



Research Article

Molecular Phylogenetic Characterization of Three Populations of *Heterobranchus bidorsalis* (Geoffroy Saint-Hilaire, 1808) from the Northeastern, Nigeria

*Nyaku¹ R. E., Jampeo J.², Mustapha F. A.¹ and Onyia L. U.²

¹National Institute for Freshwater Fisheries Research, (NIFFR) Maiduguri Zonal Office, Borno State, Nigeria

²Department of Fisheries, Faculty of Agriculture, Modibbo Adama University Yola, Nigeria

*Corresponding Author's email: raymondnyako4@gmail.com/raymond.nyaku@niffr.gov.ng

ABSTRACT

Heterobranchus bidorsalis (Geoffroy Saint-Hilaire, 1809) is an important species in tropical aquaculture. This study investigated the molecular phylogenetic relationships and genetic diversity of *H. bidorsalis* populations from three water bodies in Nigeria. Thirty fish samples (10 per location), averaging 1 kg each, were collected from Njiboliyo Reservoir, Adamawa State; Dadin Kowa Dam, Gombe State; and Lau Reservoir, Taraba State. Blood samples were collected in anticoagulant-containing EDTA bottles, and genomic DNA was extracted using the Quick-g-DNA™ MiniPrep Kit (Zymo Research, USA). The mitochondrial DNA cytochrome c oxidase (COI) gene was sequenced for all samples. Sequence analysis identified 451 conserved nucleotide positions (66.1%) and 231 variable positions (33.9%). Phylogenetic analysis showed that most individuals clustered according to their population of origin, suggesting shared maternal ancestry within populations. Genetic distances ranged from 0.000 between D6 and D5 to 0.0712 between N10 and D7. Analysis of molecular variance (AMOVA) revealed greater genetic variation among populations (62.30%) than within populations (8.5%). All pairwise FST{ST}FST values exceeded 0.070, indicating substantial population genetic structuring and potentially limited gene flow among the populations. Dadin Kowa Dam exhibited relatively high genetic diversity. These findings provide insights into the genetic differentiation and population structure of *H. bidorsalis* and highlight the importance of considering population-specific genetic variation in its conservation and sustainable aquaculture management.

Keywords: AMOVA; Cytochrome C oxidase; *Heterobranchus bidorsalis*; Molecular; Phylogenetic

Citation: Nyaku, R.E., Jampeo, J., Mustapha, F.A., & Onyia, L.U. (2026). Molecular Phylogenetic Characterization of Three Populations of *Heterobranchus bidorsalis* (Geoffroy Saint-Hilaire, 1808) from the Northeastern, Nigeria. *Sahel Journal of Life Sciences FUDMA*, 4(3): 378-386. DOI: <https://doi.org/10.33003/sajols-2026-0403-41>

INTRODUCTION

Aquaculture of catfish of the *clariidae* family remains the largest contributor and well developed facet of aquaculture in Nigeria (Tukura *et al.*, 2005; Abubakar *et al.*, 2020). The favored species of the *clariidae* family are those of the genus *Heterobranchus* and *Clarias* in the aquaculture industry in Nigeria. *Heterobranchus bidorsalis* due to their fast growth rate, body forms, hardiness, resistance to diseases, capacity for high stocking density, and utilization of

an array of food are some of the features which makes these fish species culturable in the tropics (FAO 2000; Agbebi *et al.*, 2009; Agbebi *et al.*, 2013). Observation from the field showed that problems of improper identification and indiscriminate hybridization of the *clariid* species affect the genetic make-up of the species (Yisa and Olugeagba, 2005; Abubakar *et al.*, 2018). The use of mitochondrial (mtDNA) marker offers several advantage such as mode of inheritance and relatively limited recombination that make it effectively in conducting

studies regarding phylogenetic analysis (Hurst et al., 1999; Abubakar et al., 2018) and the use of molecular markers in genetic studies have yielded tremendous success (Gao et al 2001; Fujii, 2002; Yashim et al., 2017).

Heterobranchus bidorsalis, is a large air-breathing catfish that plays an important ecological and economic role in Nigerian freshwater systems. It is widespread in Nigerian rivers and lakes and is highly valued for aquaculture and local fisheries Adaka and Olele, (2015; Froese and Pauly, 2017). It is closely related to the vundu catfish, which is well-known among fishermen in the sub-saharan Africa, as well as the Nile (Laleye et al., 2019). *Heterobranchus bidorsalis* potentials as a good aquaculture species has been described by Teugels since the early 90's (Idodo-Umeh, 2003, Teugels et al., 1990, Solomon et al., 2013). The head of *Heterobranchus bidorsalis* is shaped like an oval and has a rectangular dorsum. The snout is round and the eyes are lateral. The frontal fontanelle is long and narrow while the occipital fontanelle is relatively long and is shaped like an oval. The postorbital bones are completely united. The suprabranchial organ is well developed. The pectoral spine is smooth. The body and fins may have spots. It can reach a length of 150 cm (59.0 inches) TL. The maximum recorded weight for this species is 30.0 kg. (Idodo-Umeh, 2003; Solomon et al., 2013). The species has 40-46 dorsal (in the back) soft rays, 49-58 anal soft rays, and 62-63 vertebrae (Froese and Pauly, 2017). *Heterobranchus bidorsalis* is widely distributed in various freshwater bodies across Nigeria, including rivers, lakes, and reservoirs (Aluko, 1998). It is known for its adaptability to different environmental conditions, which has made it a preferred species for aquaculture. However, the genetic diversity within the species and its evolutionary relationships with other closely related species, such as *Heterobranchus longifilis* and *Clarias gariepinus*, keeps on evolving. This knowledge gap presents challenges in the effective management of the species, particularly in terms of breeding programs, conservation strategies (Aluko, 1998; Yisa & Olufeagba, 2005; Abubakar et al., 2018). Molecular characterization involves the use of molecular markers such as mitochondrial DNA (mtDNA), microsatellites, and single nucleotide polymorphisms (SNPs) to study the genetic variation for fish species among other living organisms. Techniques such as Polymerase Chain Reaction (PCR), DNA sequencing, and restriction fragment length polymorphism (RFLP) are employed to analyze genetic material and identify specific markers associated with desirable traits like

