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Research Article

Geographical Distance and Genetic Differentiation of Some Populations of *Tilapia guineensis* (Bleeker, 1862) in Nigerian Coastal Waters

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Abstract

Background and Objective: Assessment of genetic diversity is crucial for breeding, conservation and management programmes, especially in species of *Tilapia* being important source of animal protein in many developing countries. In this regard, the evaluation of the relationship between geographical and genetic relatedness of *Tilapia guineensis* populations is important because it allows for ascertaining the role of gene flow and other factors that shape the genetic structure of the fish populations. **Materials and Methods:** A total of 120 randomly selected fish samples from twelve coastal populations and nine microsatellite loci were considered for the present population genetic study. **Results:** Microsatellite analysis showed that the genetic distance between *T. guineensis* populations in River Ethiopie and Brass (2 km apart) was 0.30 reflecting low genetic distance. Populations in Oron and Ibaka (1 km apart) and those in Ishaka and Igbokoda (1 km apart) had genetic distances of 0.01, respectively. The highest geographical distance was between fish populations in Oron and Epe (29 km), however, the genetic distance between these populations is low (0.03). Geographical distance seems to have less influence on the genetic relationship among the studied populations. Nevertheless, the populations are highly differentiated as assessed by F_{ST} values of 0.12-0.66. **Conclusion:** This is evidenced by the low gene flow of less than one migrant per generation. Thus, the general notion that geographical proximity enhances genetic similarity but discourages genetic differentiation does not hold in *T. guineensis* populations found in Nigerian coastal waters.

Key words: Genetic differentiation, geographical distance, gene flow, populations, *Tilapia guineensis*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Any meaningful breeding, conservation and management programme should consider the assessment of inter and intragenetic diversity as the initial and the most critical step¹. This is more so for *Tilapia* is the third most important fin-fish in the world after Shark and Carp². *Tilapia guineensis* is a source of animal protein in the world, especially in poor developing countries like Nigeria³. Thus, the role of *T. guineensis* in fighting protein deficiency diseases like kwashiorkor and marasmus in such countries cannot be overemphasized. An important question in this study is: Does geographical proximity encourage genetic similarity in the population of *T. guineensis* found in Nigerian coastal waters?

A previous study⁴ has reported that no correlation exists between geographical and genetic distances among populations of the Atlantic salmon (*Salmo salar* L.) and in particular, the brown trout (*Salmo trutta* L.) based on allozyme electrophoresis. Another study⁵ also reported no correlation between genetic and geographical distances, making isolation by distance population structure unlikely.

On the other hand, species of Pacific salmon (*Oncorhynchus* spp.) populations were observed to have a positive correlation between genetic and geographical distances which suggested an Isolation-by-distance effect⁶. Similarly, Li *et al.*⁷, there is a significant correlation between genetic distance and geographical distance of *Euchiloglanis* populations. A significant correlation was also observed in tagging experiments with anadromous trout, which shows that individuals spawning in a "wrong river" are most likely to ascend a river close to the natal river⁸. The study aimed to assess the significance of geographical distance as a correlate of genetic distance between selected populations of *T. guineensis* in Nigerian coastal waters. The results of this study are hoped to reveal the relationship between geographic distance and genetic differences in *T. guineensis* populations. This will provide an important basis for the breeding, conservation and management of *T. guineensis* in Nigeria.

MATERIALS AND METHODS

Study area: This study was carried out for a period of 1 year (May, 2012 to April, 2013) in all the twelve locations.

Collection of fish samples: A total of 120 *T. guineensis* samples with a weight range of 20-35 g and length of 11.5-14.0 cm were identified and collected from twelve coastal

Table 1: Geographical locations of the sampling stations

Location	Latitude	Longitude	State
Buguma	N04°44.613 ¹	E006°57.401 ¹	Rivers
New Calabar	N04°448 ¹	E07°010 ¹	Rivers
Ishaka	N05°03.243 ¹	E005°45.332 ¹	Delta
River Ethiope	N05°53.397 ¹	E005°33.671 ¹	Delta
Epe	N06°35.832 ¹	E02°59.096 ¹	Lagos
Igbokoda	N06°21.028 ¹	E004°48.319 ¹	Ondo
Oropo	N06°25.238 ¹	E04°75.228 ¹	Ondo
Iwoama	N04°51.224 ¹	E06°28.333 ¹	Bayelsa
Brass	N04°31.500	E06°24.167	Bayelsa
Badagry	N04°25.012 ¹	E02°52.988 ¹	Lagos
Oron	N04°49.217 ¹	E008°04.625 ¹	Akwa Ibom
Ibaka	N04°27.200 ¹	E007°19.618 ¹	Akwa Ibom

