

PHENOTYPIC AND GENETIC DIVERGENCE IN SOME CICHLID POPULATIONS FROM TWO SELECTED BRACKISH WATER BODIES IN BENIN REPUBLIC AND NIGERIA

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ABSTRACT

For sustainable management of fish resources, an understanding of the adaptive capacity and population structure of species is important. However, there are still limited data on tilapine variations across West African lagoons. The morphological and genetic differences in *Oreochromis niloticus*, *Coptodon guineensis*, and *Tilapia mariae* populations were investigated in Porto-Novo Lagoon (PNL), Benin, and Epe Lagoon (EPL), Nigeria, to determine their heterogeneity. For eight months, from December 2022 to July 2023, 188 fish samples were collected (EPL: 43 *O. niloticus*, 29 *C. guineensis*, 17 *T. mariae*; PNL: 44 *O. niloticus*, 30 *C. guineensis*, 27 *T. mariae*) and analyzed for meristic attributes (MRA), morphometric attributes (MPA), phenotypic heterogeneity, and genetic diversity using cytochrome b (Cyt-b) gene sequences. For MPA, the coefficients of variation ranged from 9.33% to 33.25% in *O. niloticus*, 6.18% to 32.22% in *C. guineensis*, and 7.19% to 39.58% in *T. mariae*, pointing to substantial inter-population variability. From genetic analyses, *O. niloticus* populations in PNL were more closely related to the *Coptodon aff. louka* 'Samou' strain than EPL counterparts; PNL *C. guineensis* clustered nearer to *Oreochromis niloticus* haplotype HAP14 and *Oreochromis niloticus* Asejire12 than EPL populations; and *T. mariae* from PNL showed closer affinity to *Bathybates graueri* from Lake Tanganyika than EPL. It can be concluded that there are significant phenotypic and genetic divergence between the lagoons, affirming the need for location-specific conservation and management to sustain biodiversity.

Key Words: Phenotype, Tilapines, *Coptodon aff. Louka*, Phenotypic characteristic, Genetic structure

INTRODUCTION

One of the widely produced and economically significant aquaculture species are the Tilapias. They belong to the Cichlidae family, with *Oreochromis niloticus*, *Coptodon guineensis*, and *Tilapia mariae*, being prime examples. *O. niloticus* exhibit rapid growth, short generation time, and high fecundity, which have facilitated its extensive introduction for aquaculture across Africa (Bob-Manuel, 2013). *Coptodon guineensis* ranks as the third most important brackish water tilapia in West Africa, and supports local fisheries and ecosystems (Philippart and Ruwet, 1982). Another brackish water species is the *Tilapia mariae*: a native species that also exhibits robust growth and reproductive rates (Robins, 2018). These three tilapias play very significant roles in the fisheries, food security, livelihood enhancement and biodiversity in the West African sub-region. *O. niloticus*, *C. guineensis* and *T. mariae* thrive in transitional habitat like the lagoon; with marine and freshwater influences, because they can adapt to salinity gradients (Mahfuj *et al.*, 2023). In spite of this adaptability, these tilapia populations are faced with growing threats from habitat fragmentation, harmful anthropogenic activities, climate change-driven salinity shifts, and pollution (FAO, 2022). These threats endanger the species population structures and genetic integrity (FAO, 2022). The aforementioned challenges are exemplified in the Porto-Novo Lagoon in Benin Republic and Lagos Lagoon in Nigeria, representing trans-boundary brackish water bodies. According to Akinwumi (2019), interconnected ecosystems experience differentials in water chemistry, fluctuating tides, and varying impacts from human activities, fishing pressure

and habitat degradation. The ecosystem still sustains, through adaptation, diverse fish communities essential to local economies. These environmental adaptations, according to Mahfuj *et al* (2023) are indicated by morphometric and meristic differences, while incipient speciation signalled by varying levels of divergence are revealed by genetic studies. It is therefore advisable to combine phenotypic and genetic analyses in biodiversity status assessment in trans-boundary waters to guide sustainable management. According to WorldFish (2022), the characterization of fish population across ecological zones, have mostly been carried out using morphometric and meristic analyses that reveals adaptations to environmental variations. However, the level of divergence in a population can be further revealed by genetic studies, as it is able to point to incipient speciation, most especially in species prone to hybridization resulting from overlapping distributions (Mahfuj *et al.*, 2023). Thus, in biodiversity assessment for sustainable management, the integration of phenotypic and genetic approaches is advocated. This will enhance the understanding of intraspecific genetic variability which is fundamental for conservation efforts (Frankham, 2008).

Although there are numerous studies on *O. niloticus*, there appears to be limited works on the morphological characterization, phenotypic variations and genetic makeup of *C. guineensis* and *T. mariae*, especially with reference to trans-boundary lagoons like those in southern Benin Republic and Nigeria.

This study investigates morphological and genetic variations among cichlid populations in the Lagos

Lagoon, Nigeria, and Porto-Novo Lagoon, Benin Republic. To understand the adaptive differences, morphometric, meristic and molecular analysis were integrated, to ultimately provide evidence-based insights to support sustainable trans-boundary resource management and preserve genetic diversity in these vital ecosystems.

MATERIALS AND METHODS

Description of Study sites

Epe lagoon (Fig. 1), is a tropical water body situated at the eastern part of the Lagos lagoon complex, covering an area of 243 km² (Kusemiju 1988). A constant connection to the Atlantic Ocean is maintained through the Lagos lagoon. Freshwater from creeks and rivers contributes to the hydrology of Epe lagoon (Nwankwo 1998). The Porto-Novo lagoon (Fig. 1), is situated in the south-eastern region of Benin, between the latitudinal lines of 6°25'N and 6°30'N and the longitudinal line of 2°30'E, specifically located in the Ouémé Department. It is bounded to the north by the municipality of Porto-Novo, to the south by the villages of Djrègbé and Tohouè, to the east by the Lagos Lagoon in Nigeria, and to the west by Nokoué Lake, connected by the Totchè Channel, which spans 5 km in length and measures between 200 m to 300 m in width.