growth rate, disease resistance, and environmental tolerance (Ekerette et al., 2018; Oseni et al., 2019). Genetic distance is a measure of the genetic divergence between species or between populations within a species, whether the distance measures the time from a common ancestor or the degree of differentiation (Wei et al., 2023) Hence, genetic distance also showed significant genetic differentiation within and among groups of catfish populations and determined the population structure Genetic diversity plays a pivotal role in the conservation of species within their natural habitats (Barasa et al., 2016). Several factors, such as pollution, unfavorable habitat, and the environment, may cause a substantial change in population allele frequency (Mwakubo et al., 2007). The importance of understanding the phylogenetic relationship existing within and between species in a population cannot be overemphasized as it has become a handy method used in tracing the origin and evolution of species, physiological, biochemical and structural features of sequences obtained from organism (Ekerette et al., 2018). The cytochrome C oxidase I (COI) gene has proven reliable for genetic diversity and geographical distribution studies in various fish species (Antonioni and Magoulas 2014; Aminisarteshnizi and Moyo, 2024). Studies reported by (Abubakar et al., 2018; Aminisarteshnizi and Moyo, (2024) showed that the COI gene's slow evolution makes it a suitable DNA marker for genetic studies. The advent of genetic markers such as RAPD, RFLP, and microsatellite in conjunction with statistical tools has revolutionized population studies geared towards exploring the phylogenetic relationships within and between populations. Importantly, DNA sequence data have proven to be the most direct and reliable method of assessing the pattern of variation and relationships among species (Ekerette et al., 2018; Abubakar et al., 2020). In the present study, it was our aim to assess the molecular phylogenetic characterization of *Heterobranchus bidorsalis* from three populations in the northeastern Nigeria, using mitochondrial DNA cyt C oxidase.

MATERIAL AND METHOD

Study Area

The study was conducted on three populations of *Heterobranchus bidorsalis* (Njoboliyo samples n=10; Lau samples n=10) and Dadinkowa samples n=10. Njoboliyo Reservoir is located in eastern part of Yola-south local government area in Adamawa state 9°12'N; 12°43'E Lau Reservoir is located along the River Benue valley in the northern part of Taraba

state 9°02'N;11°0'E and Dadin kowa dam is located in Yamalyu Deba local Government area of Gombe state 10°11'N;11°01'E.; Nyaku *et al.*, 2025).

