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***Research Article***

# Genetic Diversity Assessment of Three Populations of *Chrysichthys nigrodigitatus* (Lacepede, 1803) in Southwestern Nigeria Using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

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**ABSTRACT**
*Chrysichthys nigrodigitatus*, a highly valued food fish, was examined for its genetic diversity across three different water bodies (Epe lagoon, Asejire reservoir, and Igbokoda river) in southwestern Nigeria, using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis. Samples from each population were analyzed following standard protocols. The protein bands obtained as outputs of the SDS-PAGE analysis were scored as present (1) or absent (0) to create a binary matrix which was used to determine the following indices of genetic diversity: number of alleles, percentage polymorphism, heterozygosity, and Shannon's information index using GenAlEx v. 6.5. A dendrogram was constructed by the unweighted pair group method with arithmetic average (UPGMA) using MEGA software. Results revealed a low level of genetic diversity in the three populations; however, genetic diversity was relatively higher in the Asejire reservoir population compared to the Igbokoda river and Epe lagoon. The UPGMA dendrogram indicated that the Asejire reservoir and Epe lagoon populations are genetically closer, forming a single cluster, while the Igbokoda river population is distinct from them, suggesting that this population is genetically different. The study concluded that the low genetic diversity observed in the three populations of *C. nigrodigitatus* indicates an urgent need for monitoring and conservation efforts to ensure their long-term survival and sustainable yields.

**Keywords:** Electrophoresis; Gel Electrophoresis; Genetic diversity; Polymorphism; Protein bands

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## INTRODUCTION

*Chrysichthys nigrodigitatus* (Lacepède, 1803), commonly known as the silver catfish, is a preferred fish in developing nations such as Nigeria and several other West African countries due to its affordability and high nutritional value (Onunkwor *et al*., 2023). *C. nigrodigitatus* fisheries is very significant in Nigeria and much of West Africa as they represent a sizable trophic level in the ecosystem following their introduction in numerous man-made lakes and reservoirs, including the Kivu, Kariba, and Tiga dams (Francis and Elewuo, 2012). *Chrysichthys nigrodigitatus* is one of the few fish species that possesses advantageous traits suitable for aquaculture. It can consume any food that is available in its environment, including worms, leaves, roots, crabs, insects, phytoplankton, zooplankton, and fish particles (Amachree *et al.,* 2025). Also, the species can thrive in waterbodies with different salinities ranging from freshwater to brackish (Ukenye *et al*., 2019; Adilieje *et al.*, 2020).

Despite the enormous potential of *C. nigrodigitatus* for aquaculture and its high economic value, its breeding is still struggling, and the culture largely depends on captured fry from the wild (Trushenski *et al.,* 2010). Consequently, advancing breeding and conservation strategies for *C. nigrodigitatus* populations requires a deeper understanding of their genetic diversity. Genetic diversity is the variation in alleles of different traits in a population. These variations enhance the persistence and adaptability of individuals in a population. When a species population has sufficient genetic diversity, some individuals are likely to possess alleles that will enable them to withstand pressure, survive, and reproduce, thereby reducing the risk of extinction for that population. A reduction in genetic diversity is known to limit the capacity of populations to adapt and evolve in response to environmental changes (Awodiran *et al*., 2016). Homogeneous populations with similar alleles will be equally vulnerable to biological stress and environmental changes such as major temperature fluctuations, floods, and droughts, among other factors. Genetic diversity within a population can be assessed using various metrics, such as the number of alleles, heterozygosity, Shannon's information index, analysis of molecular variance (AMOVA), percentage of polymorphic loci, etc. These measurements are derived from outputs of molecular markers that highlight variations in DNA sequences at specific locations in the genome (Avise, 2004).