Sample collection

A total of 188 individuals of *Oreochromis niloticus*,

Coptodon guineensis and *Tilapia mariae* were randomly collected from Epe lagoon and Porto Novo lagoon. Fish samples were collected monthly over an eight-month, from December 2022 to July 2023 from artisanal fishermen using gill nets, creels, and cast nets at designated landing sites in Epe Lagoon and Porto-Novo Lagoon. Fish species were identified using standard taxonomic keys, following FishBase descriptions and Volume II of The Fresh and Brackish Water Fishes of West Africa (Lévêque *et al.*, 1992). The physic-chemical parameters (temperature, pH and dissolved oxygen, nitrate, nitrite, ammonia, carbonate hardness) were taken at the two sites using Pond Lab Multi-Test Kit. The number of *Oreochromis niloticus*, *Coptodon guineensis* and *Tilapia mariae* were 43, 29 and 17, respectively from Epe lagoon while, 44, 30 and 27 were collected from Porto Novo lagoon. Immediately after collection, samples were transported in ice cooled boxes to the laboratory for phenotypic data collection. A total of seventeen (17) morphometric measurement and seven (7) meristic counts were taken from each specimen using meter ruler with millimetre (mm) markings to the nearest 0.1 cm.

Furthermore, 18 caudal peduncles were collected at a rate of 3 per species were stored in a tube with ethanol for genetic analysis. The fish tissue DNA extraction involved the initial step of transferring up to 25 mg of fresh tissue into a 1.5 ml microcentrifuge tube. Subsequently, a mixture of 200 µl of GST Buffer and 20 µl of Proteinase K

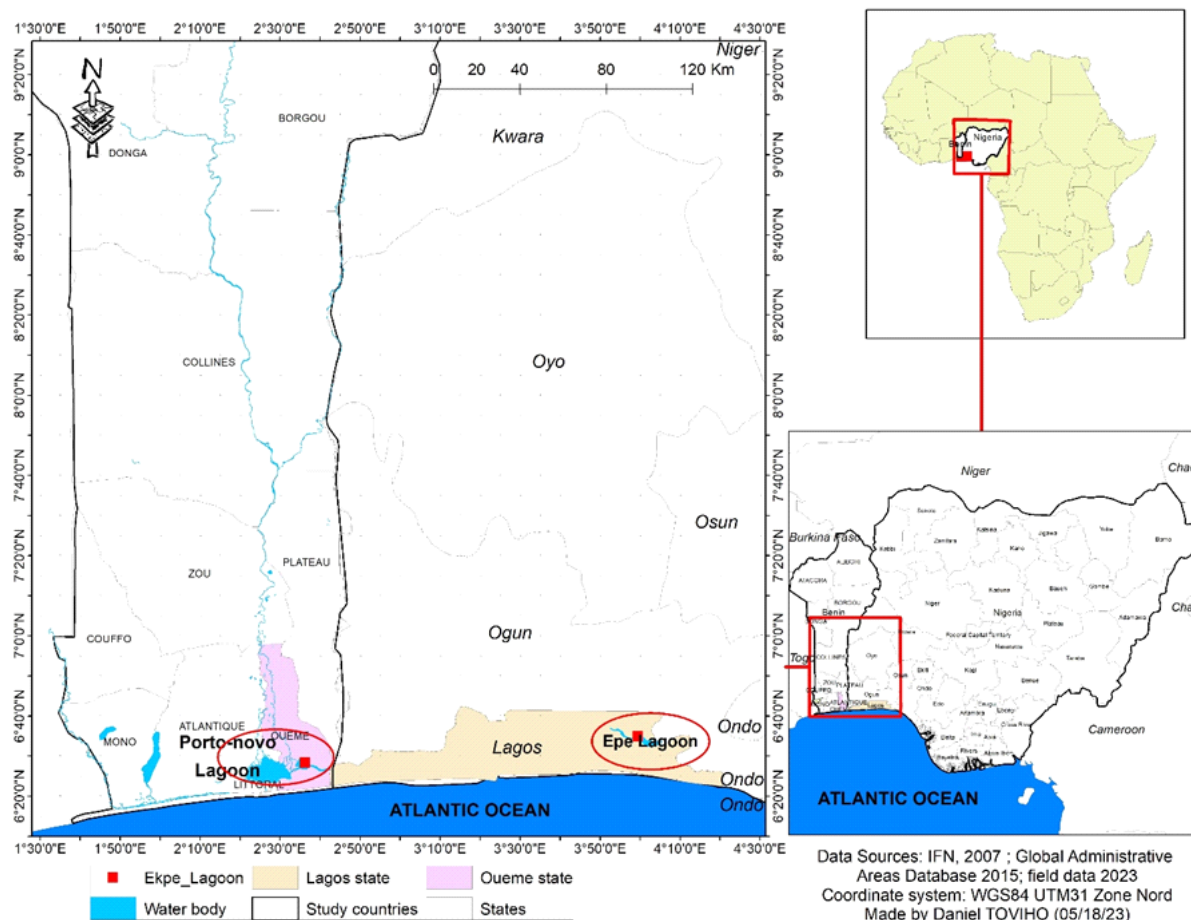


Figure 1: Map of Benin Republic and Nigeria showing sampled water reservoir and collection points

