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Molecular characterization and genetic diversity assessment of *Tilapia guineensis* from some coastal rivers in Nigeria

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Tilapia guineensis **is an important economically and nutritionally important fish commonly found in Nigerian coastal waters. Genetic diversity of the fish was assessed to obtain information that may help in developing appropriate conservation and breeding programmes for improving the economic and nutritional quality of the fish. Twelve (12) Nigerian coastal populations and nine microsatellite loci were considered. All the loci were multi-allelic giving an average of 3.1 alleles per locus. The number of alleles (Na) ranged from two to four alleles per locus while the effective number of expected alleles (Ne) ranged from 1.087 to 2.612. Buguma, Badagry and Brass populations had the highest genetic diversity as was revealed by heterozygosity (observed and expected) and shannon index of the populations. The longest pairwise genetic distance of 0.30 was between Brass in Bayelsa State and River Ethiope in Delta State. Clustering using simple sequence repeat (SSR) data gave four major clusters which did not concur with geographical location clustering. We conclude that although genetic diversity is low in some populations of *T. guineensis* in Nigerian coastal waters, some populations (Buguma, Badagry and Brass) still retain some genetic variability which may be explored for fish improvement through appropriate breeding and conservation programmes.**

Key words: Molecular characterization, genetic diversity, microsatellite analysis, *Tilapia guineensis*, coastal rivers.

INTRODUCTION

Tilapia guineensis is one of the most important Cichlid species, in view of its nutritional role in many tropical and sub-tropical countries (Saisithi, 1994). It is an important source of animal protein and income throughout the world especially in developing countries like Nigeria with many rural populations relying on subsistent farming (Sosa et al., 2005). It has continued to contribute immensely to the nutritional needs, economic growth and development of

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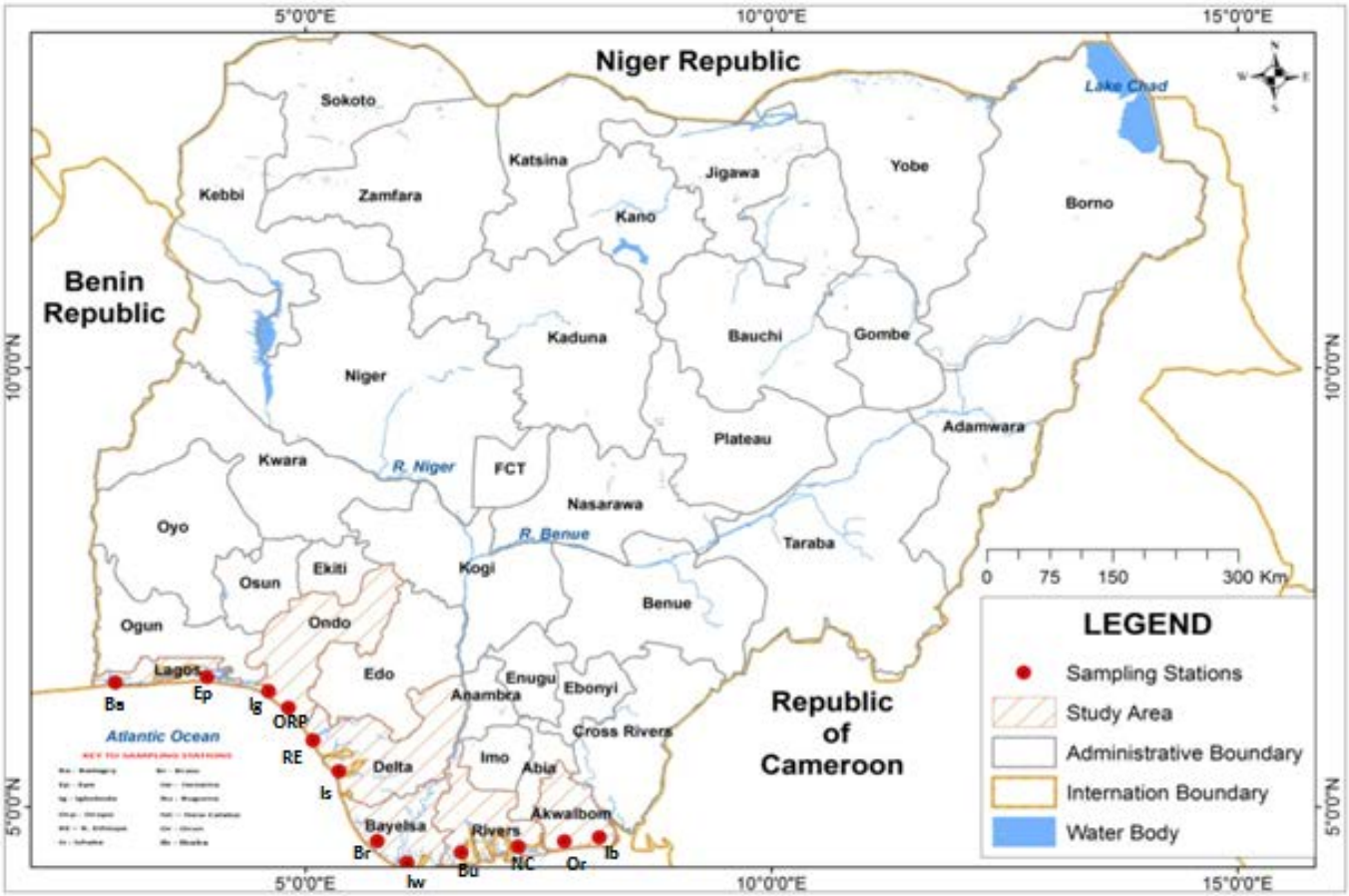