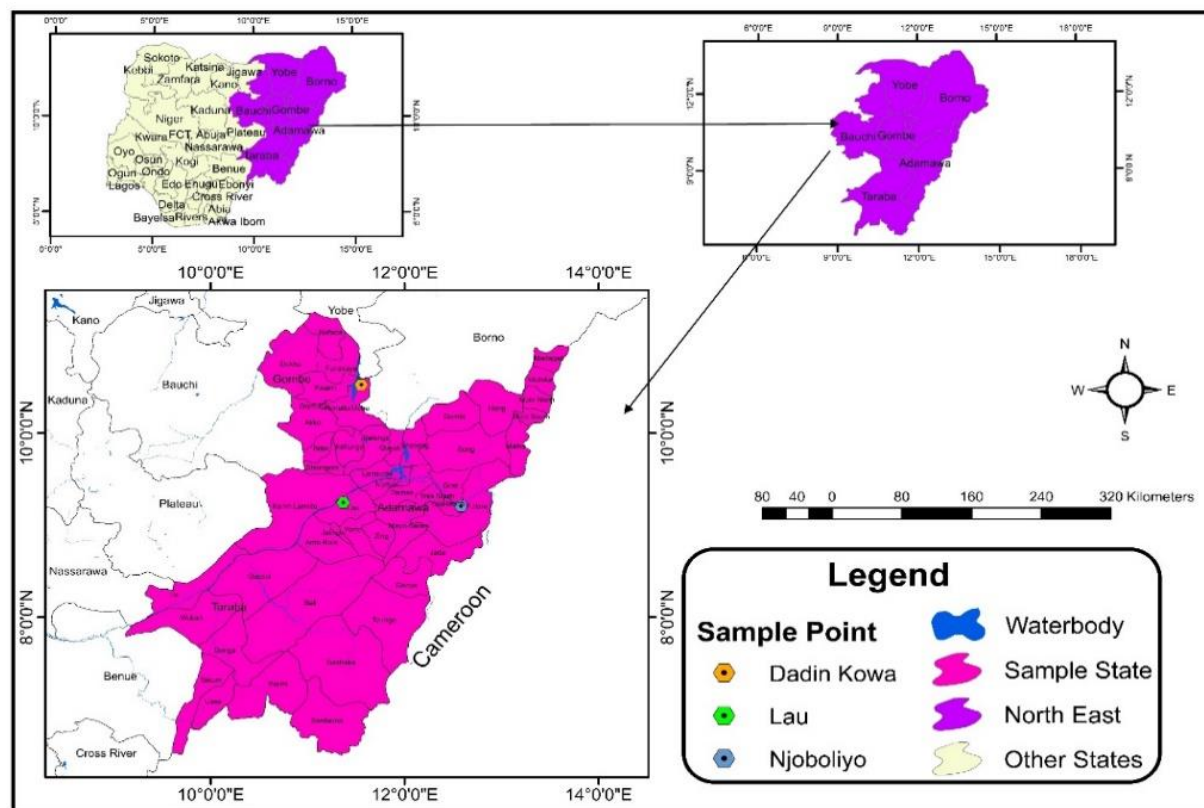


Figure 1: Map of the Study Area

Sample Collection

Thirty (30) live fish were collected randomly from fisher forks during rainy season. Ten (10) samples from each sampling site based on the availability and sizes were selected for blood collection. Blood sample (0.5ml) was collected from caudal vein/ artery via the tails fish *Aqualex* model (2004) using 2ml syringe fitted with 25G needles. The blood sample was dropped immediately in EDTA bottle that contains anticoagulant. The blood samples were transported to the Biotechnology and Genetic Center of the Modibbo Adama University Yola for mtDNA extraction.

DNA extraction, Amplification and Sequencing

DNA was extracted from the blood samples of all the fish using Quick-g DNA™ MiniPrep kit (Zymo Research, USA). The extracted DNA was obtained by ZR-96 Quick-g DNA MiniPrep kit (Zymo Research USA) 2023 D3010-D3011, extracted method and samples were conserved in 0.1% Tris-EDTA buffer at -20° C. Polymerase Chain Reaction was performed using Fish_comum_Cytochrome C_F (5' –

GGATTYTAACCCYTRCCCC - 3') L15891 as the forward primer and (5' – AGTAAAGTCAGGACCAAGCC - 3') H15891 as reverse primer (Briolay *et al.*1998; Nwafili and Gao, 2007). Four hundred and fifty-one base pairs (451bp) fragment of the cytochrome C oxidase was amplified in each sample with a DNA amplification from the Cytochrome C oxidase of the *Heterobranchus bidorsalis*. Polymerase Chain Reaction (PCR) conditions were on initial denaturation at 96°C for 15 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing was at 60°C for 30 seconds and extension at 70°C for 1-minute 30 seconds, and final extension was at 70°C for 5minutes (Ajibike, 2016)

Cytochrome C oxidase of the mtDNA were sequenced for all blood samples. Sequencing reaction was performed with AB13730 × L sequencer using 20 µl reaction comprising approximately 20 ng of purified PCR product as template DNA, 8 µl of Big DyeTerminato Reaction Mix (dNTPs,ddNTPs,buffer, enzyme and MgCl₂), 8 µl of deionized water, 2 µl of primer programmed as 25 cycles at 96°C for 10

seconds, 50°C for five seconds and 60°C for four minutes (Ekerette *et al.*, 2018). The length of the sequenced fragments ranged from 1241 to 1401 bp for cytochrome C Oxidase. A total of 15 *Heterobranchus bidorsalis* samples from three populations were analysed using the cytochrome c oxidase I (COI) marker. The populations included Dadin kowa dam (8 samples), Njoboliyo Reservoir (5 samples), and Lau Reservoir (2 samples). Sequence analysis was conducted to evaluate genetic variation, phylogenetic relationships, genetic distances, maternal linkage, and population structure.

Data Analysis

DNA sequences obtained from COI amplification were aligned and analyzed using MEGA 12 and Bioedit 7.7.1 softwares. Pairwise genetic distances were estimated using p-distance calculations. Phylogenetic relationships were inferred using hierarchical clustering based on genetic distance matrices. Population structure was evaluated from within- and between-population divergence patterns.

RESULTS

Genetic Variation among Populations

The sequence alignment revealed substantial conservation across the COI region among the analyzed populations. A total of 451bp (66.1%)

conserved nucleotide positions and 231 (33.9%) variable nucleotide positions were identified. Population Dadin kowa has the highest nucleotide diversity, 0.032 ± 0.005 indicating greater genetic variation, while population Lau has the lowest nucleotide diversity 0.018 ± 0.009 suggesting low genetic variation. The presence of variable nucleotide sites demonstrates detectable intra-specific genetic variation among the populations. Population Dadin kowa exhibited the highest level of sequence diversity because it contained the largest number of samples and several polymorphic regions.