was added to the tube, followed by thorough vortexing, and incubation at 60°C until the sample lysate became clear. Simultaneously, Elution Buffer (200 µl/sample) was preheated to 60°C for the subsequent DNA elution step. To bind the DNA, 200 µl of absolute ethanol was promptly introduced to the lysate, and the contents were vigorously shaken for 10 seconds. This mixture, including any insoluble precipitate, was then transferred to a GS Column, thoughtfully positioned within a 2 ml Collection Tube, and subjected to centrifugation at 14-16,000 x g for 1 minute. Following DNA binding, a washing step ensued, beginning with the addition of 400 µl of W1 Buffer to the GS Column, followed by a 30-second centrifugation, with the flow-through being carefully discarded. Subsequently, 600 µl of Wash Buffer was added to the GS Column, which was once again subjected to centrifugation for 30 seconds, and the flow-through was discarded. To ensure the column matrix's complete drying, the GS Column was placed back into the 2 ml Collection Tube and centrifuged for an additional 3 minutes at 14-16,000 x g. Finally, the dried GS Column was transferred to a clean 1.5 ml microcentrifuge tube, and 100 µl of preheated Elution Buffer was gently introduced. After standing for 3 minutes to allow the complete absorption of the Elution Buffer, the sample underwent centrifugation at 14-16,000 x g for 30 seconds, resulting in the elution of purified DNA. The Polymerase chain reaction (PCR) amplifications were carried out using universal primers designed for the mitochondrial DNA's Cytochrome b gene. The PCR reaction was conducted in a total volume of 50 µL, comprising 5 µL of 10 X Taq polymerase buffer, 2 µL of MgCl₂ (25 mM), 2.5 µL of each dNTP (0.05 mM), 0.5 µL of each primer (0.01 mM), 1.2U of Taq DNA polymerase, and 2 µL of genomic DNA (50 ng/µl). The specific primers used for Cytochrome b gene amplification were CYTB F (TGACTTGAAAAACCAACCGTTGTTA), CYTB R (CTTCGGTTTACAAGACCG), CYTO F (TCAACCAACCACAAAGACATTGGCAC), CYTO R (TAGACTTCTGGGTGGCCAAAGAATCA). The thermal cycling conditions consisted of an initial denaturation step of 3 minutes at 94°C, followed by 35 cycles of 45 seconds at 94°C, 50 seconds at 54°C, and 1 minute 20 seconds at 72°C. This was succeeded by a final extension step of 10 minutes at 72°C. The PCR products were purified and labeled using the BigDye Terminator V.3.1 Cycle Sequencing Kit. Bidirectional sequencing was performed using an ABI 3730 genetic analyzer, following the manufacturer's instructions. The Sequence editing and analysis were carried out using the BioEdit and Molecular Evolutionary Genetics Analysis (MEGA) 6 software packages. The sequence data underwent editing and alignment through a nucleotide blast comparison with similar samples available at the National Centre for Biotechnology Information (NCBI).

Analysis of Data

Collected data were entered into Excel for comparative analysis. To account for size effects, morphometric parameters were standardized using the following allometric formula developed by Elliott *et al.* (1995).

$$Madj = \left(\frac{Ls}{Lo} \right) M$$

Where M = measurement, Madj = size accustomed dimension, Lo = total length of fish, and Ls = mean of total length for all fish.

The coefficient of variation (CV) for both morphometric and meristic characteristics was computed by dividing the standard deviation by the mean value, following established standards (Michener 1969). Attributes exhibiting a CV exceeding ten percent (>10%) was identified as exhibiting heterogeneity Michener 1969 as cited by Oyebola (2014)

$$CV = \left(\frac{\text{Standard Deviation of Phenotypic Value}}{\text{Phenotypic Value}} \right) 100$$

Statistical analyses

Morphometric and meristic attributes were presented using descriptive statistics. Pearson correlation analysis was utilized to investigate the relationships among individual morphometric and meristic parameters. Furthermore, Student's t-test (t-test), principal component analysis (PCA), and linear discriminant function analysis (DFA) were executed to assess significant variations in morphometric characteristics among the populations. The descriptive statistics and t-test analyses were conducted using GraphPad Prism version 9.5.1 for Windows, developed by GraphPad Software in Boston, Massachusetts, USA (www.graphpad.com). PCA and DFA were performed using Statistical Package for the Social Sciences (IBM SPSS) in Version 27.

RESULTS

The analysis of water quality parameters in table 1, showed that ammonia was significantly present ($p < 0.05$) in Epe Lagoon (0.11 mg/L) but was not detected in Porto-Novo Lagoon. Nitrite and nitrate were not detected in Porto-Novo Lagoon, but present at low levels in Epe Lagoon. Carbonate hardness was significantly higher in Epe Lagoon (71.2 mg/L) compared to Porto-Novo Lagoon (26.7 mg/L). Results for dissolved oxygen, pH, and temperature values however varied marginally between the two lagoons. But was no significant difference ($p > 0.05$).

Table 2 presents the results of morphometric measurements of *Oreochromis niloticus* in Porto Novo lagoon and Epe lagoon. Generally, the results showed that the population of *O. niloticus* in Porto Novo lagoon were greater in body size compared to the one from Epe lagoon. The coefficient of variation (CV) ranged from 9.33% to 33.25%. There were significant difference ($P < 0.05$) in all morphometric parameters between samples from Epe and Port Novo Lagoons except for Caudal Peduncle Width (CPW) and Mouth Height (MH).

The morphometric measurements conducted on *Coptodon guineensis* in Porto Novo Lagoon and Epe



Lagoon, respectively were as presented in Table 3. The recorded morphometric measurements, ranged from 0.33 cm (CPW) to 14.43 cm (TL) in Porto Novo Lagoon and from 0.56 cm (CPW) to 15.15 cm (CPW) in Epe Lagoon. *C. guineensis* population within Porto Novo Lagoon exhibited smaller body sizes compared to those within Epe Lagoon. The coefficient of variation (CV) ranged from 6.18% to 32.22%.

Results of morphometric measurements of *Tilapia mariae* in Porto Novo Lagoon and Epe Lagoon are presented in table 4. The CPW ranged from 0.47 ± 0.16 (Epe) to 0.59 ± 0.23 cm (Port Novo) while TL varied from 12.59 ± 2.34 (Port Novo) to 13.09 ± 3.62 cm (Epe). *T. mariae* in Porto Novo lagoon are smaller in body size than the one in Epe lagoon, with coefficient of variation (CV) ranging from 7.19% to 39.58%, indicating variability in the measurements within each group.