ivers (ten from each river) in the Niger Delta, Nigeria which includes, the Epe lagoon, Badagry lagoon, Igbokoda, Oropo river, Ishaka Creek, River Ethiope, Buguma, New Calabar river, Iwoama river, Brass, Oron and Ibaka river. Table 1 shows the coordinates of the sampling locations used in the study. The fish samples were identified as *T. guineensis* by a fish taxonomist from Nigerian Institute for Oceanography and Marine Research Lagos, Nigeria and obtained from the fishermen at the landing sites.

Extraction of DNA and PCR amplification: Caudal fin tissue (1 cm²) was collected from each fish and placed in 95% ethanol for preservation until analysis. Genomic DNA was extracted from the caudal fin tissue using the phenol-chloroform protocol⁹. The quality of extracted DNA was checked using a Nano-drop spectrophotometer (Shimadzu Corporation Japan, MODEL UV-1800, 2000 series) at the absorbance of 260/280 nm. Amplification was carried out using nine microsatellite primers in Table 2 originally developed for tilapia¹⁰. The PCR was conducted in a reaction volume of 20 µL of the PCR ingredients which consisted of 4 µL solis biodyne (SBD) 5x fire pol (master mix with 12.5 mM MgCl), 13.1 µL dd H₂O, 0.5 µL dNTP (0.2 mM, nucleotides), 0.2 µL forward primer, 0.2 µL reverse primer and 2 µL of template DNA (10 ng) was run on a Thermocycler (Biorad, module 170-8731). The program for PCR amplification was as follows: 2 min initial 96°C denaturation, 30 cycles of 94°C for 30 sec, 30 sec at the appropriate annealing temperature (Table 2) and 30 sec at 72°C, followed by a 6 min final extension step at 72°C. The samples were stored at -20°C until separation on polyacrylamide gels (6% polyacrylamide gel, at 80 V for 2 hrs in a 1×TBE buffer). The gel was stained with ethidium bromide and visualized in a UV transilluminator. Two researchers independently scored the gel bands to reduce or rule out the error due to improper scoring.

Table 2: SSR primer code, sequences, annealing temperature and band size

Primer code	Sequence	Annealing temperature (°C)	Band size (bp)
UNH995	Forward 5' CCAGCCCTCTGCATAAAGAC 3' Reverse 5' GCAGCACAACCACAGTGCTA 3'	55	150-200
GM538	Forward 5' CAGCATGTTGTCTGGATCTTG 3' Reverse 5' TTTGTTGCTGTGGTCTGTTCTT 3'	55	150-200
GM531	Forward 5' AAAGCCAACGGTCTGAATTG 3' Reverse 5' AGCAGAGGACCCCTCAT 3'	55	100-150
GM211	Forward 5' GCAAGTTGAGAGGCTACTGT 3' Reverse 5' AAACAACCCACAACCTTAGTT 3'	55	100-150
UNH207	Forward 5' ACACAACAAGCAGATGGAGAC3' Reverse 5' CAGGTGTGCAAGCAGAAAGC 3'	55	100-150
UNH185	Forward 5' CAGACACACTAGACACATTCTA 3' Reverse 5' GTGTTCCATGTGTCTGTAC 3'	55	120-150
UNH146	Forward 5' CCACTCTGCCTGCCCTCTAT 3' Reverse 5' AGCTGCGTCAAACTCTCAAAG 3'	55	100-150
UNH123	Forward 5' CATCATCAGACAGATTAGA 3' Reverse 5' GATTGAGATTCATTCAAG 3'	55	100-150

Data analysis: Population genetic data generated were analyzed using PopGene v. 3.6 software to obtain the number of alleles per SSR locus, the effective number of alleles, Shannon information index, observed heterozygosity, expected heterozygosity, Nei's Pairwise genetic distance, pairwise population differentiation and estimated gene flow. Genetic relationships among populations were estimated by constructing a dendrogram using UPGMA (unweighted pair group method of analysis). In an attempt to compare genetic relationships with geographical location, a dendrogram based on geographical location (longitude and latitude) was generated using a clustering algorithm of SPSS version 21 software.

