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Original Research Article

Morpho-Genetic Relatedness Among Wild and Cultured *Clarias gariepinus* (Buchell, 1822) Collected from Ogbomoso, South-western Nigeria.

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ABSTRACT

Clarias species used in aquaculture are suffering from inbreeding depression with its attendant poor reproductive attributes and there is the need to explore the natural population to improve reproductive performance. Therefore, this work aimed at evaluating the morpho-genetic variability among wild and cultured *Clarias garipinus* available in Ogbomoso, South-Western, Nigeria. Fifty *C. gariepinus* samples were obtained from the wild and cultured populations and only ten were favoured for this work. The DNA was extracted from the caudal peduncles of five representatives of the two sampled populations and DNA amplification was done using cytochrome oxidase region's COX1. Predictive Analytics Software version 20.0 was used for data analyses. Results showed that virtually all morphometric parameters measured were significantly higher in the cultured populations compared to the wild except the dorsal fin length (p=0.346). The A260/280 ratio ranges from 1.85 (C1) to 2.11 (W2), with most samples having a ratio close to 2.0, indicating high-purity DNA, which is around standard recommended DNA concentration for the amplification. The DNA amplified at 700 bp from a portion of mitochondrial DNA (mtDNA) of wild and cultured *Clarias gariepinus*. The genetic distance between each strain is represented by the length of the branch that connects them. Therefore, these suggest that *C. gariepinus* breeders and farmers should source *C. gariepinus* species from the wild in order to genetically enrich the gene pool. Additionally, head region-based morphometric traits might be more informative in distinguishing wild and cultured populations of *C. gariepinus*.

Keywords: Morpho-Genetic, Wild, Cultured, Cytochrome oxidase, *Clarias gariepinus*

Introduction

Genetic diversity means different forms of the genetic makeup of organisms within the given population or species.[1] Genetic variation plays the key role in the long-term survival of the natural populations because it ensures high fitness level and allows the populations to be able to adapt to new environmental conditions. This is inherent in all organisms and is necessary for evolutionary adaptation.[2] Populations with limited genetic variation are less capable of evolving to cope with new environmental challenges, like climate change, and are at greater risk of extinction.[3] In Nigeria, *C. gariepinus* has gained popularity and attracted the interest of the aqua culturists because of its high resistance to diseases, fast growth rate, high fecundity, palatability, high stocking densities under culture conditions and ability to tolerate a wide range of environmental conditions.[4] This notwithstanding, the cultured catfish populations is faced with series of challenges such as inbreeding depression, founder effects, genetic drift that reduces genetic diversity.

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Genetic diversity in a germplasm is very cardinal and strategic to breeders as the greater the genetic diversity, the better for breeding and improvement. Morphometric analysis has proven to be useful in species, races and population differentiation and has been widely employed in identification of different fish stock[5] for breeding and genetic manipulation purposes. Fishes exhibit high phenotypic plasticity and quickly adapt themselves to environmental changes by changing certain morphometric traits.[5] The economic significance of *Clarias gariepinus* relies heavily on its species identification and genetic structure. *C. gariepinus* is the most widely grown fish in Nigeria due to its high production, fast growth rate, high stocking densities and disease-resistant capability.[6] Studies on African catfish, *Clarias gariepinus*, have used molecular markers to determine diversity. However, there is limited information on their morphological structure. To manage brood stocks effectively, it's essential to compare the genetic composition of cultured and wild populations. Factors like low reproductive active of parents can erode wild genetic diversity. This study investigates morphological and genetic variations among wild and cultured strains of *C. gariepinus* in South West Nigeria, aiming to understand the population structure in wild and farmed strains.