TABLE 1: Water Parameters Of Epe Lagoon And Porto-Novo Lagoon

Water Pararemeters	Epe Lagoon		Porto-NovoLagoon		T-test	P-value
	Mean $\pm$ SD	MIN to Max	Mean $\pm$ SD	MIN and Max		
Ammonia (mg/L)	0.11 ± 0.10	0 to 0.2	0	0 to 0	2.83	0.03*
Nitrite (mg/L)	0.04 ± 0.09	0 to 0.25	0	0 to 0	1.00	0.356
Nitrate (mg/L)	0.06 ± 0.11	0 to 0.25	0	0 to 0	1.53	0.171
Carbonate Hardness(mg/L)	71.2 ± 49.44	17.80 to 124.6	26.70 ± 19.03	17.8 to 80	2.38	0.041*
Dissolved Oxygen	8.73 ± 1.58	7.00 to 11	9.93 ± 1.70	7.2 to 12.50	1.46	0.1662
PH	7.31 ± 0.46	6.5 to 8	7.56 ± 0.42	7 to 8	1.14	0.273
Temperature($^{\circ}$ C)	29.65 ± 0.89	28 to 31	28.69 ± 1.56	25.70 to 30.50	1.51	0.158

Key: *- Significantly different ($p < 0.05$)

TABLE 2: Morphometric Measurements Of *Oreochromis Niloticus* From Porto Novo Lagoon (Benin Republic) And Epe Lagoon (Nigeria)

Mean $\pm$ standard deviation, CV (%); coefficient of variation; Rows having the same superscript are not significantly different ($P < 0.05$)

Morphometric Parameters	Porto Novo Lagoon n=44	Epe Lagoon n=43	C.V (%)	T-test	P value
T L	17.75 ± 1.97^a	15.51 ± 3.95^b	19.79	3.36	0.0012
S L	13.76 ± 1.03^a	11.97 ± 0.73^b	9.81	9.33	0.0001
HL	2.78 ± 0.43^a	2.57 ± 0.20^b	12.89	2.18	0.0323
HH	4.74 ± 0.53^a	4.09 ± 0.57^b	14.34	5.39	0.0001
BH	6.048 ± 0.49^a	5.14 ± 0.34^b	11.07	10.1	0.0001
BW	1.89 ± 0.28^a	1.44 ± 0.26^b	21.3	7.62	0.0001
MBH	4.62 ± 0.39^a	4.42 ± 0.43^b	9.33	2.27	0.0257
CFH	4.63 ± 0.64^a	4.32 ± 0.80^b	16.54	2.02	0.0466
CPL	1.76 ± 0.19^a	1.53 ± 0.27^b	15.92	4.49	0.0001
CPH	2.67 ± 0.19^a	2.06 ± 0.27^b	11.69	4.12	0.0001
CPW	0.52 ± 0.09^a	0.46 ± 0.20^a	33.25	1.55	0.1243
PelFL	4.01 ± 0.45^a	3.48 ± 0.61^b	15.92	4.59	0.0001
PelFW	1.76 ± 0.26^a	1.63 ± 0.33^b	17.68	2.08	0.0408
ERH	1.13 ± 0.20^a	1.05 ± 0.13^b	16	2.19	0.0314
MW	1.28 ± 0.19^a	1.17 ± 0.21^b	17.22	2.68	0.009
MH	1.65 ± 0.14^a	1.59 ± 0.27^a	13.33	1.26	0.2107
PFL	5.42 ± 0.45^a	4.40 ± 0.56^b	14.65	9.35	0.0001

There is difference between ($p < 0.05$) and ($p > 0.05$) so be consistent in their usage
In the text you use $p < 0.05$ while in the table you use $p > 0.05$

Total Length (TL), Standard Length (SL), Head Length (HL), Head Height (HH), Body Height (BH), Body Width (BW), Mean Body Height (MBH), Caudal Fin Height (CFH), Caudal Peduncle Length (CPL), Caudal Peduncle Height (CPH), Caudal Peduncle Width (CPW), Pelvic Fin Length (PelFL), Pelvic Fin Width (PelFW), Relative Eye Height (ERH), Mouth Width (MW), Mouth Height (MH), and Pectoral Fin Length (PFL)



Table 5 presents the outcomes of meristic counts conducted on *Oreochromis niloticus*, *Coptodon guineensis* and *Tilapia mariae*. For *O. niloticus*, the meristic counts structure ranged from 1 (PelFHR: Pelvic Fin Hard Rays) to 16.11 (CFSR: Caudal fin Soft Rays) and 1 (PelFHR) to 15.63 (DFHR : Dorsal Fin Hard Rays) in Porto Novo lagoon and Epe Lagoon, respectively. The meristic results (such as DFHR, PFSR, AFHR, PelFHR, and CFSR) showed that the population of *O. niloticus* in Porto Novo lagoon was greater in body size than the one in Epe lagoon. The coefficient of variation (CV) ranged from 0.00 % to 10.30% indicating variability in the meristic count within each group.

For *Coptodon guineensis* the meristic counts structure ranged from 1 (PelFHR) to 15.93 (CFSR) and 1 to 16.31 (CFSR) in Porto Novo lagoon and Epe Lagoon,

respectively. The meristic results (DFHR, DFSR, PFSR, and

AFSR) showed that the population of *Coptodon guineensis* in Porto Novo lagoon was smaller in body size than the one in Epe lagoon. The coefficient of variation (CV) ranges from 0.00 % to 12.42%, indicating variability in the meristic count within each group. *Tilapia mariae* meristic counts structure ranged from 1 (PelFHR) to 14.38 (CFSR) and 1 (PelFHR) to 15.07 (DFHR) in Porto Novo lagoon and Epe Lagoon, respectively. The meristic results (such as DFHR, DFSR, AFSR, and CFSR) showed that the population of *Tilapia mariae* in Porto Novo lagoon was smaller in body size than the one in Epe lagoon. The coefficient of variation (CV) ranges from 0.00 % to 17.86%, indicating variability in the meristic count within each group.

