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Genetic Variability in Cultured and Wild Population of *Clarias gariepinus* Using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

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Authors' contributions

This work was carried out in cooperation between all authors. Author OMP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AEF and IJA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The present study was undertaken to determine the genetic differences between cultured and wild populations of *Clarias gariepinus* in Southwest Nigeria using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).
Study Design: Three wild populations from River Osun, Owena, and Agbabu and three farmed populations from hatcheries in Akure, Ilesa and Ado-Ekiti, were collected for the study
Place and Duration of Study: Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Nigeria, between june 2012 and January 2013
Methodology: Two hundred and forty live specimens of *C. gariepinus* were analysed for their genetic differences. Live specimens comprising forty individual from each location were collected and kept in six concrete tanks (2x1x1) m^3 at Teaching and Research Farm of Fisheries and Aquaculture Department Federal University of Technology, Akure. Blood samples were collected and used for Sodium Dodecyl Sulphate Polyacrylamide Gel (SDS-PAGE) for the protein profiling.

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Results: The protein profile produced two clusters which indicate divergence of the species into two sub species. There was high level of genetic heterogeneity among samples of *Clarias* species and those from various locations under study. The SDS-PAGE banding patterns across the six populations studied showed that the mean heterozygosity for the six populations ranged from 0.398 to 0.425.
Conclusion: This study has established that there is genetic difference in both wild and cultured *C. gariepinus.* Further study involving RAPD, microsatellite, and AFLP can be used to maximize the efficiency of the study. Also, increased number of samples could improve the validity of this study.

Keywords: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Clarias gariepinus; wild; cultured; genetic distance.

1. INTRODUCTION

The identification of fish species is very important especially in the developing countries where diverse but unidentified species are found. Species identification will enable the genetic materials or biological potentials of individual species to be fully understood and may be used for breeding program.

Genetic variability in fishes has been proven valuable for aquaculture and fisheries management, identification of stocks, selective breeding programmes, restoration of ecology and estimating genetic contributions in stocks. Thorough knowledge of genetic variability within the species is considered prerequisite for efficient utilization of biological resources [1].

Catfish species of the genus *Clarias* originate in Africa and the Middle East [2]. The *Clarias* species reported in Africa, i.e. *Clarias gariepinus, Clarias liocephalus* (=carsonii), *Clarias werneri* and *Clarias alluaudi* [3,2,4], inhabit wetlands, lakes and their affluent rivers. Of the catfish found in Nigeria, the African catfish (*C. gariepinus*) is the most popular and relished for food. *Clarias gariepinus* commands high demand from Aquaculturists. Its high growth rate at high stocking densities under culture condition, high fecundity rate, resistance to diseases, ability to tolerate a wide range of environmental extremes has been reported [5]. Due its ability to reproduce in captivity and easy manipulation of its breeding cycle, C. *gariepinus* and other close species has been successfully bred to produce hybrid. The possibility of these hybrid escapees invading natural freshwater habitats due to flooding has been reported by [6]. It is therefore possible that the commercial hybrid escapees may have hybridized with the native catfish and contaminated the native Clariid gene pool [7].

It is important to compare the genetic composition of hatchery strains with their wild populations and also within strains from hatcheries as well as within wild strains. Studies demonstrated considerable loss of genetic variation in hatchery stocks as a result of different factors including a low effective number of parents, domestication selection or the mating design [7,8].

The interactions between farmed and wild fish can be problematic for many reasons. Selectively bred, farm-raised fish that escape from aquaculture facilities and reproduce with wild fish can cause a decrease in the genetic diversity. Also, farm-raised fish that escape into the wild can negatively affect wild populations through competition for food, habitat, and

mates. Farm environment can lead to increased levels of disease and parasites and these can be transferred to wild fish by escapees. Beside the intraspecific hybridization, interspecific gene exchanges have also been observed [9].

