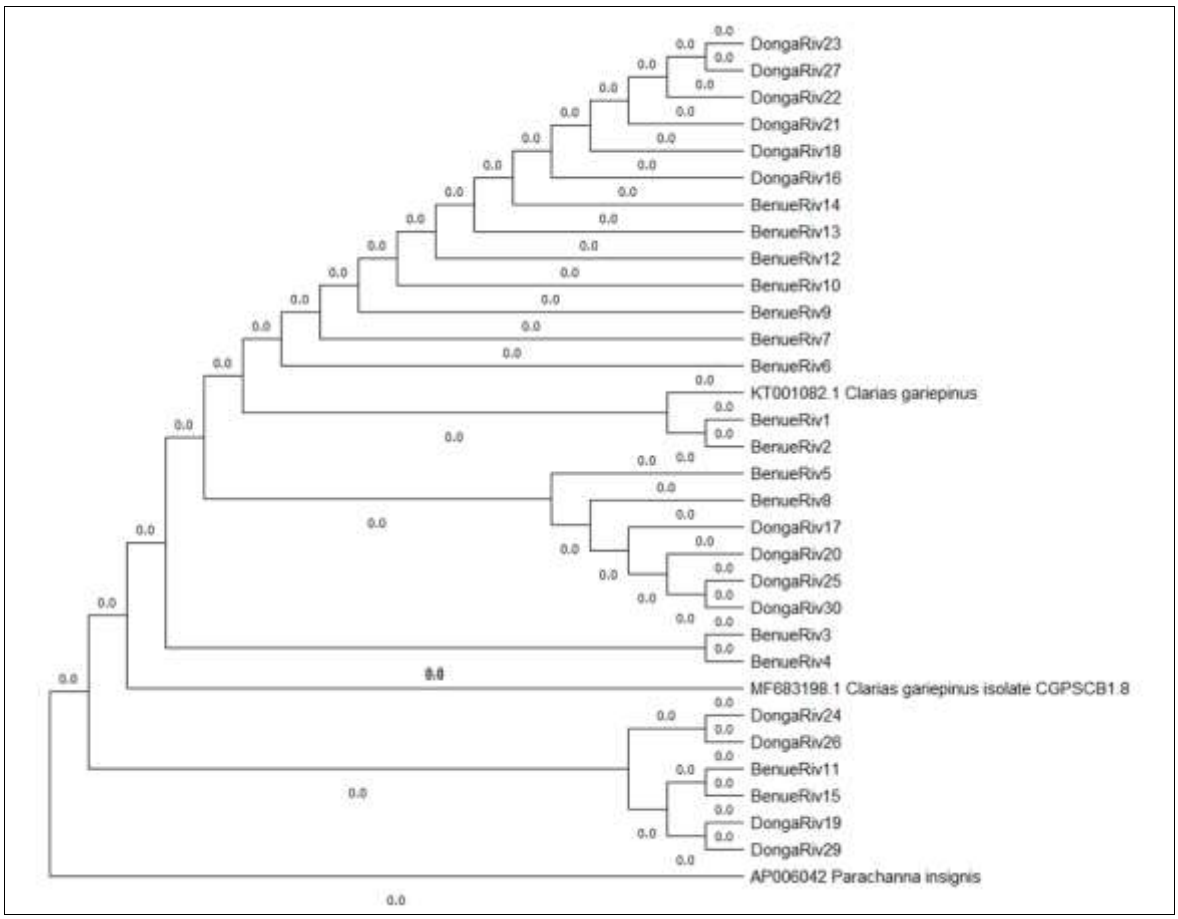

Fig 3: Tree of Life Showing the Closest Lineage of the Taxa with reference NCBI Specimens

Alignment of the nucleotides showed a T - C point mutation/single nucleotide polymorphism at Locus 430. This site separated all members of the haplotype 1 having T nucleotide complement while all members of the rest of haplotypes 2 had C nucleotide compliment (plate 2).

