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GENETIC DIVERSITY AND DEMOGRAPHIC HISTORY OF *CHRYSICHTHYS NIGRODIGITATUS* FROM NIGERIAN FRESHWATER AND BRACKISH WATER HABITATS USING MTDNA DLOOP ANALYSIS.

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ABSTRACT

Chrysichthys nigrodigitatus, the silver catfish, is an important freshwater and brackish species in West Africa, supporting artisanal and commercial fisheries. Sustaining its productivity depends on understanding its genetic diversity and population structure, which underpin resilience and adaptability to environmental change. This study investigated the genetic diversity and demographic history of *C. nigrodigitatus* from Yola (Upper River Benue) and Lagos (Epe Lagoon). Muscle tissues (±25 g) from 10 individuals per site were preserved in ethanol, with DNA extracted (Zymo Research protocol) at ACUTIG Laboratories, Abeokuta, and amplified via PCR at Stab Vida Laboratories. Sequence data were analysed in DnaSP to estimate diversity indices and assess demographic trends. Lagos (Epe Lagoon) showed 19 segregating sites, 7 haplotypes, high haplotype diversity (Hd = 0.964), moderate nucleotide diversity (π = 0.011), and a mean of 7.464 nucleotide differences. Yola recorded 11 segregating sites, 7 haplotypes, similarly high haplotype diversity (Hd = 0.964), but lower nucleotide diversity (π = 0.006) and fewer mean nucleotide differences (3.786). Neutrality tests suggested Lagos was near equilibrium (Tajima's D = 0.097, Fu's Fs = –1.031), while Yola's negative values (Tajima's D = –0.538, Fu's Fs = –2.603) indicated possible population expansion or recent bottlenecks. The higher haplotype and nucleotide diversity in Lagos suggests greater adaptive potential under environmental change. In contrast, Yola's reduced nucleotide diversity may reflect limited genetic variation, underscoring the need for conservation strategies to maintain inland river population resilience.

Keywords: Genetic Diversity, Population Structure, *Chrysichthys nigrodigitatus*, Nucleotide diversity, Demographic History

INTRODUCTION

Chrysichthys nigrodigitatus (Silver catfish) is a euryhaline fish of tropical Africa which supports successful commercial fisheries with great potentials for aquaculture in West Africa (Megbowon *et al*., 2019). It is an important source of animal protein and income throughout the world especially in developing countries like Nigeria and have shown to be better aquaculture candidates (Nwafili *et al*., 2015). This is due to their acceptance to consumers and ease of culture. The requirement for more sources of quality protein such as fish has increased over the years. This tends to replace meat from livestock in the young and aged populations. The African Silver catfish, *Chrysichthys nigrodigitatus* is widely distributed in fresh and brackish waters in West Africa where they are commercially important (Holden and Reed, 1991); with great potential for aquaculture (Nwafili *et al.*, 2015). In addition, the culture of *C. nigrodigitatus* is widely practiced in many countries of the Sub Sahara Africa and constitutes one of the largest freshwaters cultivated fish (Ouattara *et al*., 2014). It occurs in a variety of fresh and brackish water habitats such as rivers, mangrove swamps, lakes, estuaries, and low-salinity coastal areas (Ezenwa, 1981; Erondu, 1997; Moses, 2001). Bagrid catfish, *C. nigrodigitatus,* is one of the highly valuable fresh-water fish resources in West African countries including Nigeria, Senegal, and Cameroon (Azeroual *et al*., 2010; Andem *et al*. 2013). Due to the recent increased demands for the species, the sustainable production of *C. nigrodigitatus* is highly required both by aquaculture without adverse effects on the aquatic ecosystem (Kareem *et al*., 2015) and by the scientific management of native populations (Abu and Agarin, 2016). In Ivory Coast and Nigeria, *C. nigrodigitatus* is a highly valued food fish and is among the wild commercial catches as well as cultural fish species (Otémé, 1993; Hem *et al.,* 1994) found in lagoons and rivers.

The genus *Chrysichthys* is characterized by spawning migration undertaken in the rainy season from brackish waters to freshwaters where spawning occurs (Nwafili *et al*., 2012). The juveniles later move with the flood water back to the saline environment to feed and grow (Ezenwa, 1981 and Erondu, 1997). *Chrysichthys spp* is a Siluroidae belonging to the family Claroteidae (Mo, 1991 and Teugels, 1996) with great aquacultural potential (Erondu, 1997) and plays a vital role in aquatic ecology and fisheries (Ayotunde and Ada, 2013) of the River Benue and Epe Lagoom in Nigeria and other African water bodies.