TABLE 3: Morphometric measurements of *Coptodon Guineensis* from Porto Novo Lagoon (Benin) and Epe Lagoon

Morphometric Parameters	Porto Novo Lagoon n=30	Epe Lagoon n=29	CV (%)	T-test	P value
T L	14.43±2.19 ^a	15.15±4.14 ^a	22.27	0.84	0.4044
S L	11.28±0.67 ^a	11.89±0.63 ^b	6.18	3.61	0.0007
HL	1.87±0.16 ^a	2.39±0.42 ^b	19.06	6.29	0.0001
HH	3.62±0.67 ^a	4.04±0.63 ^b	17.97	2.36	0.0219
BH	5.05±0.29 ^a	5.28±0.46 ^b	7.68	2.31	0.0241
BW	1.19±0.36 ^a	1.70±0.20 ^b	26.58	6.66	0.0001
MBH	4.16±0.30 ^a	4.61±0.61 ^b	12.05	3.61	0.0006
CFH	4.01±0.51 ^a	4.10±0.58 ^a	13.42	0.66	0.5088
CPL	1.49±0.20 ^a	1.80±0.69 ^b	32.20	2.26	0.0279
CPH	1.88±0.29 ^a	2.012±0.25 ^a	14.33	1.89	0.0635
CPW	0.33±0.14 ^a	0.54±0.11 ^b	37.68	6.44	0.0001
PelFL	3.71±0.32 ^a	3.60±0.58 ^a	12.80	0.86	0.3955
PelFW	1.49±0.20 ^a	1.73±0.30 ^b	17.67	3.45	0.0011
ERH	0.98±0.14 ^a	0.99±0.17 ^a	15.64	0.2	0.8401
MW	1.31±0.19 ^a	1.19±0.21 ^b	16.89	2.38	0.0209
MH	1.31±0.19 ^a	1.58±0.17 ^b	19.09	4.15	0.0001
PFL	4.09±0.28 ^a	4.25±0.63 ^b	11.79	1.27	0.2091

Rows having the same superscript are not significantly different ($P > 0.05$)

Mean ± standard deviation, CV (%); coefficient of variation

TABLE 4: Morphometric Measurements of *Tilapia Mariae* from Porto Novo Lagoon (Benin) And Epe Lagoon (Nigeria)

Morphometric Parameters	Porto Novo (Benin) n=27	Epe Lagoon (Nigeria) n=17	CV (%)	T-statistic	P value
T L	12.59±2.34 ^a	13.09±3.62 ^a	22.15	0.55	0.5832
S L	9.85±0.67 ^a	10.41±0.70 ^b	7.19	2.57	0.0141
HL	2.17±0.38 ^a	2.08±0.29 ^a	16.31	0.82	0.4192
HH	2.69±0.36 ^a	3.00±0.78 ^a	19.89	1.82	0.0764
BH	3.74±0.35 ^a	4.37±0.54 ^b	13.04	4.57	0.0001
BW	1.68±0.50 ^a	1.74±0.62 ^a	31.83	0.35	0.7292
MBH	3.07±0.34 ^a	3.74±0.59 ^b	16.60	4.68	0.0001
CFH	3.58±0.53 ^a	3.68±0.82 ^a	17.68	0.45	0.6519
CPL	1.48±0.36 ^a	1.82±0.67 ^b	32.15	2.15	0.038



Morphometric Parameters	Porto Novo (Benin) n=27	Epe Lagoon (Nigeria) n=17	CV (%)	T-statistic	P value
CPH	1.57±0.16 ^a	1.61±0.27 ^a	12.99	0.66	0.51
CPW	0.59±0.23 ^a	0.47±0.16 ^a	39.58	1.75	0.0878
PelFL	3.09±0.64 ^a	3.47±0.48 ^a	19.09	1.97	0.0558
PelFW	1.49±0.56 ^a	1.49±0.44 ^a	34.70	0.05	0.9601
ERH	0.93±0.86 ^a	0.86±0.14 ^a	76.83	0.31	0.7586
MW	1.19±0.26 ^a	1.03±0.25 ^b	23.11	2.05	0.0472
MH	1.28±0.31 ^a	1.19±0.26 ^a	23.44	0.9497	0.348
PFL	2.32±0.56 ^a	3.41±0.89 ^b	32.81	4.905	0.0001

Rows having the same superscript are not significantly different ($P > 0.05$)

Mean ± standard deviation, CV (%); coefficient of variation

TABLE 5: Meristic counts of *Oreochromis Niloticus*, *Coptodon Guineensis* and *Tilapia Mariae* from Porto Novo Lagoon (Benin Republic) and Epe Lagoon (Nigeria)