Figure 1. Map of Nigeria showing sampling stations.

many nations including Nigeria. The species is usually found in creeks, lagoons and other coastal waters of West Africa (Philippart and Ruwet, 1982). It shows good aquaculture potential and has been successfully raised in ponds, enclosures, cages and tanks. Despite the nutritional and economic importance of *T. guineensis* worldwide, our knowledge of the genetic status in terms of genetic diversity of its natural populations is still inadequate for sustainable aquaculture practices, conservation and improvement through selective breeding (Agenes et al., 1999).

The Nigerian coastal zone comprises of eight states (Figure 1), and the majority of these populations depend on catch from the wild. This includes *T. guineensis* as a source of animal protein. Thus, Tilapia has grown to represent the third most important finfish in the world (Sofia 2012). Therefore, efforts to determine the current level of diversity and genetic structure of *T. guineensis* populations in Nigeria and many other parts of the world are useful for fishery management, aquaculture, stock conservation, and fish improvement through breeding.

Information about the genetic diversity of the wild fish populations through microsatellite analysis is essential in breeding for heterosis and effective fish management (Bo-young et al., 2005; Lee and Kocher, 1998). An extensive search on the literature revealed that much work has not been done on genetic diversity of *T. guineensis* in Nigeria. In a recent study, Abd-el-kader et al. (2013) observed a relatively high level of genetic diversity within and between three Tilapia species namely *Tilapia zilli, Oreochromis aureus* and *Oreochromis niloticus* in Egypt. In Nigeria and many other West African countries, *T. guineensis* is one of the dominant Tilapia species; in view of the importance of genetic variability in selective breeding, it is necessary to assess genetic variability of *T. guineensis* for sustainable fish improvement in Nigeria and many other African countries (Bentsen and Olesen, 2002).

Apart from the usefulness of knowledge of genetic variability in fish improvement and management, assessment and conservation of genetic variability is important in assessing risk of reduction in fitness through inbreeding and loss of species through extinction (Frankham, 2003). Consequences of these are well known (Falconer, 1989; Keller and Waller, 2002). As with all *Tilapia* species, there is a great potential for enhancing

Table 1. Geographical location of the sampling stations.