Protein electrophoresis has historically been the main method used by geneticists to evaluate genetic diversity at both population and species levels. This technique has been widely employed and remains valuable (Chauhan and Rajiv, 2010; Muhammad *et al*., 2018). It is preferred when other high-resolution markers, such as microsatellites, Single Nucleotide Polymorphisms (SNPs), and Restriction Fragment Length Polymorphisms (RFLPs), are not affordable. The SDS-PAGE electrophoretic method can reveal variations in fish genomes based on their mass or charge and does not require prior knowledge of the fish genome (Verspoor *et al*., 2005). Researchers have extensively used protein electrophoresis as a dependable approach for analyzing intra- and interspecific variation. Similar species can be easily distinguished based on their serum protein electrophoretic profiles (Akinwande *et al*., 2012). Abu-Almaaty *et al*. (2020) utilized SDS-PAGE to examine the genetic similarities among several cyprinid fish: *Pethia nigrofasciatus*, *Barbonymus schwanenfeldii*, *Puntius tetrazone*, and *Brachydanio rerio* from fish farms in Damietta. Ukenye *et al*. (2020) studied the genetic diversity of *Tilapia guineensis* and *Sarotherodon melanotheron* populations from major coastal waterbodies in Nigeria using SDS-PAGE. Reports on the genetic status of *C. nigrodigitatus* populations in key southwestern water bodies such as Epe lagoon, Asejire reservoir, and Igbokoda river remain scarce. Therefore, this study aims to use the SDS-PAGE electrophoretic technique to provide preliminary insights into the genetic diversity of *C. nigrodigitatus* populations from these three water bodies.

## MATERIALS AND METHODS

### Study Areas

Three different waterbodies representing divergent habitats of *C. nigrodigotatus* were selected for this study (Epe lagoon, Asejire reservoir, and Igbokoda river) (Fig.1).

### Fish Sampling

Being a preliminary study, ten freshly dead samples of *C. nigrodigitatus* were collected from each location with the assistance of local fishermen, making a total of thirty (30) samples. The samples were transported on ice to the Biotechnology laboratory, Animal Science Department, Obafemi Awolowo University, for further analysis. Identification of the fish samples was done using keys prepared by Paugy *et al*. (2003).

### Protein Profile

The muscle from each fish sample was carefully dissected, placed in separate, properly labelled specimen bottles, and stored at -20 °C. The SDS-PAGE technique (12%) was performed following the procedure described by Laemmli (1970). Protein molecules were separated based on their molecular weight to generate a protein profile reflecting the sample's protein content. This was achieved using a vertical electrophoresis apparatus, which separates the components based on their differential responses to an electric current (Kaimudin, 2020). A molecular weight marker kit (Novagen by Merck) (10-100 KDa) was used for the determination of the molecular mass of each protein. The electrophoresis set was operated at 100-120 V for 60-90 minutes. Coomassie Brilliant Blue R-250 solution was used to stain the gel plate,

while acetic acid was used to destain it. The protein bands were visualised and documented using a gel imager. The molecular weight of each band was determined by comparing it with standard protein bands on the molecular ladder.

