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Morphological Characterization and DNA Barcoding of Lutjanus Agennes (African Red Snapper) from Escravos and Qua Iboe Estuaries

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Abstract

Estuaries, as vital nursery grounds for marine species, are vulnerable to over-exploitation and environmental degradation. In this study, the morphological and genetic diversity of *Lutjanus agennes* (African red snapper) populations from the Escravos and Qua Iboe estuaries in Nigeria were evaluated. A total of 200 fish samples, comprising 100 randomly selected specimens from each estuary, were collected, and detailed morphometric measurements, including total length, standard length, body depth, and fin dimensions, were recorded. Muscle tissues were also collected for DNA barcoding analysis. Notably, fish from the Qua Iboe estuary were significantly larger, with an average total length of 36.91 ± 2.76 cm, compared to 32.12 ± 2.38 cm in the Escravos population. DNA barcoding, utilizing the cytochrome c oxidase subunit I (COI) gene, confirmed species identity. The results of the Basic Local Alignment Search Tool (BLAST) showed a percentage identity ranging from 95.25% to 100% and sequence lengths between 619 and 646 base pairs. These findings revealed minimal genetic divergence between the populations, consistent with the low genetic variation typically observed in estuarine fish due to gene flow facilitated by migration. Although genetically connected, the populations may experience different ecological pressures, as indicated by the larger body size of fish in the Qua Iboe estuary. This research highlights the utility of DNA barcoding for accurate species identification and emphasizes the importance of incorporating genetic and morphometric data into fisheries management to ensure the sustainable exploitation of *L. agennes* in Nigeria's estuarine ecosystems.

Keywords*: *Lutjanus agennes, Morphological diversity, Genetic diversity, Estuarine ecosystems, Fisheries management.

1.0 Introduction

Nigeria is blessed with numerous estuaries connecting different rivers to the Atlantic Ocean. Estuaries and near-coastal ecosystems play a vital role in supporting major fisheries by serving as crucial nursery grounds for young fish and invertebrates. These areas are essential for the growth and development of many marine species, contributing significantly to the overall productivity of the

world's fisheries. Estuaries exhibit high rates of primary production due to their role as recipients of land-based nutrients. Moreover, nutrient recycling is heightened in estuaries with shallow, organic-rich sediments that support diverse benthic fauna and microbial communities, facilitating efficient nutrient utilization (Jordan and Mark, 2012). These estuaries are endowed with numerous categories of fin fishes and shell fishes.

The diverse fish species in the estuaries, just like in other water bodies appear in various shapes and sizes. Traditionally, morphological characteristics such as body shape, colouration, fin counts, and scale patterns have been used to identify and classify fish species. Morphological characters in fish which are classified into morphometric [measurable structures on fish bodies] and meristic [countable body structures] are generally considered authentic and the easiest methods for the identification of specimens (Olopade *et al*., 2018; Eriegha and Eyo, 2023). By meticulously examining aspects such as body dimensions, fin configurations, and colour patterns, researchers can gain valuable insights into the phenotypic diversity and potential adaptations of fish species to their environment (Eriegha *et al*., 2024a). While morphological data can be informative, there are limitations to relying solely on visual characteristics for species identification. Therefore, it is essential to complement morphological data with genetic data in fish species identification for several reasons such as cryptic species identification, taxonomic uncertainties and species delimitation. One of such complementary genetic techniques is the use of DNA barcoding (Hajibabaei *et al*., 2007).

DNA barcoding offers a cutting-edge approach to unraveling the genetic makeup of organisms, providing a molecular fingerprint that can aid in species identification and phylogenetic analyses. By sequencing specific regions of the mitochondrial genome, such as the cytochrome c oxidase subunit I (COI) gene, researchers can discern genetic variations and delineate distinct genetic lineages among populations of fish species (DeSalle and Goldstein, 2019). DNA barcoding has gained global support for its rapid, accurate, cost-effective, and widely applicable applications, particularly in fish species (Packer *et al*., 2009). Genetic and morphological studies of fish species are essential for understanding its population structure, adaptation mechanisms, and evolutionary history. Genetic characterization offers insights into the gene flow, population dynamics, and the potential impacts of environmental changes and human activities on the species. Concurrently, morphological characterization helps in identifying variations and adaptations that may be linked to habitat-specific pressures or selective factors (Collin and Fumagalli, 2011).