RESULTS

Genetic variation among populations: In Table 3, the Badagry population had the highest mean number of alleles (2.67), followed by Buguma (2.56) and Brass (2.44) while the lowest was found in Igbokoda (1.44). The mean effective alleles varied from 1.29-2.11. In all populations, the mean effective number of the allele was lower than the mean number of alleles. Shannon information index was observed higher in Buguma population (0.77), Badagry (0.76) and Brass (0.64) while other populations had a low index. All populations showed low average observed heterozygosity. Badagry was the most variable ($H_o = 0.467$) followed by Buguma ($H_o = 0.402$) and Brass ($H_o = 0.456$) while Oron had the least observed heterozygosity ($H_o = 0.211$). The average expected heterozygosity was high in Buguma (0.503), Badagry (0.484) and Brass (0.411) and low in Oron (0.178) and Igbokoda (0.180) populations (Table 3).

Pairwise genetic dissimilarity and differentiation: The level of genetic relatedness among the studied populations was determined by the dissimilarity matrix. Nei's genetic distance between the populations ranged from 0.01-0.30 as shown in Table 4. River Ethiopie and Brass had the highest genetic dissimilarity with a genetic distance of 0.30 while Oron and Ibaka, Ishaka and Igbokoda rivers were both found to have the lowest genetic dissimilarity with a genetic distance of 0.010. Based on geographical location in Table 5, the highest distance was between Oron and Epe (29.0).

The estimate of population differentiation (F_{ST}) among the population pairs ranged from 0.120-0.664 with the Ishaka-Buguma pair being the least differentiated with the highest gene flow rate of $N_m = 0.68$ and Oropo Ilaje-Epe pair the most differentiated with the lowest gene flow rate of $N_m = 0.126$ in Table 6. The mantel test results showed a negative correlation ($r = -0.248 < 0$) between geographic distance and genetic distance

Phylogenetic relationship: Figure 1 shows that, three clusters and an out-group (River Ethiopie) were obtained based on genetic similarity among fish populations. Buguma river forms a separate cluster (cluster 1), cluster II contains six locations which include New Calabar, Ishaka, Igbokoda, Epe, Oron and Ibakathat clustered together, cluster III contains 4 locations (Oropo, Iwoama, Brass and Badagry) while River Ethiopie forms an out-group. However, clustering analysis revealed three main clusters for geographical location in Fig. 2. Analysis of tree topology in Fig. 1 and 2 revealed differences, for instance, River Ethiopie that formed an out-group in Fig. 1 clustered with River Oropo and Igbokoda in Fig. 2.

Table 3: Genetic diversity level in the twelve studied populations

Population	Na	Ne	I	Ho	He
Buguma	2.7	2.11	0.77	0.402	0.503
New Calabar	1.7	1.53	0.36	0.400	0.247
Ishaka	2.2	1.49	0.44	0.333	0.273
River Ethiopie	2.2	1.69	0.54	0.289	0.336
Epe	1.9	1.47	0.36	0.344	0.236
Igbokoda	1.4	1.35	0.25	0.300	0.180
Oropo	2.0	1.58	0.44	0.233	0.286
Iwoama	1.9	1.39	0.36	0.244	0.225
Brass	2.4	1.87	0.64	0.456	0.411
Badagry	2.7	2.09	0.76	0.467	0.484
Oron	1.7	1.27	0.27	0.211	0.178
Ibaka	1.8	1.33	0.31	0.233	0.202

Na: Number of alleles, Ne: Effective number of alleles, I: Shannon information index, Ho: Observed heterozygosity and He: Expected heterozygosity

Table 4: Genetic distance of Nei estimated between each pair of 12 populations of *T. guineensis*