Sequence Variance and Phylogenetic Relationship

The neighboring-joining phylogenetic clustering of *Heterobranchus bidorsalis* from the three populations based on Cytochrome C Oxidase is represented in Figure 1 and 2. Phylogenetic analysis showed that most samples clustered according to their population origin, indicating shared maternal ancestry within populations. Several Dadin kowa Njoboliyo population showed moderate divergence from Dadin kowa population samples, while Lau population samples occupied intermediate positions within the phylogenetic tree. These findings suggest historical genetic connectivity with slight population differentiation.

Table 1. Genetic variation of *Heterobranchus bidorsalis* from three Reservoirs in the Northeast Nigeria

Population	N	S (within pop)	H	(Hd)	(π)
D (n=8)	8	187	7	0.964 ± 0.077	0.032 ± 0.005
L (n=2)	2	45	2	1.000 ± 0.500	0.018 ± 0.009
N (n=5)	5	112	4	0.900 ± 0.161	0.028 ± 0.006

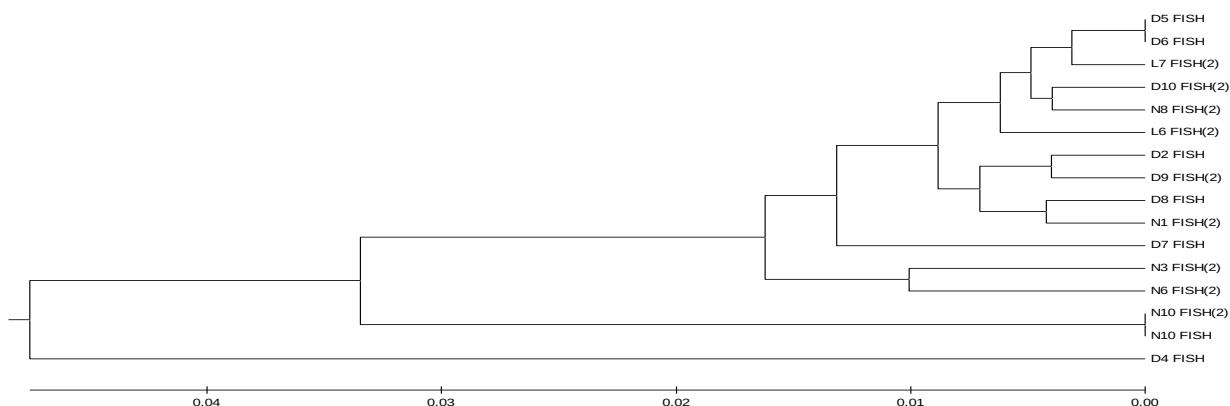


Figure 1. Hierarchical clustering of *Heterobranchus bidorsalis* samples from three Reservoirs in the Northeast Nigeria

Key: D (Dadin kowa dam), L (Lau Reservoir), N (Njoboliyo)

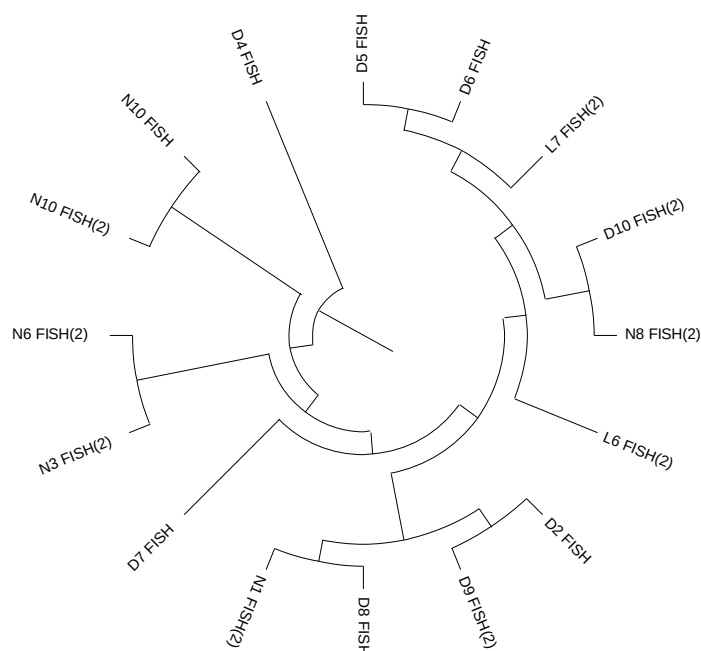


Figure 2. Neighbor-joining Phylogenetic tree showing relationship between *Heterobranchus bidorsalis* from three populations based on mtDNA cyt C oxidase

Genetic Distance of *Heterobranchus bidorsalis* based on mtDNA cyt C oxidase from the three populations.

Pairwise genetic distance Correlation matrix from the three populations as presented in Table 2. the highest genetic distance within Cytochrome C oxidase of *H. bidorsalis* from the three populations was between N10 and D7 (0.0712), while the lowest distance was between D6 and D5 (0.0). Pairwise genetic distance analysis revealed generally low genetic divergence among the populations, indicating close evolutionary relationships and probable recent common ancestry. Low genetic distances among several Dadin kowa population samples indicate strong maternal linkage and reduced sequence divergence. Higher divergence observed between some Njoboliyo and Dadin kowa samples suggests partial reproductive isolation or environmental adaptation.