The Lutjanidae family, encompassing the red snappers, is renowned for its ecological and economic significance in tropical and subtropical marine environments. Among its members, *Lutjanus agennes*, commonly referred to as the African red snapper, stands out due to its prominence in artisanal fisheries and its role as a predator within estuarine and nearshore ecosystems. *Lutjanus agennes* with their streamlined bodies and razor-sharp teeth, play a crucial role in the food web of these estuarine ecosystems, influencing the dynamics of predator-prey relationships and contributing to the overall biodiversity of the region (Martinez-Andrade, 2003). Evidence to date suggests that *L. agennes* are being over-exploited in Nigeria (Olopade and Dienye, 2018). The species' distribution spans the western and central regions of the African coast, including key estuarine systems such as the Escravos

and Qua Iboe Estuaries in Nigeria. These estuaries, characterized by their complex hydrological and sedimentological dynamics, provide crucial habitats for many marine species. The Escravos and Qua Iboe Estuaries, with their distinct ecological conditions, provide a unique opportunity to examine *L. agennes* within varied estuarine environments. The Escravos Estuary, with its brackish waters and significant freshwater inflow, contrasts with the Qua Iboe Estuary, which is influenced by different hydrological patterns and sediment deposition processes. Investigating the genetic diversity and morphological adaptations of *L. agennes* in these contrasting estuarine systems can reveal patterns of local adaptation and population differentiation. This study aims to elucidate the genetic and morphological diversity of *L. agennes* from these estuaries, utilizing molecular markers to assess genetic variation and morphological metrics to understand phenotypic diversity. By integrating these approaches, the research seeks to provide a comprehensive understanding of how estuarine environmental factors influence the genetic and morphological characteristics of *L. agennes*, contributing to broader knowledge of its biology and informing conservation and management strategies in these critical habitats.

2.0 Materials and Methods

2.1 Description of the study area

This study was carried out in two water bodies that have connections to the Gulf of Guinea, Atlantic Ocean. They are the Escravos and Qua Iboe estuaries (Figure 1). Both estuaries are vital components of the Niger Delta's estuarine system, supporting diverse ecosystems and providing essential services. While they share similarities, such as their significance as fish nurseries and their vulnerability to environmental degradation, they also exhibit distinct hydrological and ecological characteristics due to their varying geographic and environmental contexts.

The Escravos Estuary, located on the southern coast of Nigeria, is a prominent and ecologically significant estuarine system within the Niger Delta region. The Escravos Estuary extends approximately 75 kilometers inland, featuring a series of channels, mudflats, and mangrove swamps. Its hydrology is influenced by both seasonal and perennial rainfall, which impacts freshwater inflow and salinity gradients within the estuary. The estuary experiences significant tidal fluctuations, with daily tidal ranges contributing to its highly variable salinity and sediment transport processes (Nwaogbe *et al*., 2023). The estuary’s water is predominantly brackish, with salinity levels varying from oligohaline to mesohaline depending on the seasonal influx of freshwater and tidal mixing. The Escravos Estuary faces several environmental challenges, including pollution from oil and gas activities, deforestation, and habitat degradation (Eriegha *et al*., 2024b). The Niger Delta region, including the Escravos Estuary, is known for its oil-rich deposits, which has led to significant environmental impacts such as oil spills and contamination (Eriegha and Sam, 2020; Berezi *et al*., 2024). Additionally, urbanization and industrial activities contribute to water quality issues, affecting the health of the estuarine ecosystem.

The Qua Iboe Estuary is located on the southeastern coast of Nigeria, also within the Niger Delta region. It is formed by the Qua Iboe River as it flows into the Atlantic Ocean, creating another critical estuarine environment in the region (Essien-Ibok and Isemin 2020). The Qua Iboe Estuary spans

approximately 50 kilometers and features a complex network of channels, lagoons, and wetlands. It is influenced by both riverine discharge and tidal action, with water salinity varying significantly from freshwater to brackish conditions. The estuary is subject to seasonal variations in river flow, which impact the distribution of salinity and sediment in the area. Tidal patterns also play a significant role in shaping the estuarine environment, contributing to the dynamic nature of its hydrology. The Qua Iboe Estuary also faces environmental pressures similar to those experienced by the Escravos Estuary. Issues include pollution from industrial activities, deforestation, and habitat loss. The region's oil exploration activities have led to contamination and habitat degradation, impacting the estuary's ecological health. Additionally, agricultural expansion and population growth contribute to land use changes and potential impacts on water quality and habitat integrity.