Location	Latitude	Longitude	State
Buguma	N04° 44.613′	E006° 57.401′	Rivers
New Calabar	N04° 448′	E07° 010′	Rivers
Ishaka	N05° 03.243′	E005° 45.332′	Delta
River Ethiope	N05° 53.397′	E005° 33.671′	Delta
Epe	N06° 35.832′	E02° 59.096′	Lagos
Igbokoda	N06° 21.028′	E004° 48.319′	Ondo
Oropo	N06° 25.238′	E04° 75.228′	Ondo
Iwoama	N04° 51.224′	E06° 28.333′	Bayelsa
Brass	N04° 31.500′	E06° 24.167′	Bayelsa
Badagry	N04° 25.012′	E02° 52.98′	Lagos
Oron	N04° 49.217′	E008° 04.625′	Akwa Ibom
Ibaka	N04° 27.200′	E007° 19.618′	Akwa Ibom

growth and production through genetic selection. The need to assess the genetic diversity of *T. guineensis* populations using microsatellite markers for breeding and conservation purposes necessitated this study.

MATERIALS AND METHODS

Collection of fish samples

A total of 120 *T. guineensis* individuals with weight range of 20 to 35 g and length of 11.5 to 14.0 cm were identified and collected from 12 coastal rivers (ten from each river) in the Niger delta, Nigeria which includes; Epe lagoon, Badagry lagoon, Igbokoda, Oropo river, Ishaka Creek, River Ethiope, Buguma, New Calabar river, Iwoama river, Brass, Oron and Ibaka river (Figure 1). The geographical location in terms of longitudes and latitudes of the sampling stations are presented in Table 1. Experimental fish samples were identified to be *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 cm^2) was collected from each individual and placed in 95% ethanol for preservation until analysis. Genomic DNA was extracted from the caudal fin tissue using phenol-chloroform protocol (Sambrook and Russell, 2001). The quality of extracted DNA was checked using a Nano-drop spectrophotometer (Shimadzu corporation Japan, MODEL UV-1800, 2000 series) at absorbance of 260/280 nm. Amplification was carried out using nine microsatellite primers (Table 2) originally developed for tilapia by Lee et al. (2005). A total 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), 13.6 µl dd H_2O, 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: 2 min initial 96°C denaturation, 30 cycles of 94°C for 30 s, 30 s at the appropriate annealing temperature (Table 1), and 30 s 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 h 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 error due to improper scoring.

We could not observe amplification at 65°C annealing temperature unlike Saad et al. (2013) who obtained amplicons at an annealing temperature of 65°C in tilapia. In the present study, we obtained PCR amplification at 55°C through optimization of PCR conditions.

Data analysis

Population genetic data generated was analysed using PopGene v. 3.6 software to obtain the number of alleles per SSR locus, effective number of alleles, Shannon information index, observed heterozygosity, expected heterozygosity and Nei's Pairwise genetic distance (1972). Genetic relationship among populations was estimated by constructing a dendrogram using unweighted pair-group method of analysis (UPGMA). In an attempt to compare genetic relationship with geographical location, a dendrogram based on geographical location (longitude and latitude) was generated using clustering algorithm of SPSS version 21 software. Polymorphic information content (PIC), major allele frequency and gene diversity were determined using PowerMarker v. 3.6.

RESULTS

Genetic variability among microsatellite loci

All nine microsatellite loci were polymorphic in all populations (Figure 2) with polymorphic information content (PIC) values ranging from 0.07 at locus GM211 to 0.54 at locus UNH207 with an average of 0.31 (Table 3). A total of 28 alleles were found in the study. The mean number of alleles per locus was 3.1. Locus UNH207 and UNH 185 gave the highest number of alleles (four alleles, respectively) while UNH123 gave the least (two alleles). On average, 73% of the 120 individuals shared a common major allele at a giving locus ranging from 45% (UNH207) to 96% (GM211)

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

Primer code	Sequence	Annealing temperature (°C)	Molecular 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' AGCAGAGGACACCCCTCAT 3'	55	100-150
GM211	Forward 5' GCAAGTTGAGAGGCTACTGT 3' Reverse 5' AAACAACCCACAACCTTAGTT 3'	55	100-150
UNH207	Forward 5' ACACAACAAGCAGATGGAGAC3' Reverse 5' CAGGTGTGCAAGCAGAAGC 3'	55	100-150
UNH185	Forward 5' CAGACACACTAGACACATTCTA 3' Reverse 5' GTGTTTCCATGTGTCTGTAC 3'	55	120-150
UNH146	Forward 5' CCACTCTGCCTGCCCTCTAT 3' Reverse 5' AGCTGCGTCAAACTCTCAAAAG 3'	55	100-150
UNH123	Forward 5' CATCATCACAGACAGATTAGA 3' Reverse 5' GATTGAGATTTCATTCAAG 3'	55	100-150