Meristic Parameter	Porto Novo Lagoon <i>O. niloticus</i> n=44	Epe Lagoon n=43	CV (%)	T-test	P value
DFHR	15.68±0.47 ^a	15.63±0.61 ^a	3.49	0.46	0.648
DFSR	11.68±0.60 ^a	11.95±0.68 ^a	5.55	1.96	0.0531
PFSR	12.16±0.78 ^a	12.05±1.07 ^a	7.67	0.56	0.5746
AFSR	9.00±0.00 ^a	8.91±0.61 ^a	4.79	1.01	0.3146
AFHR	3.00±0.00 ^a	3.00±0.00 ^a	0.00	-	-
PelFHR	1.00±0.00 ^a	1.00±0.00 ^a	0.00	-	-
PelFSR	5.34±0.71 ^a	5.00±0.00 ^b	10.30	3.13	0.0024
CFSR	16.11±0.45 ^a	15.51±0.77 ^b	4.47	4.36	0.0001
<i>Coptodon guineensis</i> n=30					
		n=29			
DFHR	15.53±0.51 ^a	15.95±0.73 ^b	4.18	2.65	0.0105
DFSR	11.27±0.69 ^a	12.03±0.63 ^b	6.53	4.47	0.0001
PFSR	11.57±0.50 ^a	12.79±0.68 ^b	7.02	7.93	0.0001
AFSR	8.77±0.43 ^a	9.24±0.44 ^b	5.46	4.21	0.0001
AFHR	3.00±0.00 ^a	3.00±0.00 ^a	0.00	-	-
PelFHR	1.00±0.00 ^a	1.00±0.00 ^a	0.00	-	-
PelFSR	5.80±0.76 ^a	5.10±0.30 ^b	12.42	4.57	0.0001
CFSR	15.93±0.74 ^a	16.31±0.60 ^b	4.32	2.14	0.0366
<i>Tilapia mariae</i> n=27					
		n=15			
DFHR	13.60±2.72 ^a	15.07±1.16 ^a	16.86	1.97	0.0554
DFSR	11.56±0.80 ^a	11.60±0.63 ^a	6.37	0.19	0.8542
PFSR	11.63±0.56 ^a	11.53±0.83 ^a	5.73	0.45	0.6584
AFSR	8.63±0.83 ^a	8.66±0.49 ^a	8.41	0.16	0.8765
AFHR	3.00±0.00 ^a	3.00±0.00 ^a	0.00	-	-
PelFHR	1.00±0.00 ^a	1.00±0.00 ^a	0.00	-	-
PelFSR	5.15±0.95 ^a	5.27±0.59 ^a	16.06	0.43	0.6643
CFSR	14.38±3.03 ^a	14.87±1.59 ^a	17.86	0.58	0.5656

Mean ± standard deviation, CV (%); coefficient of variation; Rows having the same superscript are not significantly different ($P > 0.05$) Dorsal Fin Hard Rays (DFHR), Dorsal Fin Soft Rays (DFSR), Pectoral Fin Soft Rays (PFSR), Anal Fin Soft Rays (AFSR), Anal Fin Hard Rays (AFHR), Pelvic Fin Hard Rays (PelFHR), Pelvic Fin Soft Rays (PelFSR) and Caudal fin Soft Rays (CFSR).

The phylogenetic tree of *Oreochromis niloticus* from the Epe Lagoon, Porto-Novo Lagoon, and the NCBI Reference samples is presented in figure 2. The phylogenetic tree revealed two primary divisions at 0.886 genetic distance. The first division contained 100% of all *Oreochromis niloticus* from Epe Lagoon, Porto-Novo Lagoon, and 50% of the NCBI blasted reference sequences (*Coptodon* aff. louka 'Samou' strain, *Coptodon guineensis* strain, and *Coptodon camerunensis* and *Tilapia* sp. ONDOTG 001). With respect to rooting, at 2.915 genetic distance, POn3, POn1, and POn2 were closer to *Coptodon* aff. louka 'Samou' strain, *Coptodon guineensis* strain, and *Coptodon camerunensis* than to EOn3, EOn2, and EOn1 respectively. However, POn3 and POn1 clustered but diverged from POn2 at 0.868 genetic distance.

For *Coptodon guineensis* from Epe Lagoon, Porto-Novo Lagoon, and the NCBI Reference samples (Fig 2b), two major divisions were revealed at 0.00 genetic distance. The first division contained 100% of all *Coptodon*

guineensis from Epe Lagoon, Porto-Novo Lagoon, and 83.33% of the NCBI blasted reference sequences (*Oreochromis niloticus* haplotype HAP14, *Oreochromis niloticus* isolate Aejire12, *Sarotherodon nigripinnis* CAM01 00443, *Sarotherodon galilaeus* CAM01 452, and *Stomatepia mongo* CAM01 182). With respect to rooting, at 13.924 genetic distance, PTg1, PTg3 and PTg2 from Porto-Novo Lagoon were closer to *Oreochromis niloticus* haplotype HAP14 and *Oreochromis niloticus* Aejire12 than ETg1, ETg3 and ETg2 from Epe lagoon respectively. Figure 2c shows the phylogenetic Tree of *Tilapia mariae* from Epe Lagoon, Porto-Novo Lagoon and the NCBI reference samples. The first division contained 100% of all *Coptodon guineensis* from Epe Lagoon, Porto-Novo Lagoon, and 57.14% of the NCBI blasted reference sequences (*Bathybates graueri*, *Pelmatolapia mariae*, *Hemichromis bimaculatus*_Nigeria, *Hemichromis elongatus*_Congo). With respect to rooting, at 0.150 genetic distance, PTm1, PTm3, and PTm2 from Porto-Novo Lagoon were closer to *Bathybates graueri* than ETm1, ETm3, and ETm2 from Epe lagoon respectively.

