PROTEIN PROFILING FOR PHYLOGENETIC RELATIONSHIP IN *Tilapia guineensis* FROM SOUTH-WEST NIGERIAN WATER BODIES

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Abstract
Tilapia guineensis is one of the important Cichlid species with good aquaculture potential, economic and nutritional value commonly found in Nigerian coastal waters. Protein electrophoresis was used to determine intra and inter-specific variation among populations. The protein banding pattern of four Tilapia guineensis populations from two different states (Ondo and Lagos; two locations per state) of South-west Nigeria were used to study the phylogenetic relationship among them. The scoring of the gel images obtained after electrophoresis was done and data generated were subjected to cluster analysis. The banding pattern from sarcoplasmic protein indicated a unique profile for two populations (Pepe and Epe) and the electrophoregrams showed similarities among the populations indicating some levels of genetic affinity while Ugbonla and Badagry showed variation in their banding pattern. The molecular weight of the protein bands varied from 20 kDa - 250 kDa. Analysis of molecular variance (AMOVA) demonstrated that genetic variation was mostly among populations. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram revealed patterns of genetic variability among these populations. These results provide useful baseline genetic information for breeding and conservation of T. guineensis populations.

Keywords: *Tilapia guineensis*, Phylogenetic relation, SDS- PAGE, Protein banding

INTRODUCTION

Tilapia guineensis (Bleeker, 1862) is one of the Cichlid species usually found in creeks, lagoons, and other coastal waters of West Africa (Philippart and Ruwet, 1982). It is an important source of animal protein and continues to contribute greatly to the nutritional needs, economic growth, and development of many developing countries like Nigeria. Tilapia fish proteins are good sources of high-quality proteins and polyunsaturated fatty acids (Zibaee-Nezhad *et al*., 2010). The Knowledge of protein polymorphism in tilapia fish germplasm is essential for its efficient breeding, conservation, and management program.

Proteins/enzymes are used as genetic markers as direct products of gene action (Crick, 1963; Nirenberg *et al*., 1963). Gene controlled proteins form the structural basic source of genetic information at various levels of species organization. Electrophoretic techniques are useful in studying variation among fish species. Protein is separated according to its molecular weight in SDS-PAGE. The resolution of this technique is very high and therefore it could be used as a reliable tool for taxonomic purposes (Bartke *et al.,* 1966). Information regarding protein polymorphism in fish germplasm is explored for efficient fish conservation and management program.

Genetic studies of the distribution of protein variations are considered essential for breeding, conservation, and management of fish species (Verspoor *et al*., 2005). Hence, an attempt was made in the present study to detect the protein-based genetic diversity of four different *Tilapia guineensis* populations using the electrophoresis technique– SDS-PAGE, as a basic tool for its improvement.

MATERIALS AND METHODS

Source of Fish

Sample collection was carried out in four locations from two coastal states (Ondo and Lagos; two locations per state) in South-west Nigeria for *T. guineensis* species. The coordinates of the sampling stations are shown in Table 1. After identification, the fish were procured from the fishermen at the landing site of every station and were immediately transported to the laboratory live and acclimatized. Ten (10) samples were randomly selected from each location for protein profiling. They were filleted and skinned with a stainless steel knife and then the muscle meat was used for protein extraction.

Protein Extraction

Homogenization of 1g of fish muscle was done by grinding it in a mortar and adding 1 ml of phosphate buffer saline (PBS) containing protease inhibitor cocktail. The homogenate was centrifuged at 10,000 rpm for 15 min at room temperature and the supernatant (sarcoplasmic protein) was used for electrophoresis.

Qualitative Analysis of Proteins by SDS-PAGE

The protein was resolved by SDS-PAGE (12%) according to Laemmli (1970) using a vertical gel electrophoresis unit. A molecular weight marker

was used for the determination of the molecular mass of each protein. The marker kit was purchased from Fermentas, Lahore, Novagen by Merck (10-250 kDa). The gels were stained with Coomassi blue R-250, destained with a destaining solution (methanol, acetic acid, and distilled water), and were photographed with a digital camera.