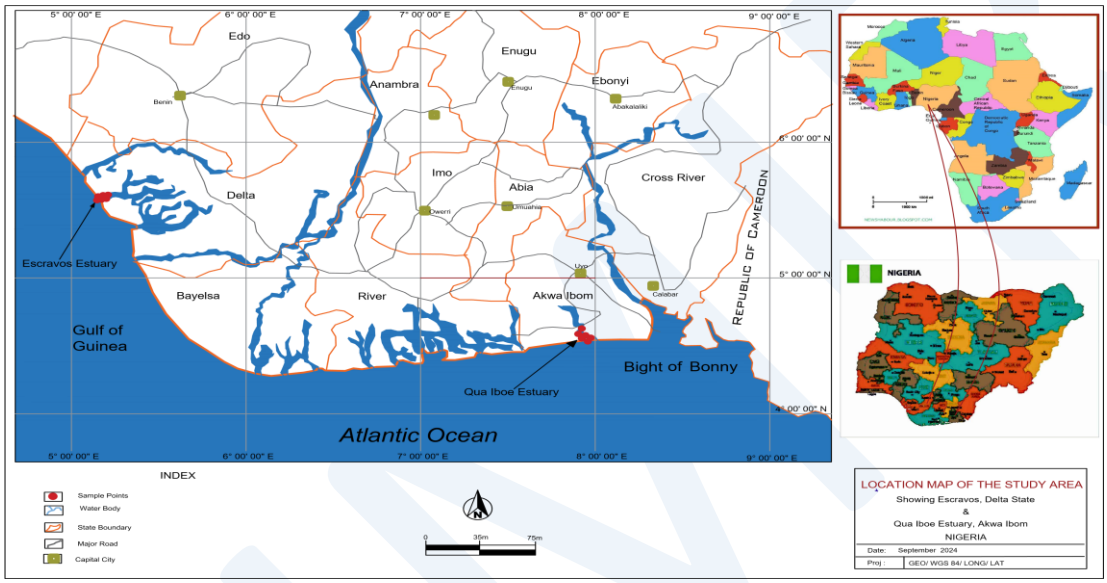


Figure 1. Map showing Escravos and Qua Iboe Estuaries

2.2 Sampling

A total of 200 fish samples, consisting of 100 randomly selected specimens from each estuary, were collected with the assistance of indigenous fishers using nets with mesh sizes ranging from 20 to 50 mm. Morphological measurements and meristic counts were conducted on fresh samples after thorough species identification, following the method outlined by Allen (1985).

2.3 Morphometric Measurements

Measurement for length (cm) and weight (g) were determined using a ruler and an electronic weighing balance (OHAUS Corporation) respectively. Seventeen (17) morphometric measurements and 8 meristic counts were made on fresh samples of *E. fimbriata* from the three water bodies. Except for weight, all other 16 morphometric measurements (described in Table 1) were measured to the nearest 0.1cm using the meter rule. Length measurements were taken parallel to the antero-posterior body axis.

Table 1. The description of the morphometric characteristics of *L. agennes* measured in the study.

S/N	Morphometric Measurement	Code	Description
1	Total Length	TL	Greatest length of the fish from its anterior extremity to the end of its tail fin.
2	Standard Length	SL	Greatest length of a fish from its anterior extremity to the hidden base of its median tail fin ray
3	Forked Length	FL	measurement taken from the anterior-most part of the fish to the end of the median caudal fin rays
4	Body Depth	BD	the greatest distance from the dorsal midline to the ventral midline of the body
5	Dorsal Fin Base	DFB	length from the start point of the dorsal fin to the posterior terminus of the base
6	Caudal Peduncle Length	CPL	This is the length from the posterior terminus of the anal fin base to the center of the caudal fin base.
7	Caudal Peduncle Depth	CPD	This is the minimum height of the caudal peduncle
8	Gape Width	GW	Distance between the anterior most upper jaw and lower jaw
9	Pre-Orbital Length	PrOL	from the snout tip to the anterior margin of the orbit
10	Eye Diameter	ED	Horizontal diameter between the fleshy margins of the orbit; expressed as parts of head length.
11	Post-Orbital Length	PoOL	The measurement taken from posterior margin of eye orbit to the end of the operculum
12	Head Length	HL	Measured from the start of the mouth or snout to the most posterior portion of the operculum.
13	Pelvic Fin Length	PFL	From anterior origin to posterior tip of pelvic-fin
14	Pectoral Fin Length	PCFL	From base to tip of pectoral-fin.
15	Anal Fin Length	AFL	From anterior origin to posterior tip of anal-fin.
16	Anal Fin Base	AFB	Length from the start point of the anal fin to the posterior terminus of the base. The notes for this length are the same as above.