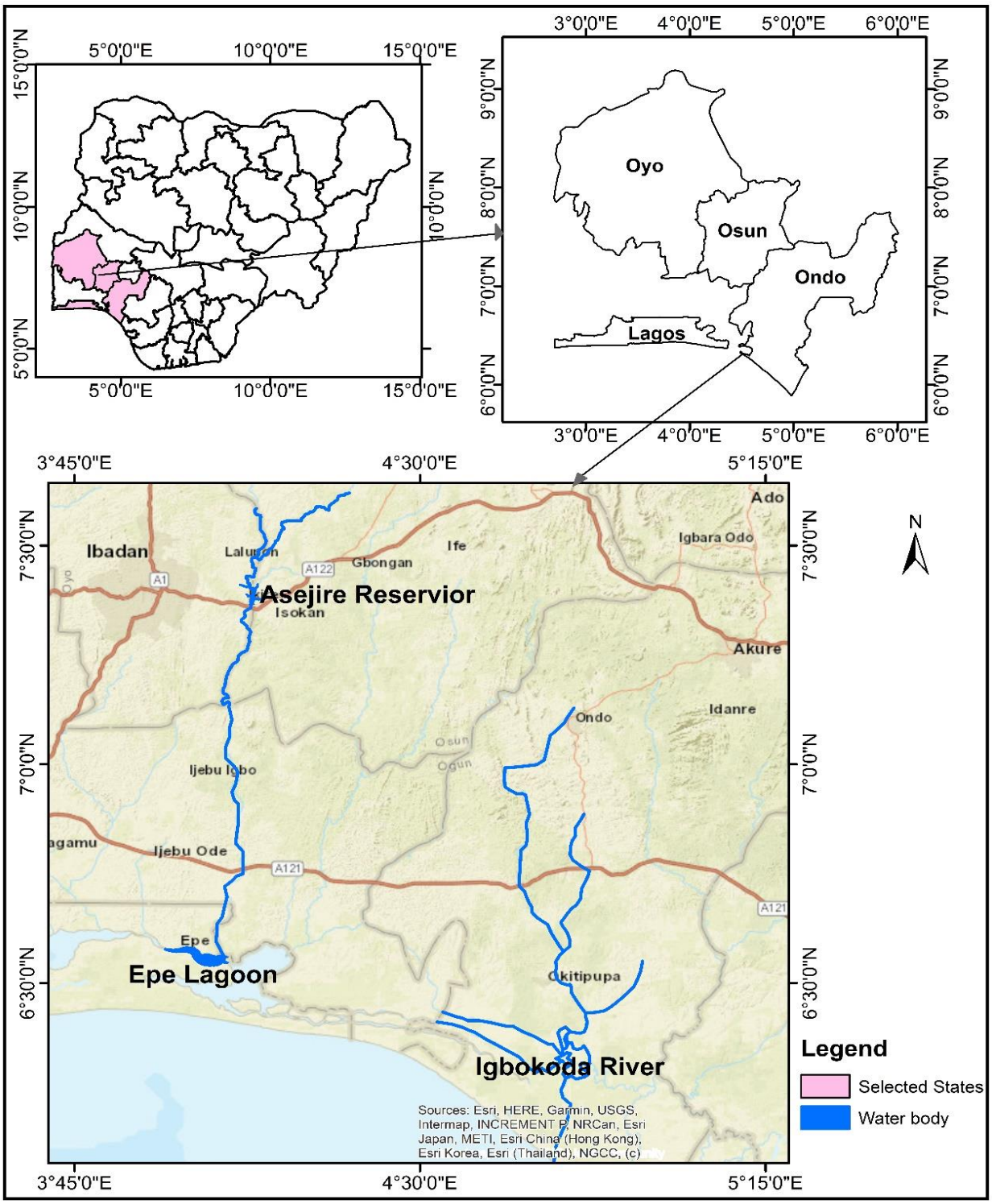


**Fig. 1: Map of Southwestern Nigeria showing the sampling locations: Epe lagoon, Asejire reservoir, and Igbokoda river**

**Data Analysis**

The data used for the genetic diversity assessment were generated from the protein bands documented in each population, which were scored as the presence of bands (1) or absence of bands (0) using PyElph 1.2 (Pavel and Vasile, 2012). The binary data obtained were used to determine the number of polymorphic bands, percentage polymorphisms, number of alleles, heterozygosity, Analysis of Molecular Variance (AMOVA), and Shannon information index using GenAlEx v. 6.5 (Peakall and Smouse, 2006, 2012). The genetic relationships among the populations were determined using the unweighted pair group method with arithmetic mean (UPGMA) based on Nei's genetic distance on MEGA software (Tamura *et al*., 2007).

## RESULTS

The electrophoresis gel images showing the protein profiles of the *C. nigrodigitatus* samples from the three study populations are presented in Figure 2. The average number of protein bands scored in the *C. nigrodigitatus* populations from the Igbokoda river, Asejire reservoir, and Epe lagoon was 12, 13, and 11, respectively. The *C. nigrodigitatus* populations from Asejire reservoir had the highest mean number of alleles (1.31) and mean effective alleles (1.77). while comparatively lower but similar values were observed in the Epe Lagoon and Igbokoda River populations, with mean numbers of alleles and effective alleles of 1.07 and 1.15, respectively. The mean heterozygosity values recorded were 0.08 for Epe Lagoon, 0.11 for Asejire Reservoir, and 0.10 for Igbokoda River (Table 1). The Shannon information index was highest in Asejire Reservoir (0.16), followed by Igbokoda River (0.13) and Epe Lagoon (0.11). The percentage of polymorphic loci across the three populations was 15.38%, 30%, and 23% in Epe Lagoon, Asejire Reservoir, and Igbokoda River, respectively. Analysis of molecular variance (AMOVA) revealed 58% genetic variation among the three populations and 42% within each population of *C. nigrodigitatus* studied (Fig. 3). The UPGMA dendrogram based on genetic distances indicated two major clades, demonstrating that the three populations of *C. nigrodigitatus* clustered into two groups. The first clade comprised the Asejire Reservoir and Epe Lagoon populations, while the Igbokoda population formed the second clade (Fig. 4).