Sita and Beatrice (2023) and Duwal *et al*. (2024) studied genetic diversity of *C. nigrodigitatus* to understand the evolutionary potential of this species as affected by environmental changes such as climate change, habitat degradation and pollution among others. Genetic variation in a species enhances the capability of an organism to adapt to the changing environment and is necessary for the survival of the species (Duwal *et al*., 2024). The genetic diversity data has varied applications in research on evolution, conservation and management of natural resources including genetic improvement programmes. Demographic history studies the past population expansion, contractions and isolation to predict future trends and risks of the population.

Nwafili and Gao (2016) reported that the use of mtDNA has a great advantage over nuclear DNA because of its ability to resist degradation and its high copy number inside the cell as compared to nuclear DNA (nDNA). The mtDNA D-loop is the only non-coding segment that exists in the vertebrate mitochondrial genome (Zardoya and Meyer, 1997). This segment is the most variable part of the mtDNA, and evolves three to five times faster than the rest of the mitochondrial genome (Li *et al*., 2017). Therefore, it is widely used as a genetic marker to assess the origin, phylogenesis, and intraspecific genetic differentiation of animal species (Rasmussen and Morrissey, 2008) including *C. nigrodigitatus*.

Several studies were carried out on genetic diversities of *C. nigrodigitatus*

(Nwafili *et al*., 2012; Ukenye *et al*., 2019; Megbowon *et al*., 2019: Adilieje *et al*., 2020; Uyoh *et al*., 2020) but did not include Upper River Benue. This study is therefore aimed at understanding the genetic diversity and demographic history of *C. nigrodigitatus* in the two water bodies for conservation and sustainability in Nigerian fisheries.

MATERIALS AND METHODS

The study was conducted on Yola and Epe Lagoon samples. Yola, River Benue (Freshwater) is the capital of Adamawa State and also the seat of Lamido Fombina. It has an average annual rainfall of 925 mm, population of 282,785 (NPC, 2018), and annual average temperature of 21.6º C- 34.5º C (Adamawa State Handbook, 2017; Adebayo *et al*., 2020). It experiences migratory fishers from all over the nation especially the Northern Nigeria. Epe Lagoon, Lagos (Brackish Water) is a town and a Local Government Area (LGA) in Lagos State, Nigeria, located on the north side of the Lekki Lagoon. It is a Yoruba town located next to the Lagos lagoon with 294 rural and 24 semi-urban communities. Epe is known for its fish market which feeds off the hard work of those men and women whose lives depend on the lagoon – and the fish inside it (Abasilim *et al*., 2020).

Table 1: Coordinates of Locations where Samples of *C. nigrodigitatus* were Collected.

LOCATION	LATITUDE	LONGITUDE	STATE
Epe-Lagos	06° 35' 02.83" N	03° 59' 00.10" E	Lagos
Upper River Benue Yola	09° 17' 14.07" N	12° 27' 51.90" E	Adamawa

Sample Collection

Freshly caught *C. nigrodigitatus* (Plate I) samples were identified according to Idodo-Umeh (2003), Olaesebikan and Raji (2013) and Duwal *et al*. (2016) and thereafter purchased from fishers at landing sites or fresh fish markets in the respective areas. Fresh muscle tissues (±25g)

Plate I. Dorsal and Lateral Views of *C. nigrodigitatus*

were cut from 10 randomly selected fish from each sample location. Each sampled tissue was placed in ten different 5 ml sample tubes containing absolute ethanol for preservation until DNA extraction. The sample tubes were labelled thus;

Lagos Sample – LA1, LA2, ….LA10, and

Yola Sample – YL1, YL2, ….YL10.

DNA Extraction

The DNA extraction was performed at ACUTIG Laboratories in Abeokuta, Ogun State, Nigeria. The extraction followed the DNA miniprep manufacturer's protocol (Zymo Reasearch ™)

The Polymerase Chain Reaction PCR.

This was carried out with the primers in Table 2 and using *Suf Hot Taq Polymerase* with a TM of 55 °C for 40 cycles. The PCR

Table 2: Primers Sequences Used for the Amplification of *C. nigrodigitatus* DNA

Name of Primer	Primer Sequence	Properties
AfricanCatFish_Fwd	CATCCTCACCTGAATCGG	52.47°C
AfricanCatFish _Rev	GGG TGCTTGCTAATGAAG	53.78°C

amplifications were performed on Biometra Gradient Thermocycler (Gottingen, Germany) in 25 µl final volume with Master Mix and DNA Surf Hot Taq Polymerase (10 U/µl) (Stab Vida, Lisbon, Portugal) with the following thermal cycling conditions as reported by Duwal *et al*. (2024).