This study is aimed at assessing the genetic variation and diversity of population of *C. gariepinus* in three rivers and three hatcheries in South West Nigeria

2. MATERIALS AND METHODS

2.1 Samples Collection and Preparation

Two hundred and forty individual samples of cultured and wild *Clarias gariepinus* with average weight of 675±2.27g were obtained from six different locations. The cultured samples were obtained from fish farms in Federal University of Technology Teaching and Research Farm (Akure), Leventis Agricultural training school, Ilesa (Osun State), Ekiti State Ministry of Agriculture, Ado-Ekiti (Ekiti state), while the wild counterparts were obtained from fishermen at Oluwa River, Agbabu (Ondo State), River Osun, Esa-Odo (Osun-State),Owena River, Owena (Ondo State) (Fig. 1). The fish were kept in six different concrete tanks for three days to acclimatize to the new environment so as to adjust to the new chemistry of the tank thus helping ensure the health of the samples in Teaching and Research Farm of Federal University of Technology, Akure.

2.2 Extraction of Soluble Cell Protein

The soluble cell protein was extracted from the blood sample obtained from the fish. 200µl of blood was collected from the caudal vein with syringe and needle containing 0.1M EDTA at a concentration of 20uL/1mL from each fish and 800µl of 0.1M Tris-HCl pH 7.6 was added followed by vortexing for 1min. The mixture was centrifuged at 10,000 rpm for 5min. The supernatant was collected and kept at 4ºC.

2.3 Gel Preparation and Soluble Cell Protein Fingerprint Analysis

Firstly, 6% of stacking gel (1000µl of 30% acrylamide, 5000 µl of stacking gel buffer, 3600µl of deionized H_2O, 25µl of 20% SDS stock, 25µl of 10% ammonium persulfate and 10µl of TEMED) and 12.5% of separating gel (8000 µl of 30% acrylamide, 630µl of resolving gel buffer, 3600µl of deionized H_2O, 25µl of 20% SDS, 25ml of 10% ammonium persulfate and 10 µl of TEMED) were prepared. The extracted soluble cell proteins from the blood together with higher and lower range of protein molecular weight marker were mixed with 2X SDS PAGE sample buffer in a 1: 1 ratio. The samples were loaded into sample well by using micro pipette followed by electrophoresis at 220 V until the bromophenol blue dye front reaches the bottom of the gel. After electrophoresis, the gel was stained overnight with Coomassie Blue R-250. The gel was then distained by soaking in distilled water for 15 min. Finally, the soluble cell protein profiles of the samples were visualized and captured using Gel Compac (Bio Rad, USA). Gel preparation, electrophoresis conditions, staining and destaining procedures were done according to [10].

2.4 Scoring of Bands and Statistical Analysis

A data matrix was prepared based on the absence or presence of bands, at each position for all soluble cell protein profiles by giving scores of 0 and 1 respectively. The matrix data was

then analysis by program for genetic diversity analysis with AFLP (and RAPD) population data *AFLP-SURV* 1.0 version 2.1 [11] based on unweighted pairgroup method with arithmetic means (UPGMA) [12].