Population Structure of *Heterobranchus bidorsalis* based on mtDNA cyt C oxidase from the three populations.

Genetic variations within and between *Heterobranchus bidorsalis* in the three populations based on mtDNA cyt C oxidase shown in Table 3, Analysis of molecular Variance (AMOVA) revealed that molecular genetic variation among populations was higher (62.30%) than within population variation (8.5%). Additionally, fixation index was 0.782, which made the variation significant ($p < 0.05$). Population structure analysis demonstrated weak-to-moderate genetic differentiation among the three populations. The generally low genetic distances suggest substantial gene flow and historical connectivity among populations. However, the existence of variable nucleotide sites and phylogenetic sub-clustering indicates emerging genetic structuring. Population Dadin kowa displayed broader diversity compared with populations Lau and Njoboliyo, suggesting either larger effective population size or greater historical admixture. All F_{ST} 0.070 showed very strong populations' structure suggesting no gene flow.

Table2. Pairwise genetic distance of correlation matrix of *Heterobranchus bidorsalis* from three populations based on cyt C oxidase in the Northeast Nigeria.

Samples	D2 FISH	D4 FISH	D5 FISH	D6 FISH	D7 FISH	D8 FISH	D9 FISH	D10 FISH	L6 FISH	L7 FISH	N1 FISH	N3 FISH	N6 FISH	N8 FISH	N10 FISH
D2 FISH	0.0	0.0826	0.0119	0.0119	0.0281	0.0099	0.0079	0.0099	0.0178	0.0159	0.0196	0.0323	0.0282	0.0139	0.0655
D4 FISH	0.0826	0.0	0.0938	0.0938	0.0855	0.0912	0.0909	0.0938	0.0881	0.0909	0.0969	0.0888	0.0914	0.0909	0.104
D5 FISH	0.0119	0.0938	0.0	0.0	0.0224	0.0234	0.0171	0.0078	0.0118	0.0078	0.0195	0.0396	0.0337	0.0098	0.0682
D6 FISH	0.0119	0.0938	0.0	0.0	0.0224	0.0219	0.0156	0.0078	0.0099	0.0078	0.0196	0.0396	0.0337	0.0099	0.0682
D7 FISH	0.0281	0.0855	0.0224	0.0224	0.0	0.0253	0.0252	0.0279	0.0224	0.0252	0.0281	0.0338	0.0281	0.0308	0.0712
D8 FISH	0.0099	0.0912	0.0234	0.0219	0.0253	0.0	0.0125	0.0265	0.0139	0.0267	0.0084	0.0372	0.0282	0.0119	0.0684
D9 FISH	0.0079	0.0909	0.0171	0.0156	0.0252	0.0125	0.0	0.0171	0.0099	0.0219	0.0139	0.0371	0.0309	0.0098	0.0653
D10 FISH	0.0099	0.0938	0.0078	0.0078	0.0279	0.0265	0.0171	0.0	0.0118	0.0125	0.0167	0.0395	0.028	0.0079	0.0708
L6 FISH	0.0178	0.0881	0.0118	0.0099	0.0224	0.0139	0.0099	0.0118	0.0	0.0158	0.0251	0.0322	0.0253	0.0138	0.0625
L7 FISH	0.0159	0.0909	0.0078	0.0078	0.0252	0.0267	0.0219	0.0125	0.0158	0.0	0.0167	0.0422	0.0309	0.0138	0.0653
N1 FISH	0.0196	0.0969	0.0195	0.0196	0.0281	0.0084	0.0139	0.0167	0.0251	0.0167	0.0	0.0394	0.0338	0.0279	0.0712
N3 FISH	0.0323	0.0888	0.0396	0.0396	0.0338	0.0372	0.0371	0.0395	0.0322	0.0422	0.0394	0.0	0.0226	0.0322	0.0653
N6 FISH	0.0282	0.0914	0.0337	0.0337	0.0281	0.0282	0.0309	0.028	0.0253	0.0309	0.0338	0.0226	0.0	0.0197	0.0655
N8 FISH	0.0139	0.0909	0.0098	0.0099	0.0308	0.0119	0.0098	0.0079	0.0138	0.0138	0.0279	0.0322	0.0197	0.0	0.0597
N10 FISH	0.0655	0.104	0.0682	0.0682	0.0712	0.0684	0.0653	0.0708	0.0625	0.0653	0.0712	0.0653	0.0655	0.0597	0.0

Table 3 AMOVA showing genetic variations within and between *Heterobranchus bidorsalis* in three populations in the Northeast Nigeria.

Source of variation	df	SS	Variance component	% variation	Fixation index
Among populations	2	892.4	62.3	78.2%	F _{ST} = 0.782
Within populations	12	102.1	8.05	21.8%	
Total	14	994.5	70.35	100%	

SOV (source of variation); df (degree of freedom); SS (sum of squares); F_{ST} (fixation index).