Materials and Methods

The Sturdy Areas

The work was carried out in Ogbomoso, Oyo State, Nigeria between June, 2023 and January, 2024. The study collected wild samples from Oba Reservoir and cultured samples from the Fisheries and Aquaculture Department of Ladoke Akintola University of Technology, Ogbomoso. Wild sampling was conducted at Latitude 8°9'N and 8°12'N and Longitude 4°9'E and 4°15'E, while LAUTECH's location is 8° 8′ 0″ North, 4° 16′ 0″ East. The dam, located within the Oba River channel, has a 5 m wide embankment, spanning 350 m long and 17 m high. The

study area is in the tropical climatic zone, with two seasons: wet (March/April-October) and dry (November-March). The temperature is high, with an annual mean of 27°C and relative humidity ranging from 60 to 80%. The vegetation is Savannah and baobab trees.[7]

Sample Collection
Nine and ten samples (N=19) of the wild and cultured *Clarias gariepinus* were collected from Oba Reservoir and the Fisheries and Aquaculture Department of Ladoke Akintola University of Technology, Ogbomoso, respectively.

Morphological Measurement
Twelve (12) morphometric measurements were investigated using Teugels' (1986) guidelines.[8] These parameters are standard length (SL), total length (TL), body weight (BW), head length (HL), head weight (HW), dorsal fin length (DFL), anal fin length (AFL), pectoral fin length (PFL), length of pectoral spine from origin to end (SPL), snout length (SNL), length of caudal fin (LCF), and length of caudal peduncle (LCP).

DNA Extraction
The gSYNCTM DNA Extraction Kit Geneaid Biotech Limited, Taiwan, was used for this protocol. To prepare the sample lysate, 25 mg of freshly homogenized caudal peduncle was micro-centrifuged and mixed with 200 µl of Glutathione S-Transferase Buffer and 20 µl of Proteinase K. The mixture was then vortexed with Vortex-Genie 2 of **Scientific Industries**, **United States** and incubated with **Heratherm Incubator** of **Thermo Fisher Scientific**, USA, overnight at 60ºC until clear. During incubation, 200 µl of elution buffer was transferred to each sample in a 1.5 ml Eppendorf's Minispin microcentrifuge tube (Germany) and heated at 60ºC. After centrifugation for 2 minutes at 14-16,000 x g, the supernatant was carefully transferred to a 1.5 ml microcentrifuge tube. 200 µl of Granular Sub Base (GSB) Buffer was added and shaken rapidly for 10 seconds. At this step, the sample and GSB Buffer are completely combined to form a homogenous solution. The RNA was efficiently degraded by adding 5 µl of RNase A (50 mg/ml) to GSB Buffer, shaking vigorously, and incubated at room temperature for 5 minutes.[9] Precisely 200 µl of absolute ethanol was added to the sample lysate, shaken vigorously for 10 seconds, and the precipitate was broken up. The mixture was then transferred to the GS Column, centrifuged, and thoroughly mixed to achieve a homogeneous solution. The GS Column was centrifuged with 400 µl of W1 Buffer, 600 µl of Wash Buffer, and discarded, then placed back in the collection tube and centrifuged again for 3 minutes to dry the column matrix. The GS Column was transferred to a microcentrifuge tube, and 100 µl of pre-heated Elution Buffer was added, absorbed, and centrifuged for 30 seconds to elute purified DNA.[9]

Quantification and DNA Purification
The sample codes W1, W2, W3, W4, W5, C1, C2, C6, C7, and C8 are unique identifiers for each sample as the letter W represents the studied wild *Clarias gariepinus* samples and C represents their cultured counterparts. The DNA samples were measured at 260, 280, and 230 nm using a Nanoquant plate on the Infinite F200 instrument. The instrument automatically measured at 340 nm to bypass contaminants. The concentration of DNA was calculated using the Lambert-Beer law, and the 260/280 ratio was used as a purity indicator.

Polymerase Chain Reaction Assay
The PCR cocktail mix includes 10× PCR buffer (2.5 µL), 25 mM $MgCl_2$ (1.0 µL), primers (2.0 µL), DMSO (1.0 µL), 2.5 mM DNTPs (2.0 µL), Taq (0.1 µL), DNA (3.0 µL), and H_2O (13.4 µL). The reaction is carried out for 36 cycles, with a final extension at 72°C and the product is stored at 10°C.