3. RESULTS AND DISCUSSIONS

3.1 Results

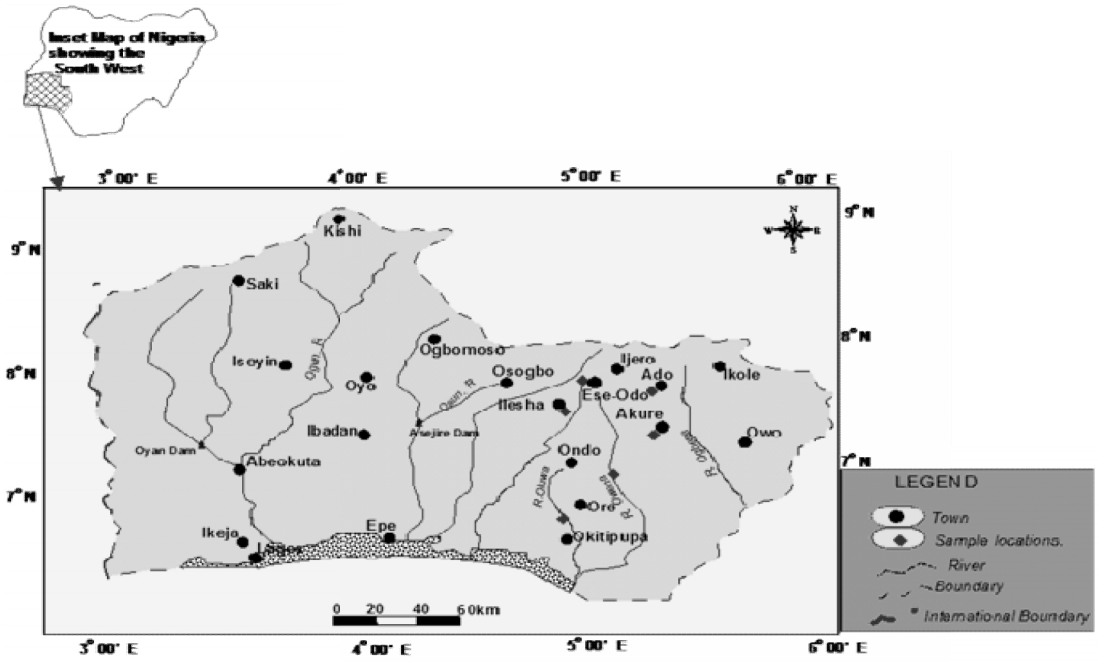

Fig. 1. Map of South West Nigeria showing the study areas

In the present study, SDS-PAGE assay was successfully generated soluble cell protein profile of the *C. gariepinus*.

Fig. 2 shows the protein gel electrophoresis bands of *Clarias gariepinus* from six populations. An examination of these bands show distinct qualitative and quantitative intra and inter specific variation in terms of the number, position and intensity of the stain. The numbers of bands produced vary from three to five and their molecular weight varies between twenty two (22kDa) to one hundred and fifty kilo Dalton (150kDa). Observed also from the profile is the overlapping in the molecular weight of some bands (22, 23, 50, 60, 70, 80, 100 and 110kDa). Band with 150kDa molecular weight is observed to common to lanes 4 and 9 while 120kDa is common only to lane 13. Protein profile of lanes 3, 12 and 16 did not shown or produce any band. This might be that protein in these were denatured thus affect the output of profiling. Bands with molecular weight of 50 and 60kDa were observed to be common to nearly all the lanes except only lane 12.

In addition to the similar protein profile displayed by some of the fish samples, the relative intensity of the band is also not similar. Bands with 21 and 100kDa produced were thick in lane 1, 2, 4, 5, and 6, but light in lanes 13, 14, 15, 17 and 18. Also, bands with 21, 45 and 55

and 150kDa produced thick band in lane 4. Some like 55kDa produced light intensity of band in lanes 5, 6, 7, 8, 14, 15, 17, and 18. This also isobserved in band with 22, 100, and 45kDa show light intensity in lanes.

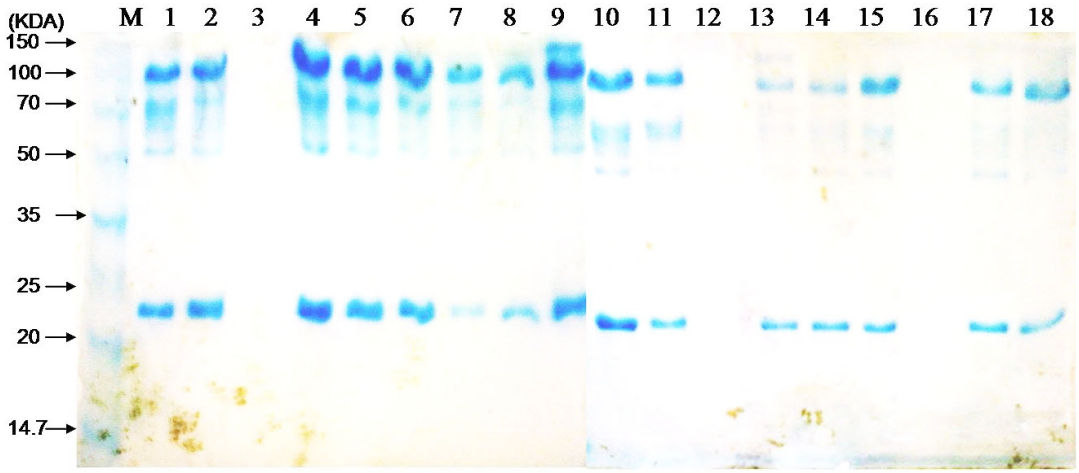

Fig. 2. Electrophoretic protein profile obtained from SDS-PAGE of *Clarias gariepinus* populations from Akure (lane1-3); Ilesa (lane4-6); Owena (lane7-9); Agbabu (lane 10-12); Esaodo (lane13-15) and Ado-Ekiti (16-18)

Table 1 shows pairwise comparison of the genetic distance (GD) of *C. gariepinus* populations from six locations using soluble protein profiling. The result shows that GD range from 0.007 to 0.034 across the six populations.