Data Analysis

Scoring of protein data across the studied *T. guineensis* populations was done by their presence as 1 or absence as 0 for each category. The binary data so obtained were used to determine the number of polymorphic bands, level of polymorphisms, Nei's Pairwise similarity, and dissimilarity matrices. A dendrogram was constructed by using the un-weighed pair group method with arithmetic average (UPGMA) with NTSYS software.

Table 1 Geographical location of the Sampling Stations

Location	Latitude	Longitude	State
Pepe	N06° 10[1] 01.3[1]	E02° 52.988[1]	Ondo
Ugbonla	N06° 08[1] 31.1[1]	E004° 47[1] 39.8[1]	Ondo
Badagry	N04° 25.012[1]	E02° 52.988[1]	Lagos
Epe	N04° 27.200[1]	E007° 19.618[1]	Lagos

RESULTS

Sarcoplasmic proteins of *T. guineensis* populations were characterized by 12% SDS-PAGE (Table 2; Plate 1). In Pepe and Epe populations, nine protein bands of 250, 130, 100, 70, 55, 50, 45,35 and 25 kDa, in Badagry population, five bands of 130, 50, 35, 30, and 20 kDa while in Ugbonla population only four protein bands of 100, 70, 50, and 25 kDa, respectively, were observed. However, Epe has no band of 100 kDa but has a band of 30 kDa. It was noted that one protein band of 50 kDa was common in all the studied populations (Table 2).

According to Table 3, Nei's genetic distance index between the populations ranged from 0.19 to 0.77. The highest genetic dissimilarity was observed between Pepe and Badagry with a genetic distance of 0.77 followed by Badagry and Epe (0.73) while the least (0.19) was observed between Pepe and Epe. Analysis of molecular variance (AMOVA) revealed 87% genetic variation among populations (Table 4). The UPGMA dendrogram based on the genetic distances revealed three clusters: Cluster-1 consists of Pepe and Epe, cluster -2 consists of Ugbonla and some Badagry samples while cluster -3 consists of Badagry that clustered separately (Figure 1).