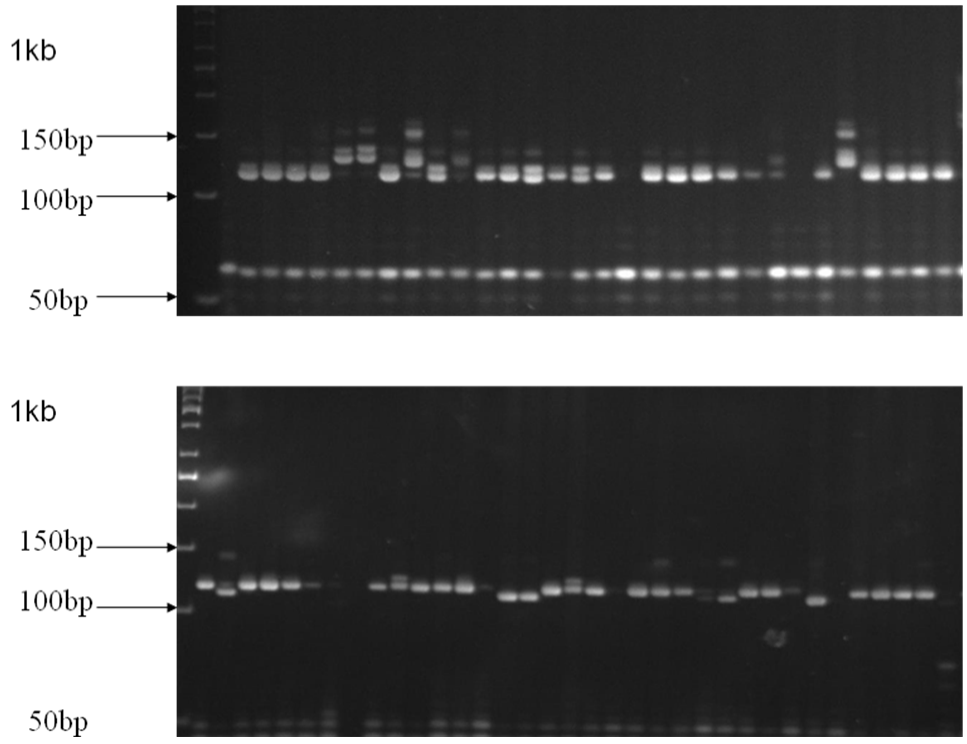

Figure 2. Electrophoresis of the amplified microsatellite loci using UNH995 primer. Upper panel: 1-60 individuals; Lower panel: 61-120 individuals.

Table 3. Characteristics of SSR Loci analyzed

Marker	Freq.	Sample size	NA	Gene diversity	PIC
UNH995	0.83	120	3	0.29	0.27
GM538	0.46	120	3	0.57	0.48
GM531	0.47	120	3	0.58	0.48
GM211	0.96	120	3	0.08	0.07
UNH207	0.45	120	4	0.62	0.54
UNH185	0.40	120	4	0.57	0.50
UNH146	0.68	120	3	0.48	0.42
UNH123	0.95	120	2	0.09	0.09
UNH104	0.88	120	3	0.23	0.21
Mean	0.73	120	3.1	0.35	0.31

Freq., major allele frequency; NA, number of allele; PIC, polymorphic information content.

Table 4. Locus specific indices of genetic diversity in the combined population.