Table 1. Genetic Distance of *C. gariepinus* from six populations from six populations based on SDS-PAGE

Akure	Ilesa	Owena	Agbabu	Esaodo	Ado-Ekiti	
-	0.024	0.034	0.029	0.019	0.024	Akure
	-	0.029	0.024	0.015	0.020	Ilesa
		-	0.020	0.019	0.014	Owena
			-	0.023	0.014	Agbabu
				-	0.007	Esaodo
					-	Ado-Ekiti

Fig. 3 shows comparison between the mean heterozygosity of the *Clarias gariepinus* from six different populations. The value recorded for mean heterozygosity across the populations ranged from 0.398 in Owena to 0.425 in Agbabu. The result suggests that Agbabu is the most variable population while Owena is the least.

A dendrogram (Fig. 4) was also generated based on the soluble cell protein of the fish samples by using PAST software version 2.16. The dendrogram consist of two clades namely 1 and 2, meaning that two broad group were identified in the populations. Clade 1 has only Akure3, while clade 2 is subdivided into A and B. Sub clade B consist of Agbabu 3 and Ado-Ekiti1 while A is further subdivided AI and AII. Sub division AI comprises Ilesa1, Akure2, Akure1, Ilesa2, Ilesa 3 and Owena 1-3. Sub division AII also noticed to have two

groups namely 1 and 2 with 1 consisting of Esaodo1-2, Ado-Ekiti 2-3. Group 2 of subdivision AII comprises of agbabu1, Esaodo3 and Agbabu 2.

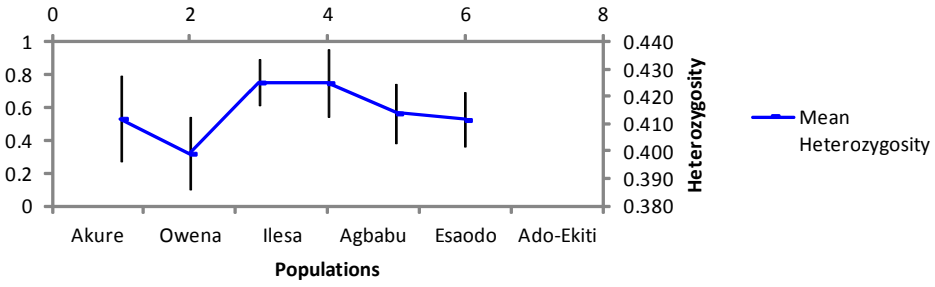

Figure 3: Protein electrophoretic bands across the six populations of *C. gariepinus* .
The bar represent heterozygosity while the linked points represent the mean heterozygosity in each population