Sequencing

After amplification, all products from the samples were purified with *Magnetic Beads* and prepared for sequencing reaction with *Big Dye Terminator v3.1 Reagent*. Finally, after the sequencing reactions were completed, the samples were again purified with *Magnetic Beads* and run on the Applied Biosystems ABI 3730XL Sequencer

Statistical Analysis

The sequenced data were analysed using DNaSP (DNA sequence polymorphism) to calculate nucleotide diversity, haplotype diversity, number of polymorphic sites, Tajima's D and Fu's Fs.

RESULTS

Diversity indices of *C. nigrodigitatus* from the two populations based on Mt-dloop (mitochondrial DNA control region) analysis is presented in Table 3. Lagos recorded 19 segregating sites, 7 haplotypes, high haplotype diversity (0.964), moderate nucleotide diversity (0.011), and an average of 7.464 nucleotide differences and Yola had 11 segregating sites, 7 haplotypes, high haplotype diversity (0.964), low nucleotide diversity (0.006), and an average of 3.786 nucleotide differences. The two populations have higher genetic diversities. **Pie Charts** comparing Epe Lagoon (Lagos) and Upper River Benue (Yola) for each **Diversity Index** of *C. nigrodigitatus* is presented in Figure 1. It shows a higher percentage of segregating sites, nucleotide diversity and nucleotide difference while number of haplotypes and haplotype diversities were equal. Table 4 show the deviation from neutrality of *C. nigrodigitatus* in the two populations. Lagos had a Tajima's D (0.097), negative Fu's Fs (-1.031), Fu and Li's D (0.071) and F (0.078) close to zero and Yola recorded a negative Tajima's D (-0.538), strongly negative Fu's Fs (-2.603), Fu and Li's D (-0.360) and F (-0.445) were also negative. The heatmap in Figure 2 also depicts the deviation from neutrality. Colour values closer to 0 are shown in grey, while increasingly negative values shift to light blue and then dark blue. Negative values often suggest population expansion or purifying selection, while positive values may indicate balancing selection or population contraction.

The Lagos population appears genetically stable and closer to neutrality, while the Yola population shows more negative values, indicating stronger signs of recent population expansion or purifying selection.

Table 3: **Diversity Indices of *C. nigrodigitatus* from the two Populations**

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Diversity Indices	Lagos	Yola
Segregating site (S)	19	11
Number of haplotypes (H)	7	7
Haplotype diversity (Hd)	0.964	0.964
Nucleotide diversity (π)	0.011	0.006
Average number of nucleotide differences (K)	7.46	3.786

Table 4: Deviation from Neutrality of *C. nigrodigitatus* from Yola and Epe Lagoon

Statistical test	Lagos	Yola
Tajima's D	0.097	-0.538
Fu's Fs	-1.031	-2.603
Fu and Li's D	0.071	-0.360
Fu and Li's F	0.078	-0.445

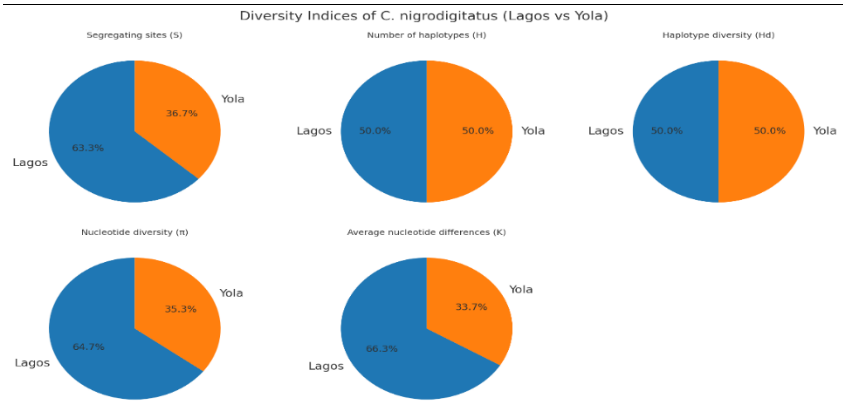

Figure 1: Pie Charts comparing Epe Lagoon (Lagos) and Upper River Benue (Yola) for each **Diversity Index** of *C. nigrodigitatus*.