Location	Buguma	New Calabar	Ishaka	River Ethiopie	Epe	Igbokoda	Oropo	Iwoama	Brass	Badagry	Oron	Ibaka
Buguma	0.00											
New Calabar	0.09	0.00										
Ishaka	0.08	0.01	0.00									
River Ethiopie	0.12	0.15	0.14	0.00								
Epe	0.09	0.03	0.01	0.11	0.00							
Igbokoda	0.1	0.02	0.01	0.12	0.01	0.00						
Oropo	0.1	0.04	0.04	0.14	0.04	0.04	0.00					
Iwoama	0.18	0.1	0.11	0.26	0.13	0.14	0.05	0.00				
Brass	0.18	0.17	0.17	0.30	0.18	0.21	0.14	0.11	0.00			
Badagry	0.08	0.08	0.07	0.18	0.08	0.09	0.06	0.09	0.05	0.00		
Oron	0.12	0.04	0.03	0.16	0.03	0.02	0.07	0.21	0.27	0.11	0.00	
Ibaka	0.11	0.03	0.03	0.18	0.04	0.03	0.07	0.19	0.25	0.09	0.01	0.00

Table 5: Geographical distance estimated between each pair of 12 populations of *T. guineensis*

Location	Badagry	Brass	Buguma	Epe	Ibaka	Igbokoda	Ishaka	Iwoama	N. Calabar	Oron	Oropo	R. Ethiopie
Badagry	0.00											
Brass	10.00	0.00										
Buguma	17.00	1.00	0.00									
Epe	9.00	13.00	20.00	0.00								
Ibaka	16.00	2.00	1.00	25.00	0.00							
Igbokoda	8.00	2.00	5.00	5.00	8.00	0.00						
Ishaka	13.00	1.00	2.00	10.00	5.00	1.00	0.00					
Iwoama	10.00	0.00	1.00	13.00	2.00	2.00	1.00	0.00				
New Calabar	17.00	1.00	0.00	20.00	1.00	5.00	2.00	1.00	0.00			
Oron	26.00	4.00	1.00	29.00	2.00	10.00	5.00	4.00	1.00	0.00		
Oropo	8.00	2.00	5.00	5.00	8.00	0.00	1.00	2.00	5.00	10.00	0.00	
River Ethiopie	8.00	2.00	5.00	5.00	8.00	0.00	1.00	2.00	5.00	10.00	0.00	0.00