Statistical Analysis
The data obtained from the morphological traits were subjected to summary statistics (Mean and error) and regression analysis to determine their length-weight relationship using SPSS version 25 and Microsoft Excel respectively. The significance of morphological differences between wild and cultured populations was assessed using an independent sample t-test at a significance level of $P < 0.05$. Morphometric measurements were also subjected to multivariate analysis based on Clustering Analysis and the Bray-Curtis Algorithm using PAST software (version 4.11) to group samples. The 2021 MEGA X software (version 10.2.6) was used for the phylogram.

Results and Discussion

Morphometric Analysis of Wild and Cultured Clarias gariepinus
The morphometric traits of the two populations (Wild and Cultured) were explored in Table 1. The summary statistics (mean and standard error) of the traits as well as an independent t-test were conducted to determine the extent of the significance on the studied groups (Cultured, Wild). It's worth noting that all morphometric parameters measured were significantly higher in the cultured populations compared to the wild except for the dorsal fin length in which there is no significant difference (p=0.346).

Table 1. Probabilities (P), t-test, and morphometric means of the two sampled *Clarias gariepinus* populations

Morphometric Parameters	P	t-statistics	Wild mean	Cultured mean
Total length (cm)	0.000*	-5.248	29.18±1.84[a]	40.46±1.20[b]
Standard length (cm)	0.000*	-5.188	25.36±1.70[a]	35.46±1.04[b]
Body Weight (kg)	0.000*	-5.441	0.18±0.03[a]	0.82±0.11[b]
Head Length (cm)	0.000*	-5.870	7.09±0.51[a]	10.88±0.41[b]
Head Weight (g)	0.000*	-5.868	61.89±9.30[a]	169.60±15.24[b]
Snout Length (cm)	0.000*	-8.198	1.73±0.04[a]	2.60±0.09[b]
Dorsal Fin length (cm)	0.346	-0.970	16.31±1.08[a]	17.64±0.86[a]
Anal Fin length (cm)	0.005*	-3.255	10.58±0.82[a]	13.88±0.62[b]
Pectoral Fin length (cm)	0.040*	-2.227	1.13±0.15[a]	1.61±0.15[b]
Pectoral Spine Length (cm)	0.000*	-4.394	2.76±0.26[a]	4.16±0.19[b]
Length of Caudal Fin (cm)	0.002*	-3.585	4.21±0.32[a]	5.71±0.28[b]
Length of Caudal Peduncle (cm)	0.000*	-4.407	2.21±0.16[a]	3.38±0.21[b]

Value = mean ±SEM: Values followed by the same superscripts in the same row are not significantly different ($P < 0.05$).

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There was a statistically significant difference in the total length (cm) across the two populations (Wild: 29.18±1.84, Cultured: 40.46±1.20, p-value = 0.000, t = -5.248). The standard length of the wild population has a mean value of 25.36±1.70 and its cultured counterparts at 35.46±1.04, exhibiting statistically significant difference (p-value = 0.000, t = -5.188). The cultured population had a significantly higher body weight mean of 0.82±0.11 kg compared to the wild's 0.18±0.03 kg (p-value = 0.000, t = -5.441). The difference in head length (cm) between the two populations was statistically significant (Wild: 7.09±0.51, Cultured: 10.88±0.41, p-value = 0.000, t = -5.870).
The head weight (g) was 61.89±9.30 for wild populations and 169.60±15.24 for cultured populations (p-value = 0.000, t = -5.868).
Snout length (cm) mean was 1.73±0.04 in wild populations and 2.60±0.09 in cultured populations (p-value = 0.000, t = -8.198). Dorsal fin length (cm) averaged 16.31±1.08 in wild populations and 17.64±0.86 in cultured populations (p-value = 0.346, t = -0.970). The mean length of the anal fins (cm) was 10.58±0.82 in wild populations and 13.88±0.62 in cultured populations (p-value = 0.005, t = -3.255). The mean pectoral fin length (cm) was 1.13±0.15 in wild populations and 1.61±0.15 in cultured populations (p-value = 0.040, t = -2.227). The mean pectoral spine length (cm) was 2.76±0.26 in wild populations and 4.16±0.19 in cultured populations (p-value = 0.000, t = -4.394). The mean length of the caudal fin (cm) was 4.21±0.32 in wild populations and 5.71±0.28 in cultured populations (p-value = 0.002, t = -3.585). Length of Caudal Peduncle (cm) mean was 2.21±0.16 in wild populations and 3.38±0.21 in cultured populations (p-value = 0.000, t = -4.407).