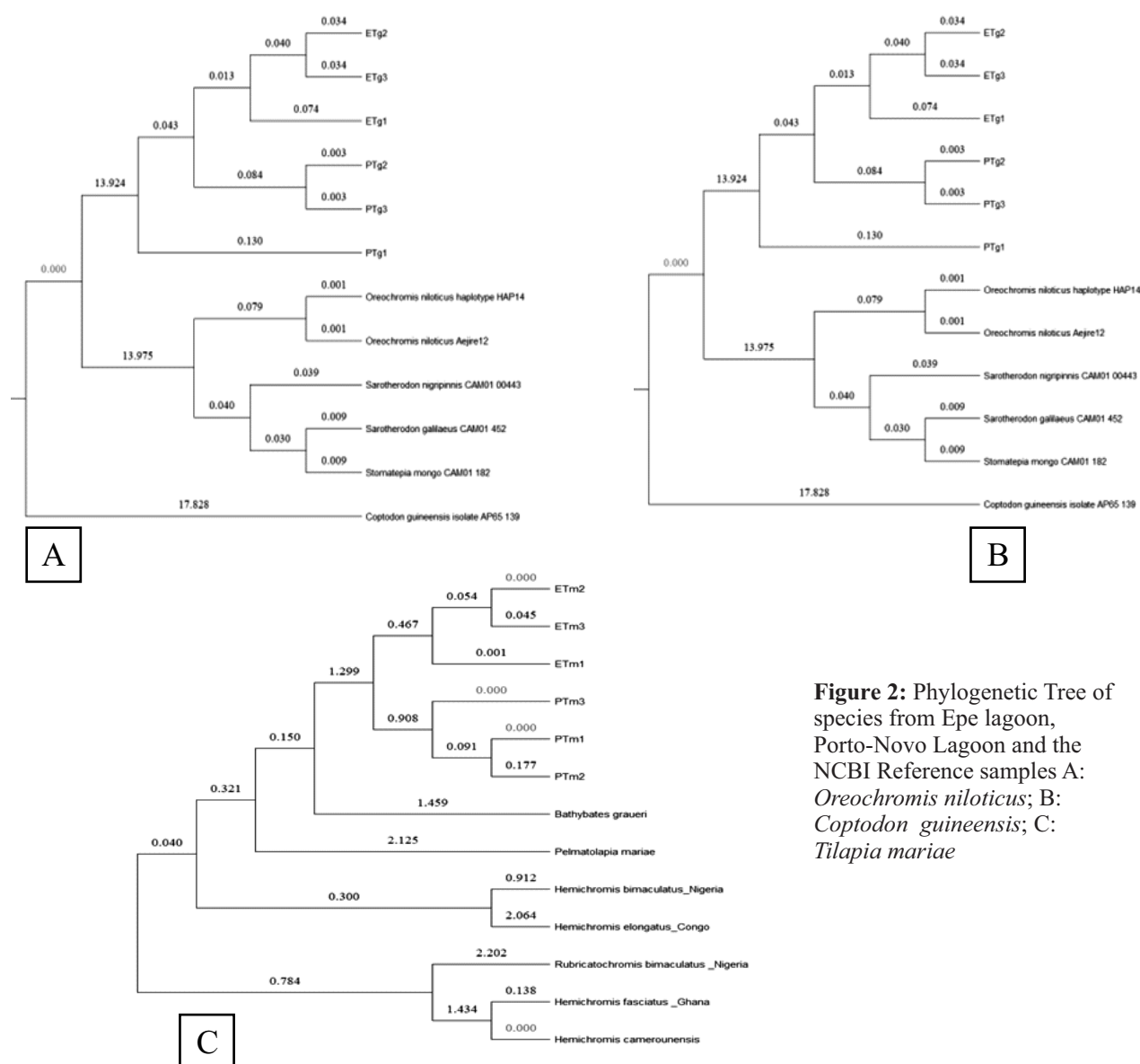


Figure 2: Phylogenetic Tree of species from Epe lagoon, Porto-Novo Lagoon and the NCBI Reference samples A: *Oreochromis niloticus*; B: *Coptodon guineensis*; C: *Tilapia mariae*

DISCUSSION

Morphological and physiological attributes in fish are significantly impacted by changes in the environment and water quality. In this present study, except for ammonia and carbonate hardness, there are no significant ($p > 0.05$) in water quality parameters from Port-Novo and Lagos Lagoons. With reference to Loh (2014), water quality parameters in the two lagoons fall within the range recommended for healthy aquatic ecosystems. Results in this present study however contradict previous reports where pollutions were observed in the lagoons of Lagos and Porto-Novo (Yehouenou *et al.*, 2013; Babalola and Fiogbe, 2017). The reported differences between the present and previous works may attributed to time variations or localized improvements in water quality management. Water quality variations have been reported to influence intraspecific variability as shown in differences in morphometric and meristic characters (Winberger, 1992; Rajput *et al.* 2013). Georgakopoulou *et al.*, (2007) stated that temperature affected the dorsal spines, soft rays, pectoral lepidotrichia, and caudal dermatotrichia in sea bass. In addition to the morphological alterations, The variation in water quality also influences aquatic organisms' composition, distribution, and abundance (Medeiros-Sousa *et al.*, 2020).

There were remarkable variations in the morphometric and meristic characteristics of *Oreochromis niloticus* and *Coptodon guineensis* populations from Porto-Novo and Epe Lagoons. The variations in meristic traits agrees with Abubakar *et al.* (2025), where complete speciation in cichlids from Zobe Reservoir was reported, as indicated by differences lateral line scales, gill rakers and the numbers of dorsal and anal rays. Meristic counts are considered more evolutionarily conserved when compared to morphometric measurements, and they provide stronger evidence for species differentiation (Fakunmoju *et al.*, 2014; Serdiati *et al.*, 2020). In this present study, meristic features such as Pelvic Fin Spine Rays (PelFSR) and Caudal Fin Spine Rays (CFSR) showed significant differences between the two populations of *O. niloticus*, agreeing with the results from previous studies by Oyebola (2014) and Makeche *et al.* (2022), which reported different levels of variability in meristic traits among fish populations. Coefficient of variation is important in comparing variability between populations. Phenotypic characters with CV greater than 10% are classified as heterogeneous (Michener, 1969). Pelvic fin soft rays (PelFSR) in *O. niloticus* had a CV of 10.30, exhibiting heterogeneity, and represents 12.5% of the meristic attributes analyzed. Although heterogeneity was only observed in PelFSR, variations shown in meristic characters may be likely caused by genetic factors (Næsje *et al.*, 2004; Koshy *et al.*, 2008). In this present study, all morphometric features displayed positive relationships indicating growth in one attribute corresponds to positive growth in another of *Oreochromis niloticus*. This is supported by reports from Safi *et al.* (2014).

Total length (TL), caudal fin height (CFH), pelvic fin length (PelFL), relative eye height (ERH), and pectoral fin

length (PFL) were significantly similar in the two *Coptodon guineensis* populations. All other morphometric attributes analyzed revealed a 70.5% difference. This finding is in agreement with Olopade *et al.* (2018), where high variability in morphometric traits were reported in *C. guineensis* from Buguma Creek and the New Calabar River, Nigeria, except for peduncle depth. Conversely, Kuton and Adeniyi (2018) identified significant variation in caudal peduncle depth (CPD) between *C. guineensis* populations in Badagry and Lagos Lagoons, highlighting potential site-specific differences.