**Table 1: Summary of the Indices of Genetic Diversity**

| Parameters | Populations (Mean±SE) | | |
|---|---|---|---|
| | **Igbokoda** | **Asejire** | **Epe** |
| Number of bands | 11 | 13 | 12 |
| Number of alleles | 1.07±0.18 | 1.31±0.13 | 1.07±0.14 |
| Effective number of alleles | 1.15±0.09 | 1.77±0.09 | 1.15±0.10 |
| Shannon information index | 0.13±0.07 | 0.16±0.07 | 0.11±0.07 |
| Heterozygosity | 0.10±0.05 | 0.11±0.05 | 0.08±0.05 |
| Number of polymorphic loci | 10 | 8 | 3 |
| Percentage polymorphisms | 23% | 30% | 15.38% |

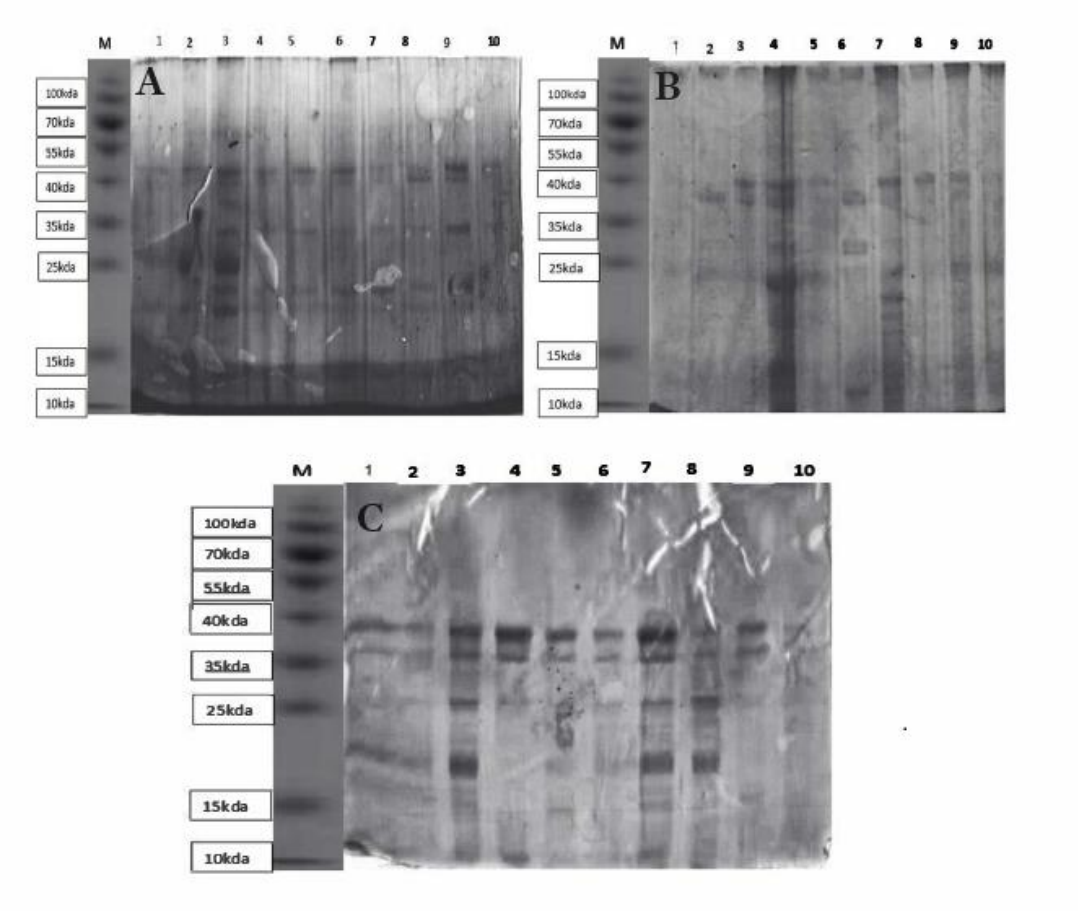


**Fig. 2: Electrophoresis gel images of ten *Chrysichthys nigrodigitatus* protein samples from (A) Epe Lagoon, (B) Asejire River, (C) Igbokoda River**