The Length-Weight Relationship of the Wild and Cultured Clarias gariepinus

The length-weight (log-transformed) relationships of the two sampled populations were determined by linear regression analysis, scatter diagrams of the total length and weight were plotted, and the hypothesis were set at 95% confidence level. The regression scatter diagram for the wild population is shown in Figure 1. The length-weight regression equation for the wild samples is Log W = 2.5825x - 1.5659, where the regression constant or intercept (a) is -1.566 and the regression coefficient (b) is 2.582. The study found a significant relationship between body weight and the total length of wild *Clarias gariepinus*, rejecting the null hypothesis (Ho_1) due to a p-value less than 0.05 (p=0.000). The wild population's coefficient of determination (R^2) is 0.9708, indicating a strong correlation between total length and body weight among the sampled populations. The regression analysis and scatter diagram are shown in Figure 2. The length-weight regression equation for cultured samples is Log W = 5.1709x - 5.4374, with a regression constant of -5.437 and a coefficient of 5.171.

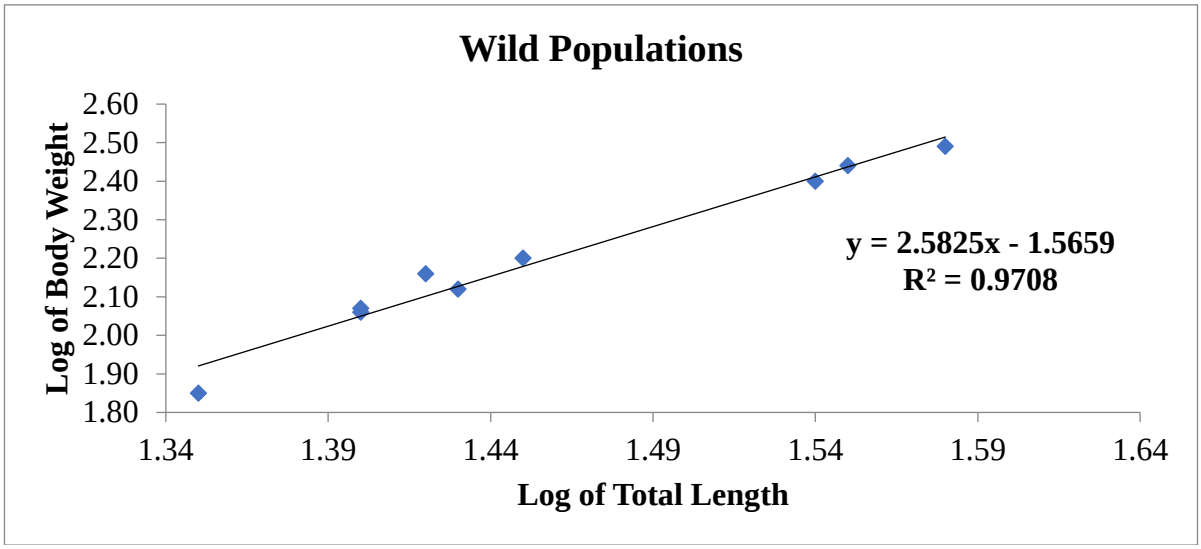

Figure 1: Log of Total Length and Log of Body Weight Relationship of the Wild *C. gariepinus* Populations