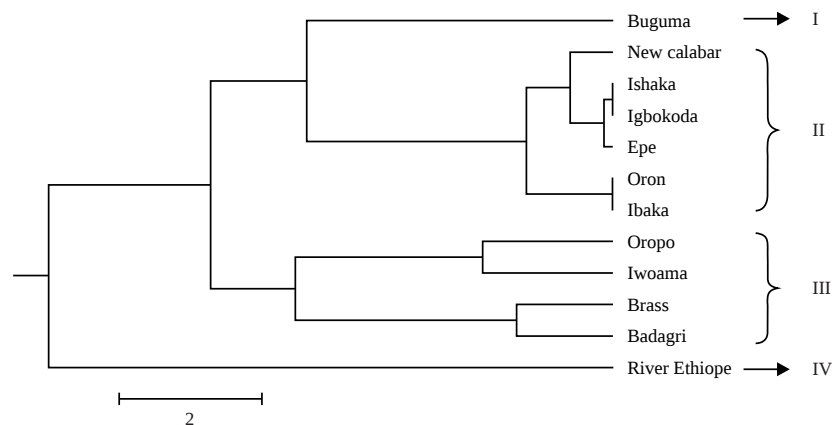


Fig. 1: Genetic relationships among the 12 populations of *T. guineensis*

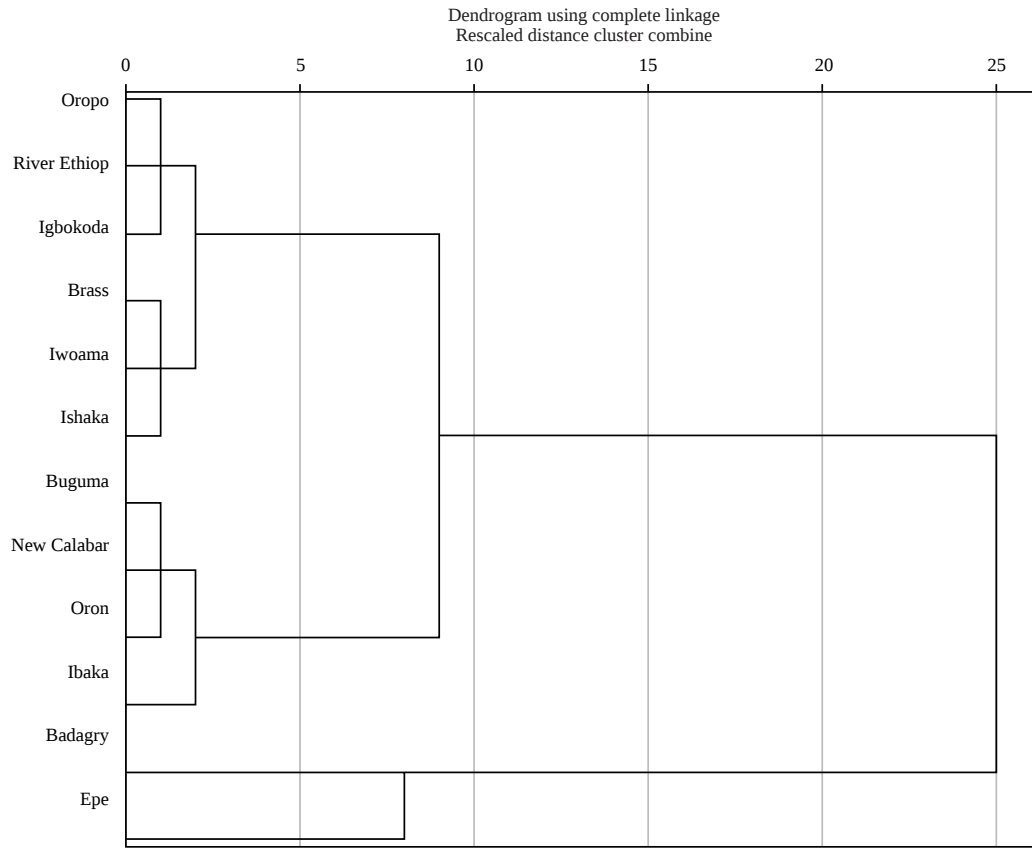


Fig. 2: Geographical relationships among 12 populations of *T. guineensis*

Table 6: Pairwise population differentiation (FST) and estimates of gene flow (Nm)

Population		Buguma	New Calabar	Ishaka	River Ethiopie	Epe	Igbokoda	Oropo	Iwoama	Brass	Badagry	Oron	Ibaka
Buguma	Fst	0.000											
	Nm	0.000											
New Calabar	Fst	0.291	0.000										
	Nm	0.608	0.000										
Ishaka	Fst	0.120	0.267	0.000									
	Nm	1.835	0.687	0.000									
River Ethiopie	Fst	0.374	0.457	0.387	0.000								
	Nm	0.419	0.297	0.396	0.000								
Epe	Fst	0.372	0.514	0.368	0.571	0.000							
	Nm	0.421	0.236	0.429	0.187	0.000							
Igbokoda	Fst	0.328	0.434	0.349	0.389	0.536	0.000						
	Nm	0.513	0.326	0.466	0.392	0.217	0.000						
Oropo	Fst	0.400	0.535	0.494	0.508	0.664	0.219	0.000					
	Nm	0.375	0.217	0.256	0.242	0.126	0.894	0.000					
Iwoama	Fst	0.273	0.397	0.290	0.386	0.557	0.215	0.298	0.000				
	Nm	0.664	0.380	0.612	0.398	0.199	0.912	0.589	0.000				
Brass	Fst	0.179	0.306	0.216	0.283	0.406	0.154	0.234	0.088	0.000			
	Nm	1.147	0.567	0.905	0.633	0.369	1.369	0.817	2.595	0.000			
Badagry	Fst	0.279	0.353	0.257	0.405	0.422	0.388	0.506	0.390	0.276	0.000		
	Nm	0.645	0.458	0.722	0.368	0.343	0.394	0.244	0.390	0.657	0.000		
Oron	Fst	0.373	0.564	0.379	0.534	0.562	0.438	0.543	0.482	0.300	0.502	0.000	
	Nm	0.421	0.193	0.410	0.218	0.195	0.321	0.210	0.268	0.582	0.248	0.000	
Ibaka	Fst	0.368	0.556	0.383	0.550	0.583	0.438	0.534	0.471	0.295	0.524	0.346	0.000
	Nm	0.429	0.200	0.403	0.204	0.179	0.320	0.218	0.281	0.597	0.227	0.473	0.000

DISCUSSION

In the present investigation, microsatellite analysis were used on 120 individuals of *T. guineensis* collected from twelve different populations encompassing almost the entire coastal states of Nigeria. Based on the mean number of alleles, Shannon's information index and heterozygosity (observed and expected), three populations namely Buguma, Badagry and Brass showed the highest biodiversity when compared to others in this study. Higher heterozygosity implies greater genetic variability¹¹ who stated that heterozygosity is an important measure of population diversity and structure at the genetic level.