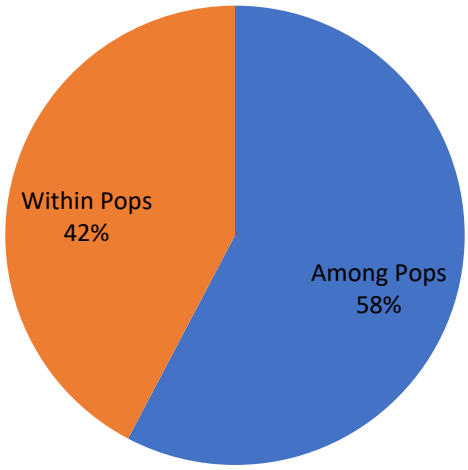


**Fig. 3: AMOVA showing percentage variation within and among the three populations of *C. nigrodigitatus***

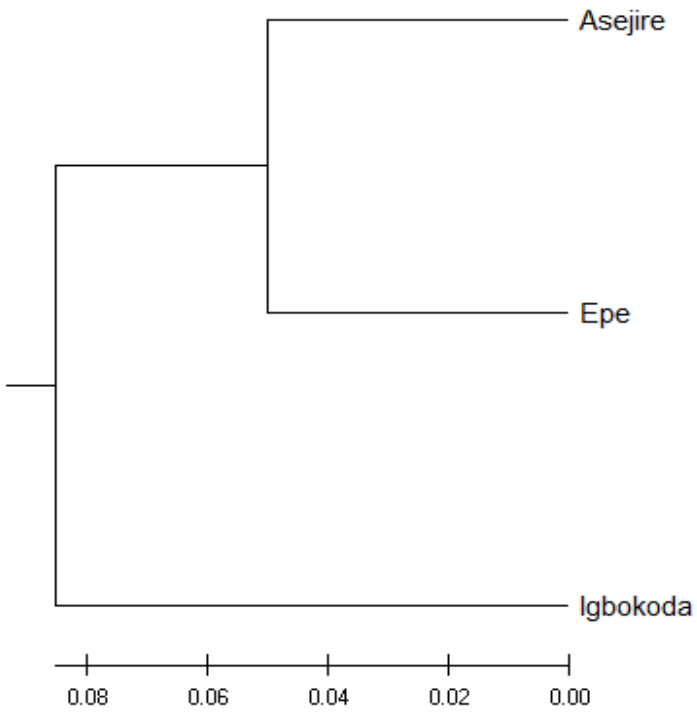


**Fig. 4: UPGMA dendrogram, showing the genetic distance among *Chrysichthys nigrodigitatus* populations**

## DISCUSSION

In this study, protein electrophoresis was used to assess the genetic diversity in three populations of *C. nigrodigitatus*. The SDS-PAGE analysis revealed intraspecific variation both within and among the three populations, as evidenced by the differences in band pattern. These differences were reflected in the variations in allele numbers, heterozygosity, and proportions of polymorphic loci, which had generally low values, indicating a low level of genetic diversity across the populations. This is further corroborated by the low values of Shannon's information index across the populations (0.11-0.16). Adilieje *et al.* (2020) pointed out that Shannon's information index serves as a measure of genetic diversity, whereby an index value of ≤ 1 indicates low diversity. The low genetic diversity recorded in the *C. nigrodigitatus* populations from Epe Lagoon, Asejire River, and Igbokoda River may be attributed to overexploitation in these waterbodies. The incidence of overfishing, which has been extensively documented in the three waterbodies (Fafioye and Oluajo, 2005; Soyinka and Ebigbo, 2012; Olawusi-Peters *et al*., 2015; Ogunremi *et al*., 2018; Omoike, 2021; Ipinmoroti and Iyiola, 2022), results in population decline, consequently eroding genetic diversity through a reduction in allelic richness (Allendorf *et al*., 2008; Gandra *et al*., 2021). The decline in genetic diversity among exploited fish species may hinder their ability to adapt to environmental changes, thereby reducing their evolutionary potential (Oladimeji *et al*., 2021). The low genetic diversity in the *C. nigrodigitatus* populations reported in this study aligns with the findings of Adilieje *et al*. (2020), who documented low genetic diversity in the Cross River populations of *C. nigrodigitatus* based on microsatellite markers. Song *et al*. (2011) also recorded low levels of genetic diversity in *C. nigrodigitatus* populations from the Niger Delta region based on AFLP analysis. Nwafili and Gao (2016) found less genetic variation in the *C. nigrodigitatus* of the Cross River compared to those from other Nigerian coastal rivers, based on the