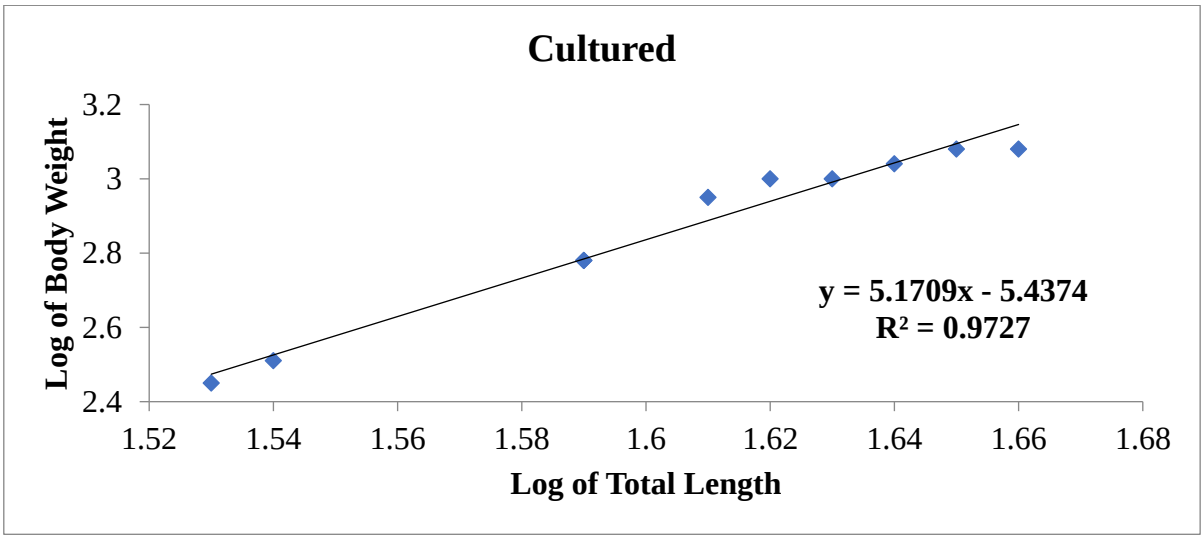

Figure 2: Log of Total Length and Log of Body Weight Relationship of the Cultured *C. gariepinus* Populations

The null hypothesis (Ho2) was rejected due to a p-value of 0.000, indicating a strong significant relationship between body weight and total length in cultured populations. The cultured population's coefficient of determination (R^2) is 0.9727, indicating a robust

correlation between total length and body weight among the sampled populations.

Multivariate Analysis of the Morphometric Traits of the Two Sampled Populations

Figure 3 shows the two-way similarity context of the morphometrics of the two sampled populations indicating Head weight (HW) and Body weight (BW) as the traits most responsible for the variations between the sampled populations. The traits are significantly higher in cultured populations compared to the wild. Two main clusters based on their similarities were noticeable from the dendrogram (Figure 4). The first clustering includes C9, W7, C10, W9, W8, W1, W6, W2, W3, W5 and W4 with 67.5% similarity. The other one includes the clustering of C4, C1, C2, C5, C6, C8, C3, and C7 with 75% similarity in their traits. C9, W7, C10, W9, and W8 have over 90% similarity index. In addition, W1, W6, W2, W3, and W5 are 90% similar in terms of their morphometric traits (Figure 4). The study found that the minimal similarity coefficient between wild and cultured populations of *C. gariepinus* was 45% , which was because of the huge variation between the HW and BW of the two populations . A strong cophenetic correlation value (0.8679) was recorded in the clustering algorithm.

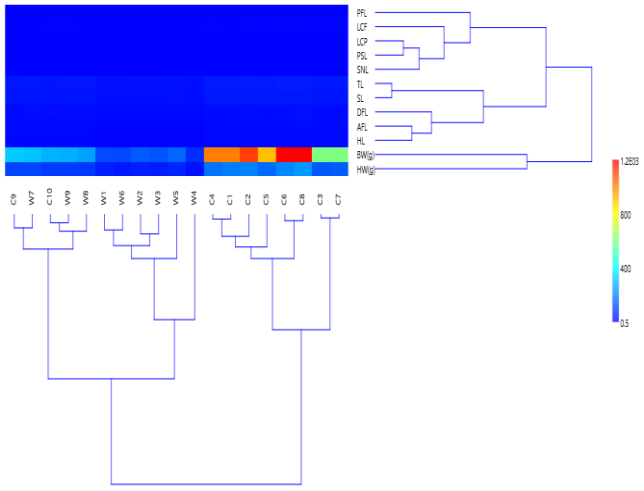

Figure 3: The dendrogram displaying a two-way similarity context of morphometrics of wild and cultured *Clarias gariepinus* using Bray-Curtis's clustering algorithm.
W1-W5= wild samples, C1-C5= Cultured Samples