2.4 Determination of length-weight Relationships and condition Factor

The raw data for all the specimens were log-transformed to reduce the effects of non-normality. The relationship between the length and weight of a fish is usually expressed by the equation described by Ricker (1973); $W = a.L^b$. The values of constant "a" and "b" were estimated from the log-transformed values of length and weight i.e. log W=log a + b log L, via least square linear regression. The condition factor (K values) was calculated, using Fulton's condition factor formula (Pauly, 1984); $K = 100W/L^3$.

2.5 Genetic analysis

A total of 10 fish samples were taken randomly from the two water bodies, and then photographed for documentation (Figure 2). Fish samples were dissected and tissues aseptically collected. The muscle tissues were preserved in saline water, kept in an iced pack, and transported to Inqaba Biotechnology, Ibadan for DNA extraction, sequencing, and sequence analysis of COI barcode gene region. DNA extraction was carried out using the Quick-DNA™ Miniprep Plus Kit (Zymo Research®) and following the manufacturer's protocol for solid tissues. The fish target region was amplified using the OneTaq Quick Load 2X Master Mix (NEB, Catalogue No: M9486), nuclease-free water, and template DNA with primers for forward and reverse reactions. Primers originally developed for marine and estuarine fish species were used for the amplification. The pair of primers is as follows (Ward *et al.*, 2009): FishF1-5′TCAACCAACCACAAAGACATTGGCAC3'and FishR1-5′TAGACTTCTGGGTGGCCAAAGAATCA3'. After thermal cycling, the amplified DNA was stored at -20°C before use as described by Shokrallan *et al.* (2010).

Gel electrophoresis was carried out using the methods described by Lucentini *et al.* (2006). Purified PCR products were directly sequenced in both forward and reverse directions with an automated DNA sequencer (ABI 3730) following the manufacturer's instruction. The nucleotide sequences were viewed and confirmed by eye using SeqManTMII (DNASTAR Lasergene 7). They were aligned in MEGA 7.0 using ClustalW (Kumar *et al*., 2018) with default parameters. The aligned sequences were translated into amino acids to check for premature stop codons and to confirm that the open reading frame was maintained in the protein-coding loci. Basic Local Alignment Search Tool (BLAST) was the program used to confirm the identity of the amplified sequences. This was achieved by comparing nucleotide sequence data that were produced from the genomic DNA to the sequences database at Genbank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Additionally, the FASTA sequences of each of the *COI* sequences were inputted into the BOLD identification request tool (http://www.boldsystems.org/index.php/IDS_OpenIdEngine). Sequences were submitted for species-level identification under the option "Species Level Barcode Record" following Hebert *et al*. (2003).

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal, 1973). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980). The generated sequence data from the DNA barcoding were

aligned and subjected to phylogenetic tree reconstruction using Maximum Likelihood (ML) and Kimura 2-parameter (K2P), with bootstrap 1000 replicates. Genetic diversity distances based on K2P were analyzed using MEGA X.



Figure 2. Showing Freshly caught sample of L. agennes

2.6 Data Analysis

Morphometric characters were expressed as mean ± standard error of the mean. Mean values of the different characters from the three water bodies were compared using a one-way analysis of variance (ANOVA) followed by Tukey's test. The significance level adopted was 95% ($P < 0.05$). Meristic counts were presented as the range. Morphometric data were further subjected to principal component analysis (PCA) to reveal patterns in measured correlated variables. Statistical analysis was performed using the Statistical Package for the Social Sciences (Version 20).