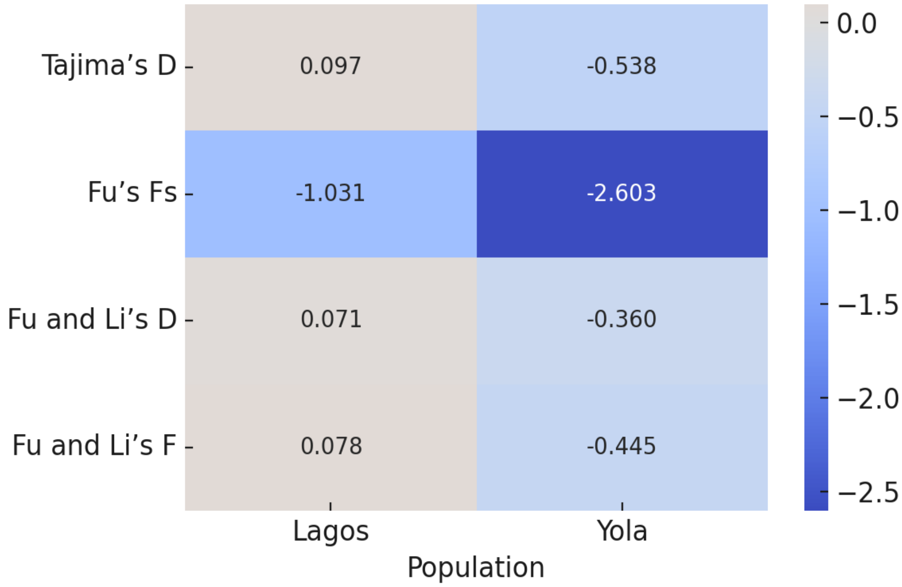

Figure 2: Heatmap of the deviation from neutrality tests for *C. nigrodigitatus* populations from **Lagos (Epe Lagoon)** and **Yola (Upper Benue River)**.

DISCUSSION

Studies of population genetic diversity and molecular evolution can provide useful information necessary for species conservation and population management (Okonko *et al*., 2022). The capability of the fingerlings, juveniles and adults to undertake migrations could contribute to shaping the genetic diversity and population structure of *C. nigrodigitatus* (Nwafili and Gao, 2016; Duwal *et al*., 2024). Chances of survival of individuals and evolutionary ability of a population rely on maintenance of genetic diversity (Hoglund, 2009; Sahoo *et al*., 2020). African Agenda 2063 encompasses in its main aims the sixth goal, which is concerned by blue economy development and promotion of sustainable use of aquatic resources (Hamed-Mohamed *et al*., 2023). This has made this study of the genetic diversity of *C. nigrodigitatus* in Upper River Benue (Yola) and Epe Lagoon (Lagos) in Nigeria timely. This is a pilot study comparing the two populations of the species using Mitochondria displacement loop (mtDNA d-loop). The two population presented a high haplotype diversity which is an attribute to long evolutionary history in a large stable population (Duwal *et al*., 2024). Nwafili *et al*. (2013) reported a similar result on *C. nigrodigitatus* and *C. walkeri* from the Lagos Lagoon. The report of Sita *et al*. (2018) on *Chrysichthys* species in Bia River, Cote D'ivoire also agreed with this report. The segregating sites presented the positions in the DNA sequence where there was variation among individuals within the two populations. Lagos population had the highest number of segregating sites (19), suggesting greater genetic variation at individual nucleotide positions. The higher number of segregating sites presented by Lagos population is an indication that it might have a higher mutation rate or more genetic mixing compared to the Yola populations, possibly due to a larger population size or greater gene flow. Recognising genetic diversity is an essential part of biodiversity conservation, enabling populations and species to survive and evolve in accordance with environmental changes

(Miller *et al*., 2015; Gu *et al*., 2021). Diversity of the Haplotype (Hd) is the probability that two randomly chosen haplotypes from the population are different. Sahoo *et al*. (2018) opined that higher number of private haplotypes might be due to independent origin of haplotypes through mutation and could be used as population specific marker for stock identification. The nucleotide diversity was 0.011 for Lagos and 0.006 for Yola. This agrees with the report of Sahoo *et al*. (2020) who studied Indian Catfish using mtDNA d-loop and obtained low nucleotide diversities of 0.00129. Similar level of genetic diversity was reported earlier for Indian Magur populations (Khedkar *et al*., 2016). The observed Hd in the present study was above the range observed in other freshwater fishes (Habib *et al*., 2012, Hd=0.876). Nucleotide diversity (π) is the average difference per nucleotide site between two DNA sequences in a population. In this report Lagos had the highest nucleotide diversity (0.011) and the least was Yola (0.006). The higher nucleotide diversity in Lagos suggested that it may have accumulated more mutations or has experienced more gene flow than the other populations. Yola's lower diversity may suggest either a smaller population size or more recent bottleneck effects (Duwal *et al*., 2024). Hamed-Mohamed *et al*. (2023) reported a similar case for the genus *Chrysichthys* in Egypt that high haplotype diversity corresponded to low nucleotide diversity. This indicated low flow of genetic information and little divergence among populations (De Jong *et al*., 2011). The Low nucleotide diversity observed here might be due to founder effect from different colonization events as well as anthropogenic activities (Khedkar *et al*., 2016). High/moderate haplotype diversity and low nucleotide diversity as evidenced in magur populations might be due to the rapid expansion and population growth after a period of low effective population leading to the retention of new mutations (Avise *et al*., 1984; Rogers and Harpending, 1992). It is a well-known fact that large population size could maintain high haplotype diversity within a population (Nei, 1987). It could now be envisaged that the two populations studied have large sizes since all of them had high haplotype diversity. However, populations with higher haplotype and nucleotide diversity, like Lagos is likely better equipped to adapt to environmental changes and Yola may have less adaptability.