analysis of 443 base pair fragments of the mtDNA control region. Uyoh *et al*. (2020) similarly observed low genetic diversity in *Chrysichthys* species using ribosomal RNA and the internal transcribed spacer region. All these reports highlight the urgency of prioritising research and monitoring efforts for this species, alongside implementing immediate conservation measures. Meanwhile, in this study, genetic diversity was relatively higher in the Asejire reservoir population compared to the Igbokoda River and Epe Lagoon, as indicated by the higher values of heterozygosity, number of alleles, and percentage polymorphism. The relatively high level of genetic diversity in the Asejire population of *C. nigrodigitatus* suggests that this population is more genetically viable than the other two populations (Igbokoda River and Epe Lagoon), although not sustainable.

The UPGMA dendrogram revealed that the Asejire reservoir and Epe Lagoon populations are genetically closer, as they formed a single cluster, while the Igbokoda River population is distinct from them. The genetic relatedness between Asejire and Epe Lagoon indicates considerable gene flow between the two populations, given that the two water bodies are interconnected. Asejire Reservoir is an impoundment of the Osun River, which discharges into the Ogun River (Oyedotun, 2011). The Ogun River, after collecting waters from various tributaries, flows southward and ultimately discharges into the Lagos Lagoon, which is closely linked to the Epe Lagoon (Agoro and Acct, 2021). Several smaller rivers and creeks interconnect the Lagos and Epe Lagoons. Therefore, although the connection is indirect, the Osun River's flow into the Ogun River ultimately contributes to the waters flowing into the Epe Lagoon through the Lagos Lagoon network (Uwadiae, 2009). Igbokoda River does not share any direct hydrological connection with either the Asejire reservoir or Epe Lagoon, thus there is no premise for gene flow among their fish. This lack of connectivity likely contributes to the genetic distinctiveness of the Igbokoda population of *C. nigrodigitatus* observed in this study. The observed pattern of genetic differentiation has important implications for conservation and management, suggesting that the distinct Igbokoda population be treated as a separate management unit, while the connected Asejire-Epe populations be managed as a single unit. The Asejire population could serve as a potential genetic reservoir for the *C. nigrodigitatus* species if managed effectively.

## CONCLUSION

The protein-based genetic analysis of *C. nigrodigitatus* populations from Epe lagoon, Asejire reservoir, and Igbokoda river revealed a low level of genetic diversity within each population. It also indicated a reasonable degree of genetic differentiation among the three populations. The Igbokoda river population of *C. nigrodigitatus* is genetically distinct, while the Epe lagoon and Asejire reservoir populations are genetically related. This study has implications for the management and conservation of the fish populations. However, further studies involving larger sample sizes and high-resolution molecular markers such as microsatellites and SNPs are recommended to establish the genetic structure of the studied *C. nigrodigitatus* populations.

### AUTHOR CONTRIBUTIONS

O.T.E. conceptualized the study; O.K.O. obtained the samples and conducted laboratory analyses; O.T.E. supervised laboratory work and wrote the original draft of the manuscript; O.T.E. and A.M.A. provided laboratory guidance; O.T.E., O.K.O., and A.O. performed data analyses, prepared figures, and interpreted results; O.T.E., O.K.O., A.O., and A.M.A. critically reviewed and edited the manuscript; all authors reviewed and approved the final draft.

### CONFLICT OF INTERESTS

The authors declare no conflict of interest associated with this study.

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