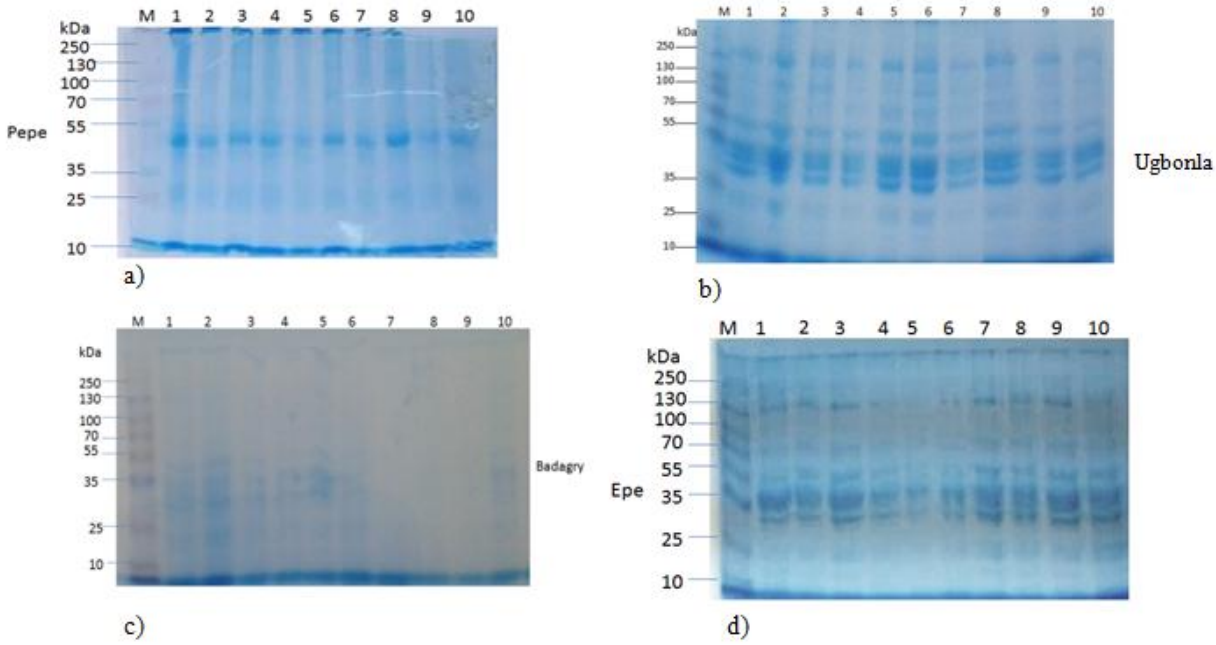

Plate 1(a-d): Electrophoregrams Showing Protein Profile of *T. guineensis* Populations

Table 2: Summary of Muscle Proteins Electrophoregram of *T. guineensis* Populations

Protein Marker (kDa)	Pepe	Ugbonla	Badagry	Epe
250	D	ND	ND	D
130	D	ND	D	D
100	D	D	ND	ND
70	D	D	ND	D
55	D	ND	ND	D
	50	50	50	50
	45			45
35	D	ND	D	D
			30	30
25	D	D	ND	D
			20	
10	ND	ND	ND	ND

Legend: D= Detected, ND= Not detected

Table 3: Genetic Similarity and Difference between *T. guineensis* Populations

Population	Pepe	Ugbonla	Badagry	Epe
Pepe	-	0.608	0.463	0.827
Ugbonla	0.497	-	0.708	0.500
Badagry	0.770	0.345	-	0.482
Epe	0.191	0.693	0.731	-

Nei's genetic similarity index (above diagonal) and genetic distance index (below diagonal)

Table 4: Analysis of Molecular Variance (AMOVA)

Source	df	MS	Est. Var.	%Var
Among Pops	3	26.350	2.596	87%
Within Pops	36	0.394	0.394	13%
Total	39		2.990	100%

Legend: df; degree of freedom; MS: mean square; Est. Var.: estimated variation; %Var: percentage variation