DISCUSSION

Advancement in molecular techniques has increased the availability of different DNA-based marker Ekerette *et al.*, (2018) which have become efficient tool in conservation genetic studies and had been successfully applied in fisheries sector. To identify closely related fish species determination of genetic diversity of fish population and species identification (Ponzoni *et al.*, 2018; Abubakar *et al.*, 2018). This study revealed high nucleotide diversity, 0.032 ± 0.005 from Dadin kowa dam indicating greater genetic variation, while population L has the lowest nucleotide diversity 0.018 ± 0.009 suggesting low genetic variation. Similarly, Abubakar *et al.* (2018) reported nucleotide and high haplotypes diversity (0.001363 ± 0.000974) from River Niger in the population of *Heterobranchus bidorsalis*, but reported similar Haplotype diversity from River Niger and Benue 0.6809 ± 0.0935 and 0.6786 ± 0.1220 using Cyt C Oxidase. High Nucleotide diversity (0.01) was recorded in two populations of *Clarias gariepinus*, while five populations showed low nucleotide diversity (0.00) Cyt C Oxidase as recorded by (Aminisarteshnizi and Moyo 2024). Similar findings based on COI, Kundu *et al.* (2023) reported 20 haplotypes with 81 segregating sites, haplotype diversity ($Hd = 0.854$) and nucleotide diversity ($\pi = 0.258$) among 63 samples collected from three sampling sites along the Nyong River in Cameroon. Popoola (2022) carried out a study in three rivers in south west Nigeria using mtDNA cytb gene, revealed 53 haplotypes among 54 individuals sampled, with greater haplotypes diversity $Hd=0.999$ and nucleotide diversity ($\pi=0.73$) the current study is consistent with the above report from Nyong River.

Genetic distance between *Heterobranchus bidorsalis* populations indicated no significant genetic distance exists in all the population. While, Abubakar *et al.* (2018) reported no significant difference in the F_{ST} in his study, but recorded (-2.96628) among two populations of *Heterobranchus bidorsalis*, but with high genetic variation 102.96628 within each population using Cytochrome C Oxidase from River Niger and Benue. The negative or low F_{ST} value indicates that there is good variability among the individual population, genetic distance between the strains appears to be decreasing (Willing *et al.*, 2012). F_{ST} value are often used to infer gene flow in which a lower value indicates low genetic divergence and high gene flow (Nyaku *et al.*, 2023). In a separate findings Aminisarteshnizi and Moyo (2024) revealed that 76% of the genetic variation existed among *Clarias*

gariepinus populations, while 23% was within populations, with a significant difference among populations at $P<0.001$ in *Clarias gariepinus* using Cyt C Oxidase.

Phylogenetic analysis showed two major clusters with several sub clusters, from the studies that most samples clustered according to their population origin, indicating shared maternal ancestry within populations which differs from the findings of Aminisarteshnizi and Moyo (2024) who reported 3 major clades from *Clarias microcephalus*. Several Dadin kowa population samples formed a close cluster, demonstrating strong sequence similarity and low divergence. Genetic diversity is critical for individual fitness, ecosystem function, and ecological and evolutionary processes (Sjogvist *et al.*, 2016). Loss of genetic diversity in cultured populations may result from strict breeding practices that isolate the stock from other populations (Suleiman *et al.* 2023). While in the wild fish populations, it may be due to overfishing, poaching, population division, genetic drift, and natural selection. The molecular variation within population may not have been uncommon with natural selection or genetic drift which are hinged on evolution via random mutation (Ekerette *et al.*, 2018). The negative or low values (non-significant) revealed within the populations may be product of sampling bias, sampling error, genetic drift or gene flow (Nyaku *et al.*, 2025). The high percentage genetic variation among the three population is an indication of the presence of high level of genetic diversity, which could be due to geographical distance (separation). Nevertheless, Gerlach *et al.* 2010; Nyaku *et al.*, (2025) suggest negative or low values be taken as a potential warning signal that sample sizes might be too small or uneven sample sizes compared to the number of alleles. Thus, further sampling may eliminate negative values and present positive values, changing possible conclusions.

CONCLUSION

The findings of the study revealed that, the genetic variation showed high nucleotide and haplotype diversity (Hd) in Dadin kowa population suggesting high genetic diversity which serves good genetic purity for conservation and improvement. The results also presented variable sites with many population specific (SNPs), while in phylogenetic relationship Dadin kowa and Lau populations are sister clades originating from single maternal ancestry. Njoboliyo population is basal no mixing across populations it stands as an entity. Genetic distance from the three populations gives high haplotype between distance,

while, within population with no shared haplotypes across populations rather produce Private haplotype only. Populations are genetically isolated with strong differentiation.

REFERENCES

Abubakar, M., Y, Ipinjolu, J., K, Esa, Y, B, Magawata, I, and Hassan, W. A. (2018). Genetic diversity, populations and differentiation of farmed and wild African catfish (*Clarias gariepinus*) in Nigeria. *African Journal of Fisheries and Aquatic Resource Management*, 3 (1) 1-10.