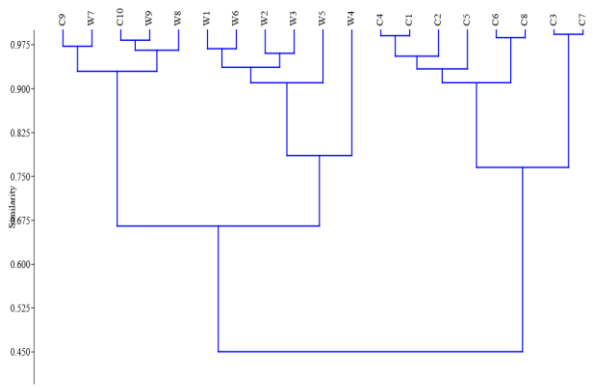

Figure 4: Similarity coefficient of the morphometrics parameters of the two sampled populations.
W1-W5= wild samples, C1-C5= Cultured Samples

Concentration and Purity of DNA Samples

Table 2 shows the results of the spectrophotometer-based DNA concentration and purity study for both the wild and cultured fish samples. The DNA concentration in the samples varies from 100.11 ng/µl (W3) to 185.08 ng/µl (W1). The A260/280 ratio ranges from 1.85 (C1) to 2.11 (W2), with most samples having a ratio close to 2.0, indicating high-purity DNA. The 260/230 ratio, which is another measure of purity, ranges from 1.89 (C8) to 2.44 (W5), with most samples having a ratio close to 2.0, indicating high-purity DNA. The spectrophotometer set 50 as the dilution factor, which is constant across the samples.

Visualization in Agarose Gel

The DNA was successfully extracted from the caudal peduncles of five representatives of the two sampled populations. The amplification of the cytochrome oxidase region's COX1 gene (700 bp) from a portion of mitochondrial DNA (mtDNA) of wild and cultured *C. gariepinus* was observed. The primers used were Cox1F: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and Cox1R: 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'. The gel electrophoresis shows the polymerase chain reactions for the samples (Plate 1).

Phylogenetic Evaluation

The amplicons display diversity in genotypes of wild and cultured Clarias species, with closely related genotypes clustered together and their neighbours joined closely (Figure 6). Eight strains of the sampled populations are used to build the phylogram. Strain C2 and strain C6 were found to be closely related while strains W1, W2, C1, W5, W3, and W4 were also clustered together showing their close relatedness (Figure 6). The genetic distance between each strain is represented by the length of the branch that connects them. The branch length between *C. gariepinus* strain C1 and *C. gariepinus* strain W2 is 0.000, which means they are very closely related genetically. *C. gariepinus* strain C1 is most closely related to *C. gariepinus* strain W2 and *C. gariepinus* strain W1. In addition, *C. gariepinus* strain W5 and *C. gariepinus* strain W3 are the next most closely related strains. *C. gariepinus* strain C8 is the most genetically different strain out of the eight.

Significant differences were observed in total length, standard length, body weight, head length, snout length, anal fin length, pectoral fin length, pectoral spine length, caudal fin, and caudal peduncle ($p<0.000$) with dorsal fin length not statistically significant. This indicates a pronounced impact of cultural conditions on the physical characteristics of these fish. Solomon, *et al* [10] also found that cultured fish display larger morphometrical features than wild ones, highlighting the impact of intensive fish culture practices on diverse species. Morphometric studies involving shape variation and other components aid in identifying new species of fish, and detecting fish population modifications.[11] Morphometric analysis is crucial for studying fish biology due to their sensitivity to environmental changes and their rapid adaptation to changes in morphometry.[12, 13]

The studied cultured fish population shows significantly larger dimensions compared to wild counterparts, indicating a positive impact of aquaculture on fish morphology, as per previous studies. Agbolade, *et al* [13] found similar trends in size-related traits between wild and cultured fish populations, attributed to selective breeding, altered feeding regimes, and environmental conditions. Genetic factors and growth-promoting substances also influence fish morphology. The study found significant differences in the means of all morphometric traits between the two studied populations of *C. gariepinus*, thus revealing the existence of heterogeneity between the two populations. There is a significant body weight increase among the cultured populations, and this echoes the findings of Ikpeme *et al* [14] in different species, suggesting a commonality in the impact of culture conditions on the growth patterns of different fish species. The observed differences in anal fin length are consistent with Langer *et al* [15] observations, supporting the notion that cultural environments contribute to the development of specific morphological features.