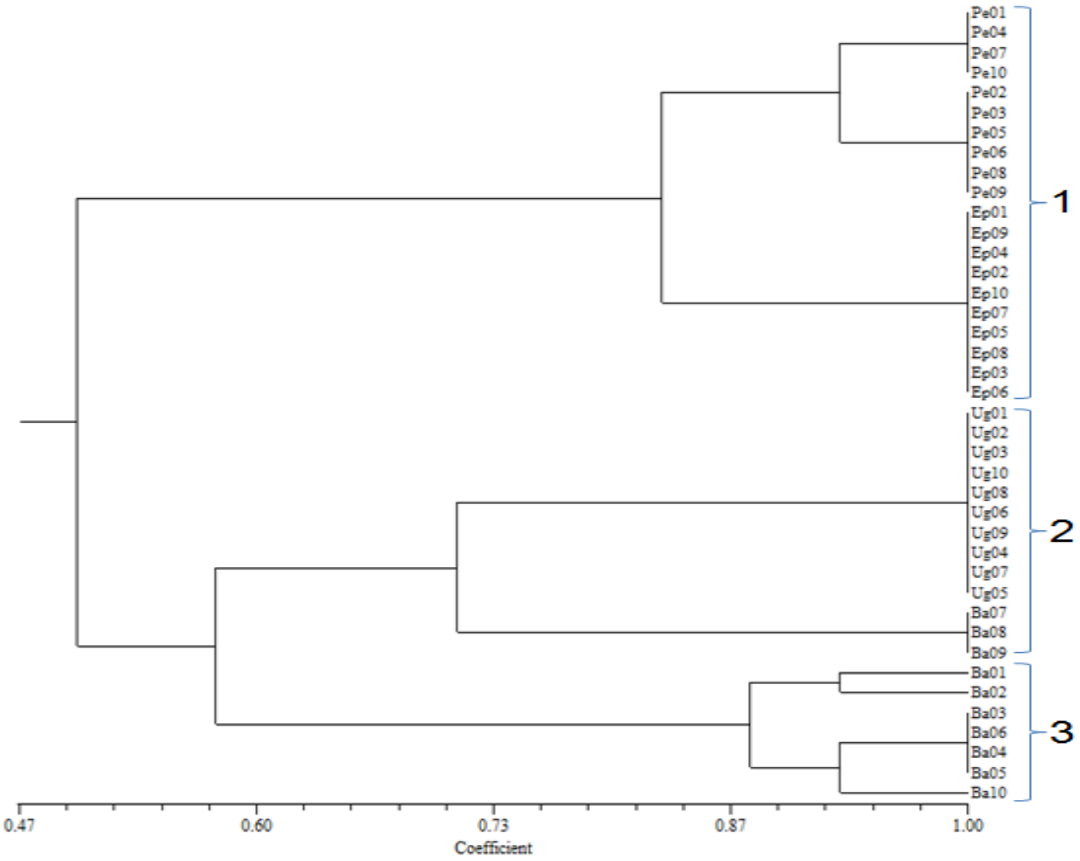

Figure 1: Dendrogram of Protein Profile for *T. guineensis* Populations Studied.

DISCUSSION

Sarcoplasmic protein electrophoregram revealed by SDS-PAGE was used as a tool for phylogenetic analysis. Electrophoretic sarcoplasmic protein profiles for all *T. guineensis* samples were noted based on the absence (0) and presence (1) of protein bands in the fish samples. Based on band pattern information, data were tabulated. Data revealed that Pepe and Epe populations have more proteins (9 bands) as compared to Badagry (7 bands) and Ugbonla (4 bands). The variations in protein bands observed in Pepe and Epe could be attributed to genomic changes and might suggest higher diversity than other populations. The unique profile in the banding pattern of the sarcoplasmic proteins of Pepe and Epe populations is an indication that a very close genetic relationship exists between them and this could be a result of belonging to the same species. This unique band could be used as a marker in delineating these *T. guineensis* populations. This result is in agreement with the report of Haniffa *et al*. (2017) on the protein profile of snakehead species. However, Pepe and Badagry populations were found to be genetically distinct which could be used for its improvement. Thus, protein electrophoresis is a useful tool for *T. guineensis* discrimination. The presence of band weight 50 and 25 kDa in all the studied populations strongly suggests that the gene coding for the protein band is an adaptive gene and has been fixed in *T. guineensis* from South-west Nigeria. This, therefore, suggests common phylogeny and ancestry among the studied populations. Similar observations were made by Popoola *et al*. (2014) in genetic variability in the cultured and wild population of *Clarias gariepinus* using SDS-PAGE. This finding is further strengthened by the earlier report of Akinwusi and Illoh (1995) who stated that when a band appears in every individual in a population, it is assumed that the gene which codes the enzyme or protein does not vary. Analysis of molecular variance (AMOVA) demonstrated that genetic variation was mostly among populations.

Clustering based on the genetic distance gave three major clusters indicating some level of genetic variability between the studied populations which could be attributed to genetic heterogeneity among the studied *T. guineensis* populations. The existence of genetic diversity in this study can therefore be used to exploit the expression of heterosis among the studied populations. The variation in the protein banding pattern of these studied populations is consistent with the report of Haniffa *et al*. (2017) on protein profiling for the phylogenetic relationship in snakehead species. Among all the four populations studied, Pepe, Badagry, and Ugbonla populations are highly diversified from each other. Hence these populations are recommended for future breeding programs for the development of improved *T. guineensis* species.

CONCLUSION

The present study revealed that genetic diversity existed among *T. guineensis* populations investigated and are recommended to be utilized in future breeding programs for the development of improved *T. guineensis* species. Furthermore, other molecular techniques like microsatellite analysis should be encouraged to discriminate against the studied populations to complement the findings of this study.

ACKNOWLEDGEMENT

We acknowledge our Head of Department, Dr. Oresegun A, and the management team of the Nigerian Institute for Oceanography and Marine Research (NIOMR) Lagos, Nigeria in funding this project. The support and assistance of other Biotechnology Department staff of NIOMR in conducting this research are highly appreciated.

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