3.0 Results and Discussion

3.1 Morphometric Characteristics of *L. agennes*

The mean and standard error of the mean of 17 morphometric characteristics of *L. agennes* from Escravos and Qua Iboe estuaries is shown in Table 2. The analysis of the morphometric parameters from the two eater bodies reveals variations across the two populations. However, statistical analysis using Student's t-test indicated that these differences were not significant ($p > 0.05$). These findings align with similar studies on the morphological plasticity of estuarine fish populations, suggesting that the observed variations may be influenced by environmental factors (Eriegha *et al*., 2024a). The average weight of samples in the Qua Iboe estuary was significantly higher (1009.98 ± 121.14 g) compared to those from the Escravos estuary, which averaged 763.07 ± 97.26 g. This marked difference in body weight suggests that *L. agennes* in the Qua Iboe region may have access to more abundant or nutritionally superior food resources, better water quality, or more favourable environmental conditions that support greater growth. The difference in weight could also be attributed to ecological factors such as reduced predation pressures or more stable conditions in the Qua Iboe estuary (Eklöv and Svanbäck, 2006).

The total length of fish from the Qua Iboe estuary was also greater, with an average of 36.91 ± 2.76 cm, compared to the 32.12 ± 2.38 cm recorded in the Escravos estuary. This trend was consistent for forked length and standard length, where Qua Iboe snappers averaged 33.27 ± 2.53 cm and 30.80 ±

2.19 cm, respectively, while their Escravos counterparts averaged 29.90 ± 2.47 cm for forked length and 27.32 ± 2.46 cm for standard length. The larger body sizes observed in Qua Iboe snappers may be indicative of better living conditions in this estuary. These conditions might include factors such as higher primary productivity, greater prey abundance, or better oxygen levels, all of which could contribute to the enhanced growth and body size of the fish. Furthermore, larger body sizes could also be an adaptation to local environmental pressures, such as stronger currents or deeper waters, which may require larger and more streamlined body shapes for efficient movement. Several studies on fish populations in estuarine environments have highlighted the role of ecological factors such as salinity, food availability, and habitat structure in shaping fish morphology (Pelage *et al*., 2021). A significant ($p < 0.05$) variations in morphological parameters of Red Snapper have been reported in the Northern Coast of Aceh waters of Indonesia (Rahayu *et al*., 2023). Although the present study found no significant differences between morphometric parameters of *L. agennes* populations from Escravos and Qua Iboe, the variations observed may similarly reflect differences in environmental conditions between the two estuaries. However, it appears that these variations are not substantial enough to result in statistically significant differences, possibly due to the inherent plasticity of estuarine fish populations.

The body depth of Qua Iboe snappers was recorded as 11.51 ± 0.90 cm, compared to 10.23 ± 0.89 cm in the Escravos population. Body depth is an important morphological trait, often linked to swimming efficiency, buoyancy control, and habitat utilization. A greater body depth could suggest that *L. agennes* are better adapted to environments requiring more streamlined body shapes for efficient swimming, particularly in more open or fast-flowing waters. Additionally, body depth is also related to the overall robustness and health of the fish, with deeper bodies often indicating better nutrition and more favorable environmental conditions (Rubio-Gracia *et al*., 2020).

Fin dimensions are crucial for understanding the swimming performance and ecological adaptations of fish species. In this study, red snapper from Qua Iboe showed larger dorsal fin base (DFB) lengths, averaging 14.85 ± 1.09 cm, compared to 13.25 ± 1.11 cm for those from Escravos. This trend was observed across other fins as well, with Qua Iboe snappers having longer pelvic fin lengths (6.07 ± 0.37 cm) compared to Escravos snappers (5.52 ± 0.38 cm), and longer pectoral fin lengths (8.29 ± 0.65 cm vs. 7.37 ± 0.65 cm). The larger fin sizes observed in Qua Iboe snappers suggest adaptations to environmental conditions that may require more efficient or powerful swimming, such as areas with strong currents or varying water depths. Larger fins could also contribute to better maneuverability and stability in the water, allowing fish to navigate their environment more effectively (Webb *et al*., 2015). Fin size could also be influenced by feeding behaviors; fish in more open or competitive environments may require larger fins for fast bursts of speed to capture prey or evade predators. The caudal peduncle, which connects the body to the tail fin, plays an essential role in propulsion. Qua Iboe snappers had both longer and deeper caudal peduncles, with lengths and depths averaging 5.57 ± 0.44 cm and 5.55 ± 0.51 cm, respectively, compared to the 4.89 ± 0.50 cm and 4.45 ± 0.59 cm recorded in the Escravos population. The larger caudal peduncle in Qua Iboe snappers might contribute to more powerful swimming capabilities, enabling faster movements through the water. This morphological trait could reflect the need for enhanced swimming performance, either to cope

with strong currents or to capture fast-moving prey. In addition, a larger caudal peduncle may indicate greater energy storage in the caudal muscles (Blickhan and Cheng, 1994), which could be advantageous for fish living in environments where food availability fluctuates.