The dendrogram shows that body weight and head weight are the most important characteristics influencing variation between the two sample populations. The cluster analysis revealed stronger morphometric correlations (similarity index) between wild and cultivated *C. gariepinus*. This shows that despite their differences, the two groups are the same species. These findings are consistent with the similarities

between the cultured and wild *C. gariepinus* reported by Solomon *et al* and Ikpeme *et al* . [10, 14] The study recorded a Cophenetic correlation coefficient of 0.8679, indicating high preservation of pairwise distances between data points. The length-weight relationship in the wild fish shows a strong correlation between body weight and total length. The regression analysis shows a substantial association between body weight and total length, which is consistent with earlier research that focused on growth characteristics and the development of weight-length relationships.[14]

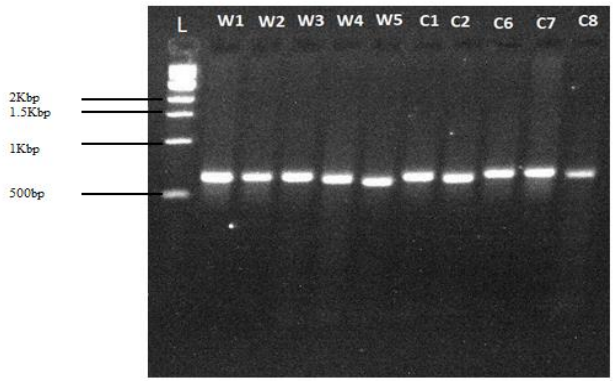

Plate 1. Agarose (1.5 %) Gel Electrophoretic Profile of PCR Amplification of COX1 Gene (700bp).
M is marker (500bp), W1, W2, W3, W4, and W5 are caudal peduncle samples of the wild *Clarias gariepinus* and C1, C2, C6, C7, and C8 are the same trait profiles of the cultured sample populations

The study demonstrates a significant relationship between body weight and total length in cultured populations, with a strong correlation observed, indicating a good predictor of fish body weight. The regression model accurately predicts body weight based on fish length with 97.27% coefficient of determination. The findings align with prior research on fish morphometrics, which have consistently shown strong correlations between length and weight across various fish species.[14] Fish traits evaluation is crucial for fisheries management and aquaculture, as highlighted in a study on European and African catfish, highlighting growth parameters and features.[14] Researchers can assess fish populations' health, growth, and quality by understanding genetic, environmental, and cultural factors, and their relationship between total length and weight for predictive purposes.[14]
The study indicates that all DNA samples are of high quality and purity, with minimal protein or substance contamination, as most have a ratio close to 1.8, which is the most acceptable ratio for good concentration. Agarose gel electrophoresis from the result reveals a single, high molecular weight DNA band with minimal shearing and RNA contamination and this corroborates the findings of Wright *et al*.[16] Another study found that the quality of DNA isolated from processed food and feed via different extraction procedures varied, with some methods producing DNA with higher purity and yield than others.[17] For pure DNA, the A260/280 ratio is typically around 1.8, indicating minimal contamination by substances like proteins and phenols.[18]

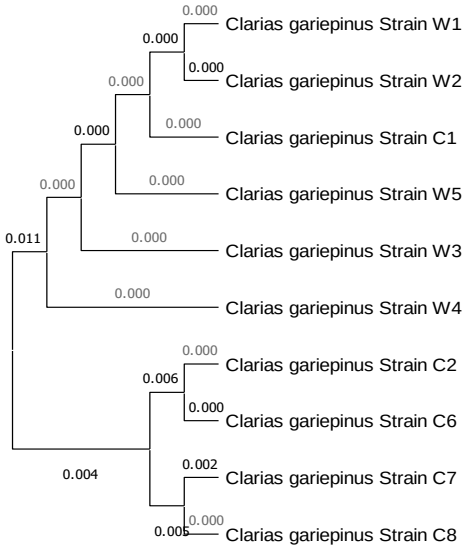

Figure 6. Phylogram of wild and cultured samples of *Clarias gariepinus*
W1-W5= wild samples; C1, C2, C6, C7 & C8= Cultured Samples