For morphometric variables, 88.2% exhibited a coefficient of variation (C.V.) greater than 10, implying high heterogeneity, with caudal peduncle width (CPW) showing the greatest variability. The variability may be attributed to the combination of genetic and environmental factors, as well as their interactions (Poulet *et al.*, 2004). Although water quality parameters, like carbonate hardness and ammonia showed minimal differences between the two lagoons, other factors like migration distance, geographic isolation, and food availability may have contributed to morphometric differences, also reflecting their adaptation to local environmental conditions (Solomon *et al.*, 2015).

There were positive correlations for all morphometric characters, as an increase in one trait corresponds to proportional growth in others. This is in agreement with the report in Safi *et al.* (2014) on *Coptodon guineensis*. Total length (TL) and head height (HH) showed the strongest correlation, and this underscores a robust linear relationship. Strong linear relationship was also reported in Yazici *et al.* (2020) between total length and head length in *Nemipterus randalli* from Iskenderun Bay. For meristic traits, the strongest correlation was exhibited between pelvic fin soft rays (PelFSR) and pectoral fin soft rays (PFSR), indicating a negative relationship. In contrast, Ukenye *et al.* (2015) where dorsal fin count (DFC) and anal fin count (AFC) were the most correlated meristic variables in *C. guineensis* populations from some coastal waters in Nigeria.

In this study, a comparison of meristic counts in *Tilapia mariae* from the two lagoon populations showed no significant differences. This is consistent with reports in Oladimeji *et al.* (2020), on fish from selected reservoirs in southwest Nigeria. However, significant differences in meristic characters were reported among *Labeo ariza* populations (Ahammad *et al.*, 2018).

Coefficient of variation between the two *Tilapia mariae* populations was 35.30% for morphometric traits. C.V in all traits except standard length (SL) was greater than 10, indicating heterogeneity. In a previous study, Simon *et al.* (2010) reported limited differentiation in morphometric features between two congeneric archerfish species. Importantly, this present study revealed strong positive correlations between caudal peduncle height (CPH) and head length (HL), suggesting coordinated development of these features. However, meristic attributes showed both low positive and negative correlations, aligning with the findings of Simon *et al.* (2010) and Ahammad *et al.* (2018).

Understanding the patterns and degree of genetic divergence of wild and cultured fish populations is crucial



for effective management. The *Oreochromis niloticus* samples from Porto Novo Lagoon (POn3, POn1, and POn2) rooted more closely to *Coptodon* aff. louka 'Samou' strain than samples from Epe Lagoon, Nigeria (EOn3, EOn2 and Eon1). These results indicated that these two populations are potentially divergent in genotypes but closely related to the species of *Coptodon* aff. louka 'Samou' strain. EOn1 and EOn2 clustered but diverged from EOn3, implying a divergent genotype within *Oreochromis niloticus* population from Epe Lagoon, Nigeria. Earlier findings by Lind et al (2019) also noted a genetic divergence in *O. niloticus* across the West Africa region. *Coptodon guineensis* samples PTg1, PTg3, and PTg2 from Porto Novo Lagoon rooted more closely to *Oreochromis niloticus* haplotype HAP14 and *Oreochromis niloticus* Asejire12 than ETg1, ETg3 and ETg2 from Epe lagoon respectively. This result indicates that the two populations are potentially divergent genotypes closely related to the two species. The diverging of PTg1 could indicate that this sample is a diverging genotype from the rest. In addition, the formation of two subdivisions (0.043 genetic distance) by the rest of the specimen could indicate the presence of genotypes composed of PTgt3 and PTgt2, ETg1, and ETg2 and ETg3 genotypes. While, the phylogenetic trend indicated that the samples were heterogenous, *Coptodon guineensis* from Epe Lagoon, Nigeria (ETg1, ETg2, and ETg3) are potentially close divergent of PTgt3 and PTgt2 from Porto Novo Lagoon, Benin Republic. Similar results were obtained by Ukenye et al., (2019), which showed that *C. guineensis* from Gabon displayed low genetic diversity. In addition, Gu et al., (2014) also found that *C. guineensis* had lower genetic diversity than *O. niloticus*. *Tilapia mariae* samples taken from Porto Novo Lagoon, namely PTm1, PTm3, and PTm2, exhibited a closer genetic affinity to *Bathybates graueri* and *Pelmatolapia mariae* when compared to the samples collected from Epe Lagoon, specifically ETm1, ETm3, and ETm2. This closeness suggests that the two populations may represent divergent genotypes closely related to *Bathybates graueri*. In contrast, earlier study by Megbowon (2019) utilizing RAPD markers from Epe Lagoon, Nigeria revealed highest similarity between *Tilapia mariae* and *Coptodon guineensis* (0.52992) and lowest between *Tilapia mariae* and *Sarotherodon melanotheron* (0.28925). Further, analysis using mitochondria DNA cytochrome oxidase subunit 1 by 'Megbowon and Fashina-Bombata (2019) revealed that *T. mariae* was closer to 'Wesafu', an ecotype Cichlid of Epe Lagoon (Fashina-Bombata and Hammed 2010).

CONCLUSION

This study revealed minor differences in physicochemical properties of Porto-Novo Lagoon (Benin Republic) and Epe Lagoon (Nigeria), but all were within recommended limits. Morphometric and meristic characteristics analysis demonstrated significant variations between fish populations, likely influenced by genetic, geographical, environmental, and productivity factors. Lastly, genetic analysis revealed the presence of multiple genotypes within *Oreochromis niloticus*, *Coptodon guineensis*, and

Tilapia mariae populations, emphasizing the need for conservation efforts to preserve their genetic diversity. These findings underscore the complexity of Cichlid populations and provide valuable insights for future research and conservation strategies.

AUTHORS CONTRIBUTIONS:

KOK: Conceptualization, project administration, supervision, and revision

OO: Design, Analysis and revision

DATG: Experimentation, analysis and first draft

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