Demographic history is closely related to genetic diversity and the study is especially critical for *C. nigrodigitatus* since it inhabits both freshwater and brackish water. Tajima's D is widely used to detect departures from neutrality, particularly in cases of demographic changes or selection. It is used to compare two estimates of genetic diversity in a population: the pairwise differences (π) and the number of segregating sites (S). Under neutrality, these two estimates should be equal. This study discovered that Tajima's D values were greater than zero for Epe Lagoon populations (0.097) while Upper River Benue populations recorded negative value (-0.538). In a study conducted by Okonko *et al*. (2022), Tajima's D and Fu's Fs values were used to determine the demographic expansion in *C. nigrodigitatus*. They reported positive Tajima's D value of 2.091 for freshwater samples and 1.292 for brackish water samples, while Fu's Fs values were 1.667 for freshwater samples and 1.108 for brackish water samples. This could mean that Lagos population is experiencing balancing selection or recent population bottleneck. This present study does not agree with their report since the fish species from Upper River Benue (freshwater) recorded a negative Tajima's D. It is also consistent with the reports of Nwafili *et al*. (2012), Nwafili and Gao (2016) and Sita *et al*. (2018) who opined that the brackish water fish population was not subject to demographic expansion. Cognetti and Maltagliati (2000) presented a similar report to this study where negative selection was recorded in freshwater and brackish water having high negative site index. This suggested that the high negative selection pressure observed in this study is acting to remove the effects of deleterious mutations in the populations.

CONCLUSION

In conclusion, high haplotype diversity was shown in *C. nigrodigitatus* populations in both Epe Lagoon and Upper River Benue but greater overall genetic variation and demographic stability is displayed by Epe Lagoon population, while Upper River Benue population exhibits lower nucleotide diversity and a mild signal of recent expansion. These differences likely reflect variations in historical population size, gene flow, and habitat connectivity. Continuous habitat monitoring and management is therefore recommended to maintain genetic health, particularly for the Yola population.

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REFERENCES

Abasilim, C. F., Samuel, O. B. & Onyewuchi, I. V. (2020). Economic Analysis of Artisanal Fisheries Value Chain in Epe Lagoon, Lagos State, Nigeria. *Ife Journal of Agriculture* 22(2); 91-101

Abu, O.M.G. & Agarin, O.J. (2016). Length- Weight Relationship and Condition Factor of Silver Catfish (*Chrysichthys nigrodigitatus*) from the Lower Reaches of the New Calabar River, Niger Delta. *International Journal of Innovative Studies in Aquatic Biology and Fisheries*, 2(4): 1-7. https://doi.org/10.20431/2454-7670.0204001

Adamawa State Government Handbook, (2017). *Know and Speak to your Leaders in Adamawa State*. Adamawa State Government, Yola. pp32

Adebayo, A. A., Tukur, A. L. & Zemba A. A. (Eds). (2020). Adamawa State in Maps. *Paraclete Publishers*, Yola-Nigeria, 203p

Adilieje, C. M., Uyoh, E. A., Ntui, V. O. & Ama-Abasi, D. (2020). Morphological and Genetic Diversity Studies of *Chrysichthys nigrodigitatus* from the Cross River, Nigeria, Using Microsatellite Markers. *Annual Research and Reviews in Biology*. 35(5): 42-52:

https://doi.org/10.20431/2454-7670.0204001.