Differences were also observed in the head and eye dimensions of the fish from the two water bodies. Qua Iboe snappers had larger head lengths (11.26 ± 0.92 cm vs. 9.97 ± 0.91 cm) and greater post-orbital lengths (6.11 ± 0.54 cm vs. 5.37 ± 0.53 cm). Similarly, their pre-orbital lengths and eye diameters were larger, averaging 3.86 ± 0.30 cm and 2.21 ± 0.12 cm, respectively, compared to 3.43 ± 0.31 cm and 2.01 ± 0.14 cm in Escravos samples. These larger head and eye sizes in Qua Iboe snappers could reflect ecological adaptations to their feeding strategies or habitat conditions. Larger eyes might be an adaptation to environments with lower light availability, allowing for better visual acuity in murky or deep waters. Similarly, larger head dimensions could be related to feeding strategies, with bigger jaws and greater bite force allowing Qua Iboe snappers to exploit a broader range of prey items or larger prey species (Gidmark *et al*., 2019).

The findings of this study suggest that despite the observed morphometric variations between the two estuarine environments, the lack of significant differences may indicate that *L. agennes* populations in Escravos and Qua Iboe share similar ecological niches. This is consistent with studies by Langerhans *et al*. (2003), which reported that fish populations within the same geographic region often exhibit minor morphological differences due to overlapping ecological habitats. The ability of this species to thrive in diverse environments likely reflects their adaptability and resilience to different environmental pressures, which could explain why no significant morphometric differences were found in this study. However, the observed variations, though not statistically significant, may still hold biological importance. Subtle morphological differences could be indicative of local adaptations that are not yet fully expressed or detectable at the sample size used in this study.

Table 2. Mean values of the morphometric characteristics of *L. agennes* from Escravos and Qua Iboe estuaries (Mean ± S.E)

Measurement/Count	Variable Code	Escravos	Qua Iboe
Weight	WT	763.07 ± 97.26	1009.98 ± 121.14
Total length	TL	32.12 ± 2.38	36.91 ± 2.76
Forked length	FL	29.90 ± 2.47	33.27 ± 2.53
Standard length	SL	27.32 ± 2.46	30.80 ± 2.19
Body depth	BD	10.23 ± 0.89	11.51 ± 0.90
Dorsal fin base	DFB	13.25 ± 1.11	14.85 ± 1.09
Caudal peduncle length	CPL	4.89 ± 0.50	5.57 ± 0.44
Caudal peduncle depth	CPD	4.45 ± 0.59	5.55 ± 0.51
Gape Width	GW	3.62 ± 0.26	3.92 ± 0.28
Pre orbital length	PrOL	3.43 ± 0.31	3.86 ± 0.30
Eye diameter	ED	2.01 ± 0.14	2.21 ± 0.12
Post orbital length	PoOL	5.37 ± 0.53	6.11 ± 0.54
Head length	HL	9.97 ± 0.91	11.26 ± 0.92
Pelvic fin length	PFL	5.52 ± 0.38	6.07 ± 0.37

Pectoral fin length	PCFL	7.37 ± 0.65	8.29 ± 0.65
Anal fin length	AFL	4.85 ± 0.44	4.47 ± 0.49
Anal fin base	AFB	3.38 ± 0.28	3.67 ± 0.28

*Means with different superscripts in the same row are significantly different ($p < 0.05$).

3.2 DNA Barcoding of *L. agennes*

The BLAST (Basic Local Alignment Search Tool) predictions affirmed that all fish samples collected from both estuaries corresponded to *L. agennes*. This molecular confirmation serves as a crucial step in species verification, as morphological identification methods often encounter challenges such as phenotypic plasticity and overlap in distinguishing features among closely related species. This is especially true in juvenile stages or environments where environmental stressors may cause morphological variations. Thus, the high accuracy of DNA barcoding proves advantageous in confirming species identity beyond visual characteristics (Imtiaz *et al*., 2017). The percentage identity of the COI sequences obtained in this study ranged from 95.25% to 100% when compared with reference sequences in the BLAST database. This high similarity score indicates a strong match between the genetic sequences of *Lutjanus agennes* from the Escravos and Qua Iboe estuaries and their corresponding species reference data. The relatively narrow range of variation further supports the hypothesis that the populations from both estuaries are genetically similar, with minimal divergence. These findings are consistent with prior research, where percentage identities for fish species typically fall within the 95%–100% range, confirming species-level identification (Eriegha *et al*., 2024c). A percentage identity above 95% is generally considered robust evidence for correct species assignment in barcoding studies (Ward *et al*., 2005). The relatively high sequence similarity across populations in the two estuaries suggests a lack of significant genetic differentiation, which may be attributed to ongoing gene flow facilitated by migration or larval dispersal between estuaries.