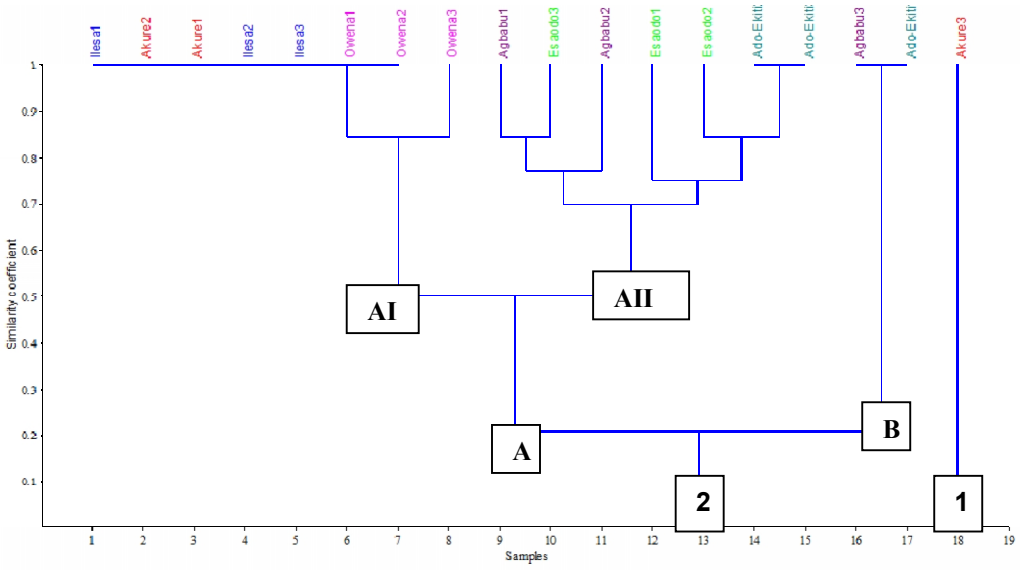

Fig. 4. Dendrogram generated from data matrix from SDS-PAGE of six populations of *C. gariepinus*

3.2 Discussion

This study reveals some degree of variability in the soluble protein profiling. The discriminating protein bands between wild and cultured *C. gariepinus* obtained in this study was very low. Only the 150kda bands present in lane 9 which were absent in the other

samples can be used as the diagnostic marker for the biochemical identification of the Owena and Esaodo (wild) populations.

The overlapped molecular weight of some of the bands indicate that some proteins are common to the individual fish sample. This could be a result of belonging to the same species or taxa. The presence of unique band in lane 9 and 13 give an indication that certain soluble protein is common to these samples. The lanes that produced this unique bands are from wild population (Owena and Esaodo), while no such difference was noticed in cultured populations. This unique band could be used as a marker in delineating wild population of *C. gariepinus*. Thus, the non-variable features (protein profiles and bands intensity) shown by wild type *C. gariepinus* fish is beneficial for monitoring of the protein composition of cultured *C. gariepinus* fish. The soluble protein profile resolved through SDS-PAGE was able to reveal intraspecies variation, as some of the profiles were not all uniform. This was in contrary to [13] who reported that in *A. salmonida* from 28 Finnish strains uniform SDS pattern was observed. [14] also has reported the use of SDS PAGE in Protein fingerprinting of *A. hydrophila* and also revealed the genetic similarity between strains and reference strain (MTCC 646). They observed unique clear and distinct bands in different local and reference strains of *A. hydrophila,* which was suggested as suitable for molecular identification. The consistent presence of some similar bands (100, 70, 50 and 22.5) in all the samples from six locations confirms that they were close in ancestral relationship. Similar observations were made by [15] in muscle protein electrophoretic studies between *O. niloticus* and *S. galileus*.

The high mean heterozygosity observed in the study confirmed high genetic variability in C*. gariepinus* both within and between populations. The mean heterozygosity recorded in this study was high (0.398-0.425). This disagreed with [16] who observed 0.095 in *C. anguillaris* and 0.152 in *C. gariepinus*. Despite the high genetic variability, no diagnostic loci were found. Also, the same author had reported 0.80 genetic distance for 8 loci. The genetic difference recorded in this study was low (0.007 to 0.034). The result of this finding disagreed with report of [17] on genetic distance of *C. anguillaris* and *C. gariepinus.* The difference might be as a result of species difference.

The dendrogram generated from this study shows that zero value for Akure3 and other samples. It thus means that based on soluble blood protein, this sample is different form the other samples.

In this study, high similarity coefficient (0.85) was recorded. This was lower to what Akinwande et al reported (0.92) between hybrid of *H. longifilis*, *C. gariepinus* and *C. angularis*. The high value in similarity coefficient of *C. gariepinus* from six locations also shows the very low genetic diversity. The SDS-PAGE pattern of the *C. gariepinus* samples from various locations shows consistency in many similar protein bands, thus showing close ancestral relationship.

4. CONCLUSION

This study has established that there is genetic variability in both wild and cultured C. gariepinus. Further study involving more definitive and sensitive like RAPD, microsatellite, and AFLP can be used to maximize the efficiency of the study. In addition, increase in number of samples could also be used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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