Locus	No. of allele	Effective alleles	Observed heterozygosity	Expected heterozygosity	Fis	D
UNH995	3	1.410	0.100	0.292	0.656	-0.658
GM538	3	2.341	0.942	0.575	-0.644	0.389
GM531	3	2.358	0.817	0.578	-0.418	0.414
GM211	3	1.087	0.583	0.808	0.275	-0.278
UNH207	4	2.612	0.442	0.619	0.284	-1.286
UNH185	4	2.428	0.308	0.586	0.316	-0.819
UNH146	3	1.919	0.283	0.481	0.408	-0.412
UNH123	2	1.105	0.050	0.954	0.474	-0.948
UNH104	3	1.293	0.117	0.228	0.485	-0.487
Mean	3.1	1.706	0.324	0.324	0.066	-0.409

NA, number of alleles; NE, effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; Fis, inbreeding coefficient; D, heterozygote deficiency calculated as D = (Ho-He)/He.

common allele per locus. The level of diversity revealed by the studied loci ranged from 0.08 to 0.6 with an average of 0.35 (Table 3). Locus UNH207 had the highest effective number of alleles (2.6) while GM211 gave the least (1.0) (Table 4). The highest observed heterozygosity was obtained by locus GM538 (0.94) while locus UNH123 had the lowest (0.050). The expected heterozygosity ranged from 0.186 to 0.954 with a mean of 0.349 (Table 4). The inbreeding coefficient (Fis) was positive across seven loci in all populations reflecting excess of homozygotes while two loci were negative indicating an excess of heterozygotes (Table 4).

Genetic differences among populations

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 to 2.11. In all populations, the mean effective number of 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) reflecting high genetic diversity while other populations had low index. All populations showed low average observed heterozygosity. Badagry was the most variable (Ho = 0.467) followed by Buguma (Ho = 0.402) and Brass (Ho = 0.456) while Oron had the least observed heterozygosity (Ho = 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 as shown in Table 5.

According to Table 6, Nei's genetic distance between the populations ranged from 0.01 to 0.30. The highest genetic dissimilarity was between River Ethiope and Brass with a genetic distance of 0.30. Based on geographical location, the highest distance was between Oron and Epe (29.0) (Table 7). Thus, genetic distance did not concur with geographical distance in this study.

The UPGMA dendrogram based on the genetic distances revealed four clusters: cluster 1 consists of

Table 5. Summary of the 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 Ethiope	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
Bdagry	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; He, expected heterozygosity.

New Calabar, Ishaka, Igbokoda, Epe, Oron and Ibaka, while cluster 3 consists of Oropo, Iwoama, Brass and Badagry and finally, cluster 4 consists of River Ethiope that formed an out-group (Figure 3). Three clusters were obtained from dendrogram based on geographical location (Figure 4). The tree topology based on genetic distance showed that Oron clustered with Ibaka while Igbokoda clustered with Ishaka. However, based on the geographical location, Oron clustered with Buguma while Ishaka clustered Brass.

DISCUSSION

Nine microsatellite markers were utilized to characterize and investigate genetic variation in some coastal populations of *T. guineensis* in Nigeria with a view to stimulating interest and giving insights into possibilities of improving nutritional and economic qualities of *T. guineensis* through breeding and conservation programmes. We felt that nine SSR markers were sufficient to characterize the fish populations in view of the work of Abdul et al. (2012) who also used nine microsatellite markers to assess diversity in rice. Moreover, our results in which a total of 28 alleles were revealed is similar to that of Abdul et al. (2012) who got a total 27 alleles in rice. In contrast, the study of Hesham and Gilbey (2005) revealed 80 alleles in six loci from five populations of *O. niloticus.* An earlier report by Hesham and Gilbey (2005) is comparable to a more recent report of Gu et al., (2014) in which 10 microsatellite loci revealed 75 alleles in *O. niloticus* populations. These results suggest that *T. guineensis* had lower genetic diversity than *O. niloticus.* Urgent steps are therefore necessary to arrest further reduction in diversity of *T. guineensis* through various breeding and conservation programmes.

Electrophoresis of PCR–amplified DNA gave one or two bands. This is expected in microsatellite analysis where one band represents homozygosity and two bands represent heterozygosity. Nevertheless, null and multiple alleles were obtained for a few loci in this study. Occurrence of null alleles may indicate over-stringent PCR conditions and poor primer annealing due to nucleotide sequence divergence in one or both flanking regions resulting in non-amplification. Presence of multiple alleles might suggest aneploidy or/and existence of paralogy in the genome of *T. guineensis* in Nigerian coastal waters. Considering rarity of aneuploidies and its adverse effect on genome balance and survival in animals, paralogy seems to be a more attractive explanation for the existence of multiple bands in this study. Nevertheless, there is need for selective optimization for primer annealing coupled with cytogenetic analysis in future studies. The observed number of alleles (Na) and the effective number of alleles (Ne) varied among *T. guineensis* populations in the present study. The average number of alleles observed in Buguma, Badagry and Brass were higher than that of other populations indicating more allelic polymorphism in Buguma, Badary and Brass populations.