Andem, A. B., Okorafor, K. A., Eyo, V. O. & Ekpo, P. B. (2013). Ecological Impact Assessment and Limnological Characterization in the Intertidal Region of Calabar River Using Benthic Macroinvertebrates as Bioindicator Organisms. *International Journal of Fisheries and Aquatic Studies* 1(2):8-14.

Avise, J. C., Neigel, J. E. & Arnold, J. (1984). Demographic Influence on Mitochondrial DNA Lineage Survivorship in Animal Populations, *Journal of Molecular Evolution*, 20(2): 99-105. https://doi.org/10.1007/BF02101942

Ayotunde, E. O. & Ada, F. B. (2013). Silver Catfish *Chrysichthys nigrodigitatus* (Lacepede, 1803), an Endangered Fish Species in Cross River, Cross River State, Nigeria. *International Journal of Agricultural Science Research*, 2(3): 083- 089

Azeroual, A., Da Costa, L., Laleye, P. & Moelants, T. (2010). Chrysichthys nigrodigitatus. the IUCN Red List of Threatened Species 2010: https://doi.org/10.2305/IUCN.UK.2010-3.RLTS.T182997A8019531.en

Cognetti, G. & F. Maltagliati. (2000). Biodiversity and Adaptive Mechanisms in Brackish Water Fauna. Marine Pollution Bullettin, 40: 7-11. https://doi.org/10.1016/S0025-326X(99)00173-3

De Jong, M. A., N. Wahiberg, M. Van Eijk, P. M. Brakefield & B. J. Zwaan. (2011). Mitochondrial DNA Signature for Range-Wide Populations *Bicyclus anynana* Suggests a Rapid Expansion from Recent Refugia. *PLoS ONE*, 6(6): e21385. https://doi.org/10.1371/journal.pone.0021385

Duwal, S. D., Sanda, M. K., Amadi, G. C., Onyia, L. U. & Ja'afar, N. J. (2016). Preliminary Identification and Phylogenetic Study of *Chrysichthys* Pisces: Claroteidae, From Upper River Benue, Nigeria Using Microsatellites DNA. *International Journal of Innovative Research and Development, 5(9): 154-160*

Duwal, S. D., Peter, M.B., Sogbesan, O.A. & Onyia, L.U. (2024). Genetic Diversity among *Chrysichthys nigrodigitatus* from Fresh and Brackish Waterbodies in Nigeria. *Journal of Agriculture, Environmental Resources and Management*, 6(7)1-800; pp71-80

Ezenwa B. (1981). Reproductive Biology of the Catfish, *Chrysichthys nigrodigitatus* (L) in Nigeria. *Ph. D thesis, University of Lagos*; Nigeria.

Erixon, P. & B. Oxelman. (2008). Whole Gene Positive Selection, Elevated Synonymous Substitution Rates, Duplication and Indel Evolution of the Cloroplast *clpP1* Gene. *PLoS ONE* Vol 3, 10.1371/journal.pone.0001386. https://doi.org/10.1371/journal.pone. 0001386

Erondu, E.S. (1997). Aspect of the Biology of *Chrysichthys nigrodigitatus* in the New Calabar River and its Aquaculture. *Ph. D Thesis of the University of Nigeria*, Nsukka.

Gu, S., Yi, M. R., He, X. B., Lin, P. S., Liu, W. H., Luo, Z. S., Lin, H. D. & Yan, Y. R. (2021). Genetic Diversity and Population Structure of Cutlassfish (*Lepturacanthus savala*) along the Coast of Mainland China, as Inferred by Mitochondrial and Microsatellite DNA Markers, *Regional Studies in Marine Science*. 43: https://doi.org/10.1016/ j.rsma.2021.101702

Habib, M., Lakra, W. S., Mohindra, V., Lal, K. K., Punia, P., Singh, R. K., ... (2012). Assessment of ATPase 8 and ATPase 6 mtDNA Sequences in Genetic Diversity Studies of *Channa marulius* (Channidae: Perciformes). *Proceedings of the National Academy of Sciences, India, Section B: Biological Sciences*, 82; 497-501. https://doi.org/10.1007/s40011-012-0044-7

Hamed-Mohamed, D., AbouelFadl, K. Y., Younis, A. M. & Mohammed-Geba, K. (2023). Preliminary Analysis for Genetic Structure and Interspecific Relationships Within Genus *Chrysichthys* in Lake Nasser, Egypt. *Scientific African*, 19: https://doi.org/10.1016/j.sciaf.2022.e01494

Hem, S., Legendre, M., Trébaol, L., Cissé, A., Otémé, Z. & Moreau, Y. (1994).
L'aquaculture Lagunaire. In: *Environnement et ressources aquatiques de*
Côte d'Ivoire: Les milieux lagunaires. Durand, J.R., Dufour, P., Guiral, D.,
Guillaume, S., Zabi, F. (Eds), ORSTOM, Tome II, Paris, 1-46.