The sequence lengths of the DNA barcodes ranged from 619 to 646 base pairs (bp), a typical range for COI sequences in fish species barcoding studies (Fontes *et al*., 2024). The standardized target region for fish DNA barcoding is approximately 600–650 bp, as this length has proven sufficient for species-level resolution in most cases (Hebert *et al*., 2003). The query coverage of the sequences, which varied from 96% to 100%, further substantiates the quality of the sequences obtained in this study. High query coverage is essential in ensuring that the entirety of the sequence is aligned, providing a reliable comparison to reference data. Compared to earlier studies on marine fish species, the sequence lengths reported here are well within the expected range. For instance, Hajibabaei *et al*. (2007) demonstrated that sequences of approximately 600 bp yield high success rates for species discrimination in fish, which is corroborated by the findings in this study. The high query coverage (near 100%) ensures that the sequence data encompass the full target region, thus allowing for precise taxonomic resolution.

The findings of this study align with previous research on DNA barcoding of marine fish, particularly within the *Lutjanidae* family, where species identification through molecular means has proven to be more accurate than morphological methods alone (Rahayu *et al*., 2023). Studies by Steinke *et al*. (2009) have similarly reported percentage identities ranging from 95% to 100% for species-level

identifications in marine fish, particularly when using the COI gene as the barcode marker. This comparison underscores the utility of DNA barcoding in confirming species identity, especially in environments where morphological variation may hinder accurate identification. In terms of genetic divergence, the minimal variation observed in the percentage identity (95.25%–100%) between *L. agennes* populations from the two estuaries is consistent with other studies of estuarine fish species (Bolaji *et al*., 2023; Eriegha *et al*., 2024c). Other studies focusing on geographically isolated populations of fish have documented higher genetic divergence due to restricted gene flow and ecological differentiation (Ruzzante *et al*., 1998). However, the results of this study indicate that *L. agennes* populations in the Escravos and Qua Iboe estuaries are likely part of a larger, genetically connected metapopulation, which could explain the low variation in sequence data between the two sites. The high percentage identity across both populations reinforces the view that these estuaries are not acting as isolated ecosystems for *L. agennes* but are part of a broader network of connected habitats. The molecular confirmation of *L. agennes* in both the Escravos and Qua Iboe estuaries has several implications for the ecology and management of these populations. The lack of significant genetic divergence between populations suggests that the fish in these two estuaries may be treated as a single management unit for conservation purposes. This is important for developing sustainable fisheries management plans, as the interconnectedness of these populations implies that overexploitation in one area could impact the genetic diversity and long-term viability of the species across a broader geographic range.

4.0 Conclusion

This study provided valuable insights into the genetic and morphological characteristics of *Lutjanus agennes* from two key estuarine systems in Nigeria—the Escravos and Qua Iboe estuaries. Using DNA barcoding, the molecular analysis confirmed the morphological identification of the fish samples, showing a high degree of genetic similarity between the two populations, as reflected in the percentage identity and minimal sequence length variation. These results suggest limited genetic divergence, likely due to gene flow across the two estuaries. The genetic homogeneity observed implies that these populations may form part of a broader metapopulation that is ecologically connected. Despite the genetic similarities, there were notable differences in the morphometric characteristics of fish from the two estuaries. Fish from Qua Iboe were generally larger in weight and body length, which may be due to better environmental conditions or differences in prey availability, water quality, or predation pressures. These morphological differences, though not statistically significant, could indicate local adaptations to the distinct ecological conditions of each estuary. The findings of this study have important implications for the management and conservation of *L. agennes* populations. The genetic connectivity between populations suggests that they should be managed as a single unit to prevent localized overfishing from affecting the broader population. Furthermore, the integration of molecular tools like DNA barcoding into fisheries management practices can help ensure accurate species identification, contributing to better regulation and conservation strategies for estuarine ecosystems. This research demonstrates the value of combining genetic and morphological data in understanding population dynamics and guiding sustainable exploitation of marine resources.

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