Technical issues with the isolation stages, such as improper sample destruction that prevents DNA in cells from lysing during isolation, can have an impact on the purity and concentration of DNA.[19]

Table 2: Nanodrop Spectrophotometer of Quantity and Quality of the Genomic DNA extracted from the Sampled *Clarias gariepinus*

Sample Code	Accession No	ng/ul	A260	A280	260/280	260/230	Constant
W1	PP479593	185.08	3.702	1.792	2.07	2.13	50
W2	PP479594	155.66	3.113	1.472	2.11	2.33	50
W3	PP479595	100.11	2.002	1.018	1.97	2.34	50
W4	PP479596	133.93	2.679	1.424	1.88	2.36	50
W5	PP479597	132.96	2.659	1.391	1.91	2.44	50
C1	PP479598	120.63	2.413	1.304	1.85	2.09	50
C2	PP479599	112.09	2.242	1.149	1.95	2.15	50
C6	PP479600	136.32	2.726	1.39	1.96	2.32	50
C7	PP479601	100.81	2.016	1.014	1.99	2.37	50
C8	PP479602	120.08	2.402	1.207	1.99	1.89	50

Cytochrome C oxidase (COX) is a crucial enzyme in mitochondria's electron transport chain, reducing oxygen to water and generating an electrochemical gradient for ATP synthesis.[20] The cytochrome oxidase region is the genomic region that encodes the COX subunits or their functional domains. The COX complex is composed of multiple subunits, with the catalytic core formed by COX1, COX2, and COX3, which are encoded by the mitochondrial genome.[21] In this study, the COX1 region was used for the DNA sequencing. The COX1 gene (700 bp) was amplified from the mitochondrial DNA of wild and cultured *C. gariepinus.* The Cox1 region is useful for identifying vertebrate and

invertebrate species, but it is not appropriate for plants or certain groups, notably those with heterozygous hybrid lineages or mitochondrial introgression events.[22]

Phylogenetic trees are the most direct representation of the principle of common ancestry—the very core of evolutionary theory.[23] Phylogenetic trees are used to depict evolutionary relationships among biological entities. The tips of the tree represent the *Clarias gariepinus* strains, and the lengths of the branches represent the genetic distance between the strains. Phylogenetic trees are a fundamental tool in evolutionary theory, illustrating shared ancestry and representing the evolutionary relationships between biological entities.[23] In this study, the tree's tips reflect the *C. gariepinus* strains, while the branch lengths represent their genetic distance. The phylogenies reveals that all *C. gariepinus* strains are genetically closely linked due to their relatively short branch lengths and have a similar level of inherent genetic diversity with their wild counterparts, and this is in the same trend with the findings of Suleiman *et al.* [24] The study reveals a strong correlation between body weight and total length in wild fish and this is consistent with previous research on growth characteristics. In cultured populations, the regression model accurately predicts body weight based on fish length, with a 97.27% coefficient of determination. Understanding genetic, environmental, and cultural factors can help assess fish populations' health, growth, and quality for predictive purposes.

Conclusion

The present research work therefore suggests that *C. gariepinus* breeders and farmers should source *C. gariepinus* species from the wild in order to genetically enrich the gene pool. There is a need to deliberately inject new breeds from the wild into the cultured population in order to boost their evolutionary potentials. To avoid trading one woe for another, the introduction of these new genotypes must be done systematically, because out-breeding depression is an imminent risk, if the genetic distance between the populations and the new breeds is too large. This study also suggest that genetic variations among fish species stands to expand the understanding of fish farmers on the diversity, environmental impact and cultural factors which can help to assessed fish populations' health, growth and quality for future predictive purposes.

Conflict of Interest

Authors declared no competing of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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