Hoglund, J. (2009). *Evolutionary Conservation Genetics*, Oxford University Press.

Holden, M. & Reed, W. (1991). *West African Freshwater Fish*. Longman Publishers Ltd., Singapore. 68p.

Idodo-Umeh, G. (2003). *Freshwater Fishes of Nigeria (Taxonomy, Ecological Notes, Diet and Utilization).* Idodo-Umeh Publishers Ltd, Benin City, Nigeria. 232p.

Kareem, O. K., Ajani, E. K., Orisasona, O. & Olarenwaju, A. N. (2015). The Sex Ratio, Gonadosomatic Index, Diet Composition and Fecundity of African Pike, *Hepsetus odoe* (Bloch, 1794) in Eleyele Lake Nigeria, *Journal of Fisheries and Livestock Production*, 3: 139. https://doi.org/10.4172/2332-2608.1000139

Khedkar, G. D., Tiknaik, A. D., Shinde, P. N., Kalyankar, A. D., Ron, T. B. & Haymer, D. S. (2016). High Rate of Substitution of the Native

Catfish *Clarias batrachus* Indian Magur by *Clarias gariepinus* in India. *Mitochondria DNA Part A*, 27(1); 569-574. https://doi.org/10.3109/19401736.2014.89937

Li, L., H. Lin, W. Tang, D. Liu, B. Bao & J. Yang. (2017). Population Genetic Structure in Wild and Aquaculture Populations of *Hemibarbus maculates* Inferred from Microsatellites Markers. *Aquaculture and Fisheries* 2: 78-83. https://doi.org/10.1016/j.aaf.2017.04.003

Megbowon, I., E.A. Ukenye, M.M.A. Akinwale, A.B. Usman, H. Jubrin, B.A. Sokenu, I. Chidume, N.C. Eze, R.O. Adeleke, J.B. Joseph, G.E. Olagunju & O.J. Ayokhai (2019). Molecular Characterization and Genetic Diversity of *Chrysichthys nigrodigitatus* From Some Coastal Rivers in Nigeria. *Journal of Agriculture and Environment* Vol. 15 No. 1: 157-164

Miller, Steven J., Van Genechten, Daniel T., Cichra & Charles E. (2015). *Length–weight Relationships and an Evaluation of Fish–size and Seasonal Effects on Relative Condition (Kn) of Fishes from the Wekiva River, Florida,* vol. 78. Florida Academy of Sciences, Inc., pp. 1–19. No. 1 (Winter, 2015). https://www.jstor.org/stable/24321839

Mo T. (1991). *Anatomy, relationships and systematic of the Bagridae (Teleostei: Siluroidei) with a hypothesis of siluroid phylogeny* – Thesis Zoologie 17, Koeltz Scientific Books, Koenigstein, 216 p

Moses, B.S. (2001). The Influence of Hydroregime on Catch, Abundance and Recruitment of the Catfish *Chrysichthys nigrodigitatus* (Bagridae) and the bonga *Ethmalosa fimbriata* (Clupeidae) of South-Eastern Nigeria's Inshore Waters. *Environmental Biology and Fisheries*.;61: 99–109. https://doi.org/10.1023/A:1011009414217

Nei, M. (1987). *Molecular Evolutionary Genetics*, Columbia University Press, New York, USA pp10-88

Nwafili, S. A., O. O. Soyinka & X. G. Tian. (2012). Levels and Patterns of Genetic Diversity in Wild *Chrysichthys nigrodigitatus* in Lagos Lagoon Complex. *African Journal of Biotechnology*, 11:15748-15754. https://doi.org/10.5897/AJB12.2037

Nwafili, S. A., Eminue, B. O. & Jamabon, N. (2013). Genetic Differentiation of Two *Chrysichthys* Species Using Mitochondrial DNA Sequencing, *Lasbela University Journal of Science and Technology*, 36-43

Nwafili, S. A., Ren Guijing & Gao Tian Xiang (2015). Microsatellite Analysis of Six Populations of *Chrysichthys nigrodigitatus* from Nigeria. *Annual Research and Review in Biology* 5(6): 544-552.

https://doi.org/10.9734/ARRB/2015/13737

Nwafili, S. A. & T. X. Gao (2016). Genetic Diversity in the mtDNA Control Region and Population Structure of *Chrysichthys nigrodigitatus* from Selected Nigerian Rivers: Implication for Conservation and Aquaculture. *Archives of Polish Fisheries*, 24:85-97. https://doi.org/10.1515/aopf-2016-0010

Okonko, L. E. Ikpeme, E. V. & Ozoje, M. O. (2022). Molecular Evolution and Genetic Analysis of Silver Catfish (*Chrysichthys nigrodigitatus*) in Nigeria. *Asian Journal of Biological Sciences*. 15(2): 93-100. https://doi.org/10.3923/ajbs.2022.93.100

Olaosebikan, B.D. & Raji, A. (2013). Field Guide to Nigerian Freshwater Fishes. *Nigerian Freshwater Fisheries Research Institute,* New Bussa.