Abubakar, M., Y, Ipinjolu, J., K, Esa, Y, B, Magawata, I, Hassan, W, A., and Turaki, A, A. (2020) Genetic diversity of wild population of *Heterobranchus* spp , Based on Mitochondria DNA Cytochrome C Oxidase Subunit 1 Gene Analysis. *International Journal of Agricultural and Bio Systems Engineering* 14(4) 20-35.

Adaka, G. S. & Olele, N. F. (2015). Length-weight relationship and condition factor of *Heterobranchus bidorsalis* (Geoffroy St-Hilaire, 1809) caught in Njaba River, Southeast, Nigeria. *International Journal of Fisheries and Aquatic Studies*. 3(2): 279-282.

Agbebi O, T., Ilaboia, D, E., and Adebambo, A, O. (2013) Preliminary characterization of genetic strain in *Clarias* species, *Clarias gariepinus* and *Heterobranchus bidorsalis* using microsatellite markers. *African journal of Biotechnology* 12 (4) 354-369.

Agbebi OT, Ajagbe O, Makinde L, Afolabi O (2009). Production, growth and effect of varying stocking density of *Clariobranchus fry* *Journal of fisheries International* 4(4):73-78.

Agnese, J. F., Teugels, G. G., Galbusera, P., Guyomard, R, and Volckaert, F. (1997). Morphometric and genetic characterization of sympatric populations of *Clarias gariepinus* and *Clarias anguillaris* from Senegal *Journal of Fisheries Biology*, 50: 1143-1157.

Ajibike, A.B. (2016) Genetic Diversity of Nigerian Indigenous Chicken using Microsatellite Markers and Mitochondrial D-loopDNA Sequences. M. Agric. Dissertation, Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta

Aluko, P, O. (1998) Growth characteristics of the parental F₁ F₂ and backcross generation of the hybrids between *Heterobranchus longifilis* and *Clarias angularis*. *West African Journal of Biological Science* 8; 16-21.

Aminisarteshnizi, M., and A.G. Moyo A. G, N. (2024) Genetic and Haplotype diversity of *Clarias gariepinus* (Burchell, 1822) Based on Cytochrome C Oxidase (COI) gene. *Journal of Hydrobiology*, 3(4): 337-349.

Antoniou, A.; Magoulas, A. *Application of Mitochondrial DNA in Stock Identification. Stock Identification Methods*, 2nd ed.; Academic Press: Cambridge, UK, 2014; pp. 257–295

Barasa, J.E.; Abila, R.; Grobler, J.P.; Agaba, M.; Chemoiwa, E.J.; Kaunda-Arara, B. (2016). High genetic diversity and population differentiation in *Clarias gariepinus* of Yala Swamp: Evidence from mitochondrial DNA sequences. *Journal of Fish Biology* 89, 2557–2570.

Briolay, J., Galtier, N., R. Miguel, Brito and Yvette Bouvet (1998) Molecular phylogeny of *Cyprinidae* inferred from Cytochrome b DNA sequence. *Molecular phylogenetic Evolution* pp 67-70.

Ekerette, E., Ikepeme, K., Durosaro S, and Wheto, M. (2017). Phylogenetic and Molecular Divergence of tilapia Fish (*Oreochromis* species) Using Mitochondrial D-Loop and Cytochrome b Regions *American Journal of Molecular Biology* 8, 39-54.

FAO. 2000. *FAO Year Book of Fisheries Statistics. Aquaculture Production*, 90(2): 209-213.

Froese, R. and Pauly, D. (2017) World Wide Web electronic publication. www.fishbase.org, version 06/ 2017.

Fujii, T. (2002). Tracking released Japanese Flounder *Paralichthys olivaceus* by mitochondrial DNA sequence. In: Nakamura Y., McVey, J. P. Leber, K. Neidig, C. Fox, S. Churchhill, K. (Eds.) *Ecology and Aquaculture species and enhancement of stocks. Proceedings of the 13th US-Japan meeting on Aquaculture*, pp. 51-53.

Gao, T., Zhang, X., Dagang, C., Meizhao, Z., Yiping, R. and Yaping, Z. (2001). Study on mitochondrial DNA cytochrome b gene of Chinese seabass, *Lateolabrax* sp. *Journal of fisheries international* 12(2) 354-374

Gerlach G, Jueterbock A, Kraemer P, Deppermann J, Harmand P. (2010) Calculations of population differentiation based on GST and D: forget GST but not all of statistics! *Molecules Ecology*, 2010 19(18):3845-52. doi:10.1111/j.1365-94X.2010.04784.x.

Hurst, C. D., S, E. Barlett, W., S. Davidson and I. J. Bruce (1999) the complete mitochondrial DNA sequence of the Atlantic Salmon, *Salmo Salar*, *Genetic* 239:239-242

Hvilsom, C.; Segelbacher, G.; Ekblom, R.; Fischer, M.C.; Laikre, L.; Leus, K.; O'Brien, D.; Shaw, R.; Sork, V. (2022). Selecting Species and Populations for Monitoring of Genetic Diversity; *International Union for Conservation of Nature (IUCN) Gland, Switzerland*.

Idodo-Umeh, G. (2003) *Freshwater Fishes of Nigeria (Taxonomy, Ecological Notes, Diets and utilization)* Idodo Umeh Publishers Ltd. Benin City. pp. 232.