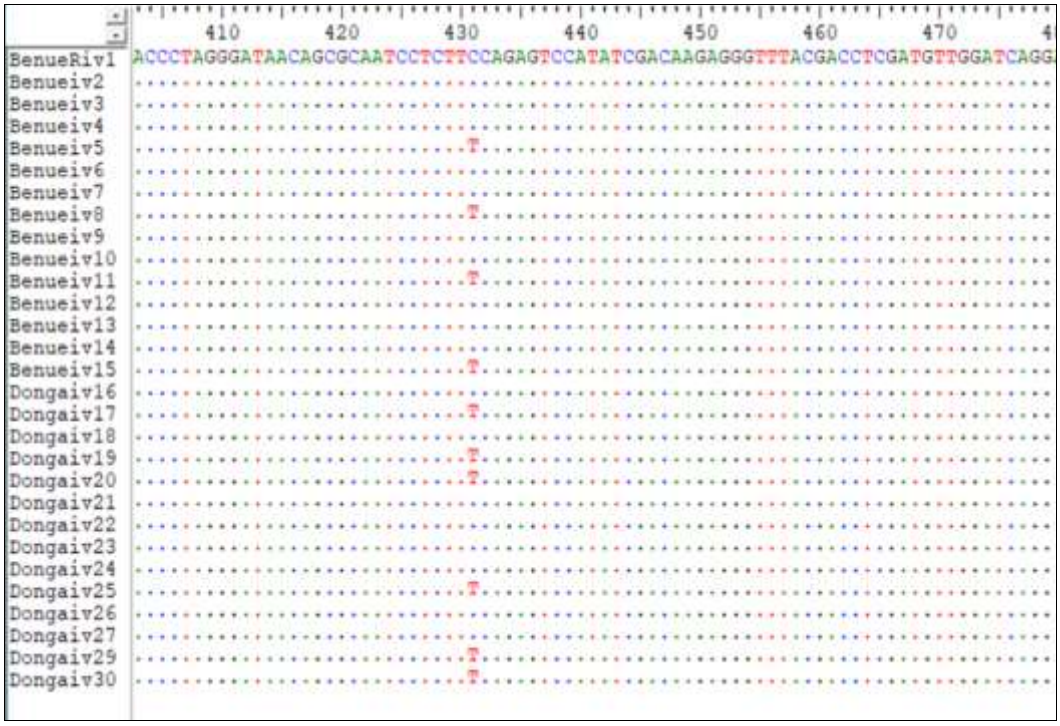

Plate 2: Nucleotides alignment showing C/T SNP position at Locus 430

4. Discussion

The genetic diversity of *C. gariepinus* haplotype was high, but comparable to values reported by other studies [13, 14]. This shows that genetic variation in the populations could be accounted for by within population variation, which was comparatively higher than values reported for populations of *C. macrocephalus* from Malaysia [13], whose nucleotide and haplotype diversities were 0.003 in the populations. The presence of shared haplotypes is attributed to gene flow among the populations, when fish migrate between water bodies, this could lead to gene flow and homogeneity of geographically isolated populations of the species.

However, Benue population formed a distinct cluster of its own, unlike samples from the Donga River. This finding, reporting the highest number of haplotype and nucleotide diversities among the two sampling sites seem to suggest that catfish at Benue and Donga can be sourced from multiple sites. A combination of genetically distinct stocks is reported to increase genetic variation in farmed *C. gariepinus* [15, 16]. However, samples of Benue River appeared in the Donga River cluster. This could be attributed to assisted translocation of the population by water drift, especially in the raining season. In addition, Donga river populations had lower haplotype diversities than Benue populations, this could be attributed to genetic drift and cannibalism among the fish, which caused a reduction in gene frequencies in the samples and also higher fishing pressure, especially in the Donga population could have reduced its genetic diversity, through fishing mortality [17]. Small and isolated populations also suffer lower genetic diversities due to genetic drift that results from founder effects and lower effective population sizes [18]. This reflects a high divergence of the population, which could be attributed to the possibility of multiple sources of the *C. gariepinus*.

5. Conclusion

Genetic diagnosis is an effective tool in fish identification, and for genetic conservation and management purposes. Analysis of the genetic diversity of Benue and Donga population revealed clearly different strains and their respective population structures, which the study suggests that fish farmers can source for their brooders from the two water bodies to genetically enrich the gene pool.

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