Oteme, Z. J. (1993). Sexual Cycle and Fecundity of the Catfish Clarias nigrodigitatus in Ebrie Laggon Cote d'Ivoire. *Journal Ivoirien d'Oceanologie et de Limnologie* 2 (1); 61-67

Ouattara, T.A.S., Konan, K.M., Konan, K.J., Adepo-Gourene, A.B., Arse, B.C. & N'guetta, A.S.T. (2014). Morphological Identification and Taxonomic Relationship of Farmed Fish of Genius Chrysichthys. *International Journal of Research and Environmental Sciences,* 1(3):29-39

Rasmussen, R.S. & Morrissey, M.T. (2008). DNA-based methods for the identification of commercial fish and seafood species. *Comprehensive Reviews in Food Science and Food Safety*, 7, 280-295. https://doi.org/10.1111/j.1541-4337.2008.00046.x

Rogers, A. R. & Harpending, H. (1992). Population Growth makes Waves in the Distribution of Pairwise Genetic Differences, *Molecular Biology and Evolution*, 9(3): 552-569. https://doi.org/10.1093/oxfordjournals.molbev.a04072

Sahoo, L., Mohanty, M., Meher, P. K., Murmu, K., Sundaray, J. K. & Das, P. (2018). Population Structure and Genetic Diversity of Hatchery Stocks as Revealed by Combined mtDNA Fragment Sequences in Indian Carp, *Catla catla. Mitochondrial DNA Part A*. 30(2); 289-295. https://doi.org/10.1080/24701394.2018.1468656

Sahoo, L., Barat, A., Sahoo, S. K., Sahoo, B., Das, G., Das, P., Sundaray, J. K. & Swain, S. K. (2020). Genetic Diversity and Population Structure of Endangered Indian Catfish, *Clarias mogur* as Revealed by mtDNA D-loop Marker. *Turkish Journal of Fisheries and Aquatic Sciences* 21(1), 09-18. https://doi.org/10.4194/1303-2712-v21_1_02

Sita, O. T. A., B. A. Carole, K. K. Mexmin, A. G. A. Beatrice & G. Germain (2018). Morphological and Genetic Characterisation of Chrysichthys Species from the Bia River (Cote d'Ivoire). *International Journal of Fisheries and Aquatic Studies,* 6:116-121

Sita, T. A. & Beatrice, A. (2023). Morphological and Genetic of Fish of the Subgenus *Chrysichthys chrysichthys* from Ivorian Rivers. *Open Journal of Applied Sciences* 13, 1336-1347. https://doi.org/10.22271/fish

Teugels, G.G., (1996). Taxonomy, Phylogeny and Biogeography of Catfishes (Ostariophysi, Siluroidae), An Overview: *Aquaculture Living Resources* 9: 9-34.

Ukenye, E. A., Akinwale, M. M. A., Megbowon, I., Usman, A. B., Jubrin, H., Sokenu, B. A., Chidume, I. & Eze, N. C. (2019) Genetic differentiation and structure of *Chrysichthys nigrodigitatus* populations in some Nigerian coastal waters. *International Journal of Fisheries and Aquatic Studies* 7(2): 254-257

Uyoh, E. A., Ntui, V.O., Ibor, O.R., Adilieje., C.M., Udo, M., Opara, C., Ubi, G. & Ama-Abasi, D., (2020). Molecular Characterization of Two Catfish Species (*Chrysichthys nigrodigitatus* and *Chrysichthys auratus*) from the Cross River, Nigeria, Using Ribosomal RNA and Internal Transcribed Spacers. *Annual Research and Review in Biology*. 35(2): 13-24. https://doi.org/10.9734/jalsi/2020/v23i130144

Zardoya, R. & Meyer, A. (1997). The Complete DNA Sequence of the Mitochondrial Genome of a "Living Fossil," the Coelacanth (Latimeria chalumnae). Genetics Society of America *Genetics* 146; 995-1010