- Kundu, S., P. S. de Alwis, J. D. Binarao, et al. 2023. "Mitochondrial DNA Corroborates the Genetic Variability of *Clarias* Catfishes (Siluriformes, Clariidae) from Cameroon." *Life* 13: 1068.
- Laleye, P., Tweddle, D., Azeroual, A., Gentahun, A. Hassens, M. Kazembe, J. Marshall, B., Moelants, T. (2019) *Heterobranchus longifilis* International Union for Conservation of Nature (IUCN) Red list of threatened species.
- Mwakubo, S.M.; Ikiara, M.M.; Abila, R. Socio-economic and ecological determinants in wetland fisheries in the Yala Swamp. *Wetland Ecology Management*, 15: 521–528
- Nwafili, S. A., Gao, T. (2007) Is the Dutch domesticated strain of *Clarias gariepinus* (Burchell, 1822) a hybrid. *African Journal of Biotechnology*, 6 (8) 1072-1078.
- Nyaku, E. R., Diyaware, M, Y., Sulieman, S, B., and Nwafili, S, A. (2023). Mitochondrial DNA D-loop Genetic Relatedness and characterization of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) from Lake and Bako, Northeast, Nigeria, *Journal of Biotechnology* 2(1), 22-33.
- Nyaku, E. R., Onyia, U, L., Mustapha, F, A., and Haruna, U, N, (2025) Genetic diversity of Nile tilapia (*Oreochromis niloticus*) strains from the northeast, Nigeria based on mtDNA Cyt B sequence. *Nigeria journal of Fisheries Science and technology* 3(2) 89-102.
- Oseni, T. A., Akande, T. A., and Adeyemo, T. J. (2019). Adaptation Mechanisms of African Catfish in Hypoxic Environments. *Journal of Fish Ecology*, 32(4), 403-412.
- Oseni, T. A., Akande, T. A., and Adeyemo, T. J. (2019). Adaptation Mechanisms of African Catfish in Hypoxic Environments. *Journal of Fish Ecology*, 32(4), 403-412.
- Ponzone, R.W.; Hamzah, A.; Tan, S.; Kamaruzzaman, N. (2018). Genetic parameters and response to selection for live weight in the GIFT strain of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 247, 203-210.
- Popoola, O. M. (2022). "Genetic Differentiation and Molecular Phylogenetic of North African Catfish from Three Distinct Waterbodies." *Croatian Journal of Fisheries* 80: 123–132.
- Sjoqvist, C.O. and Kremp, A. (2016). Genetic diversity affects ecological performance and stress response of marine diatom populations. *ISME Journal* 10, 2755–2766.
- Solomon, S. G., Okomoda V. T., and Ochai L. (2013) Growth of pure bred *Heterobranchus bidorsalis*, *Clarias gariepinus* and their intergeneric crosses fed commercial diet. *Banat's Journal of Biotechnology*, 4 (8): 71-76.
- Suleiman, I.O.; Moruf, R.O. and Usman, B.I. (2023) Population Genetic Structure of Feral and Cultured African catfish (*C. gariepinus*) inferred from Random Amplified Polymorphic DNA in Kano, Nigeria. *Tropical Journal Natural Production Resources* 7, 2650–2654.
- Tamura, K., Stecher, G., Peterson, D., Filipski, K. and Kumar, S. (2013) MEGA6; Molecular Evolutionary Genetic analysis version 6, *Molecular biology and Evolution* 30, 2725-2729
- Teugels, G, G., Denayer, B., and Legengre, M. (1990) A systematic revision of the African catfish genus *Heterobranchus* (Pisces, Clariidae) *Zoology Journal of Linnaeus* 98; 237-257
- Tukura, B. W., Kagbu, J. and Gimba, C. E. (2005). Bioaccumulation of trace metals in fish from Ahmadu Bello University Dam. *Nigerian Journal of Scientific Research*, 5(1): 23-30.
- Wei, H.; Geng, L.; Shang, X.; Li, L.; Ma, B.; Zhang, Y.; Li, W.; Xu, W. (2023) Comparison genetic diversity and population structure of our *Pseudorasbora parva* population in Heilongjiang River Basin based on mitochondrial COI gene. *Frontier Mathematics*, 10 (11) 115-120.
- Willing, E., Motia, Dreyer C, Oosterhout, C, (2012) Estimates of Genetic diversity of differentiation Measured by F_{ST} Do not necessarily require Large Samples Sizes when using SNP marker *PLOS* 7 (8) e4269doi:10.1371/journal.pone.0042649
- Yashim, E. Y., Aken Ova, T. and Auta, J. (2017) Molecular characterization of wild and cultured species of *Clarias* Scopoli, 1777 and *Heterobranchus* (Geoffroy Saint-Hilaire, 1808) in Zaria *Zoological Society of Nigeria* 15:1-5 December.
- Yisa, M., and S, O., Olufeagba (2005) An exposition on field identification of Clariid catfishes as an important tool in fish breeding and genetics *Proceeding of the 19th Annual Conference of the Fisheries Society of Nigeria* (FISON) 29th Nov 2004, Ilorin 189-193.