Based on Nei's genetic distance, the low genetic diversity between the populations indicated a high genetic similarity with Buguma and River Ethiopie populations having the highest genetic distance suggesting a narrow genetic base among the different populations. Reducing fishing pressure and introducing *T. guineensis* from other populations may be considered as a conservation strategy to boost the narrow genetic base. Based on geographical location, the highest distance was between Oron and Epe which did not agree with the genetic distance of 0.30 between Buguma and River Ethiopie populations reflecting a negative correlation between genetic and geographical distance. Thus, genetic distance did not concur with geographical distance in this study. This is in agreement with the findings of another study, Garg *et al.*¹², who reported no significant correlation between the genetic and geographical distance of *Sperata seenghala* genotypes collected from different water bodies. On contrary, Carlsson and Nilsson¹³ observed a significant correlation between geographical and genetic distances of sea trout (*Salmo trutta* L.) populations.

Geographical distance did not correlate with genetic distance in the present study as evidenced by the Mantel test that revealed a negative correlation between geographic and genetic distance. This suggests that geographical distance between populations within the coastal states studied had little influence on the genetic structure of *T. guineensis* populations in Nigerian coastal waters. It could be deduced that the lack of correspondence between gene flow and geographical distances between populations could be the result of strong drift.

Overall, the genetic differentiation between the investigated populations (0.120-0.664) is very high with low gene flow (0.126-0.608). This concurred with other study, Balloux and Lugon-Moulin¹⁴, who stated that population differentiation of more than 0.15 are considered high rather

than moderate and are associated with low gene flow of less than one migrant per generation among the populations. Low gene flow among populations ($N_m = 0.91$) implies that the populations are out breeding due to the possible presence of large numbers of fish which may be the reason for the observed high genetic differentiation among the populations. The high F_{ST} obtained in this study hence signify that the populations are distinct. According to another study¹⁵, the migration rate of $N_m \geq 1$ leads to considerable homogeneity among populations but population divergence and structuring occur when $N_m \leq 1$. The populations in the present study have an overall migration rate of $N_m \leq 1$ rendering them to structuring and divergence.

However, the pairwise F_{ST} values between the Ishaka-Buguma pair and a few other population pairs are low (<0.15) and must have attributed to an increase in the level of gene flow among the populations. These populations are less differentiated which must have allowed gene flow between the populations concurring with the work of another study¹⁶ that there is possibly breeding in close natural populations hence narrowing the genetic differentiation between them. This high genetic similarity may have resulted in high homozygosity that was observed in this population pair.

However, clustering based on the genetic distance gave four major clusters indicating some level of genetic variability between the studied populations while three clusters were obtained from dendrogram based on geographical location. The tree topology based on genetic distance showed that Oron clustered with Ibaka while Igbokoda clustered with Ishaka while on the contrary, based on the geographical location, Oron clustered with Buguma while Ishaka clustered Brass indicating that genetic clustering due to microsatellite analysis did not concur with clustering based on geographical location. Therefore, proximity may not be a significant factor favouring gene flow between these populations. It could also be deduced that the low relationship between genetic and geographical distance indicates that the populations do not fit into the isolation by distance model. This model states that gene flow is highest between close populations and it is expected that close populations should show similar genetic composition but it is not the case with these studied populations.

CONCLUSION

The study revealed genetic differentiation between *T. guineensis* inhabiting Nigerian coastal waters. Moreover, geographical distance seems to have less influence on the

genetic relationship among these populations. Thus, the isolation by distance model could not account for the genetic structure of *T. guineensis* in this study.

SIGNIFICANCE STATEMENT

The present study discovered that *T. guineensis* from Nigerian coastal waters have some level of genetic variability and structure that could be explored through appropriate breeding for Tilapia improvement in Nigeria. It also established that geographical proximity does not encourage genetic similarity between the studied *T. guineensis* populations.

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