The polymorphic information content (PIC) of 0.31 obtained in our study suggests that the microsatellite loci considered were moderately informative with good discriminating power in accordance with the view of Bostein et al. (1980). Thus, these markers had good merits for detecting DNA identity and diversity in these populations and are therefore suitable for use in the characterization of natural populations and determination of genetic differentiation in *T. guineensis*. In all investigated populations, only Buguma population demonstrated polymorphism for all loci while others showed lower polymorphism. This result is contrary to the result obtained by Corujo et al. (2004) in nine populations

Table 6. Nei's genetic distance between twelve *T. guineensis* populations revealed by nine microsatellite loci.

Location	Buguma	New calabar	Ishaka	River Ethiope	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 Ethiope	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 7. Distance matrix based on geographical (longitude and latitude) location.

Location	Badagry	Brass	Buguma	Epe	Ibaka	Igbokoda	Ishaka	Iwoama	N. Calabar	Oron	Oropo	R. Ethiope
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				
N. 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	
R. Ethiope	8.00	2.00	5.00	5.00	8.00	0.00	1.00	2.00	5.00	10.00	0.00	0.00

of brown trout in Spain with as many as seven populations having all loci polymorphic.

Three populations namely Buguma, Badagry and Brass were identified as having considerable biodiversity in this study. This was based on Shannon's information index and heterozygosity (observed and expected) which were higher in these populations when compared to others. Higher heterozygosity implies greater genetic variability according to Mu et al. (2011) who stated that heterozygosity is an important measure of population diversity at the genetic level. Thus, in order to embark on a meaningful breeding and conservation programme for *T. guineensis* in Nigerian coastal waters, the identified populations

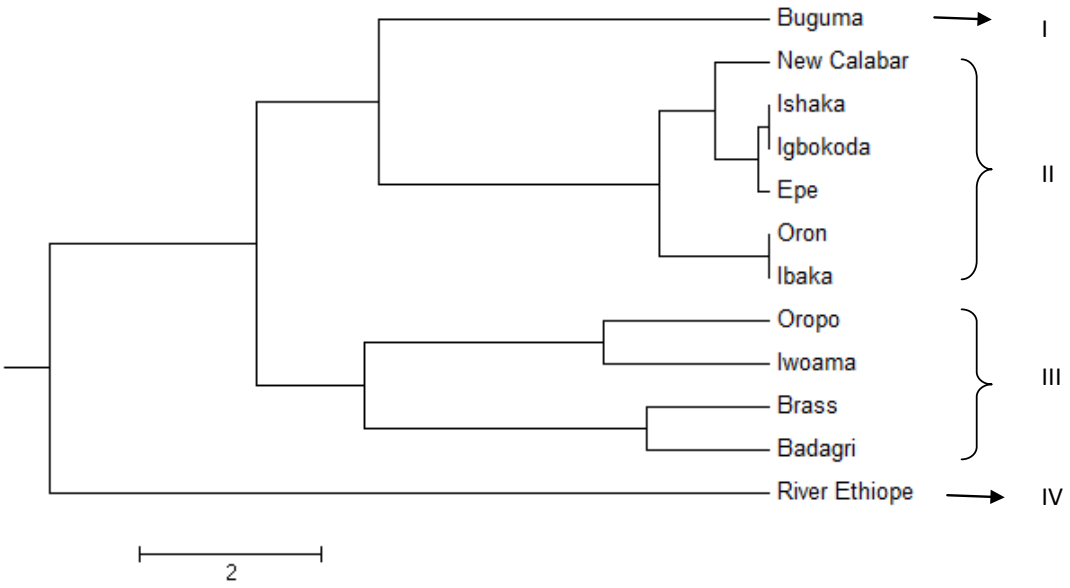

Figure 3. UPGMA dendrogram showing the genetic relationships among 12 populations based on Nei's genetic distance.

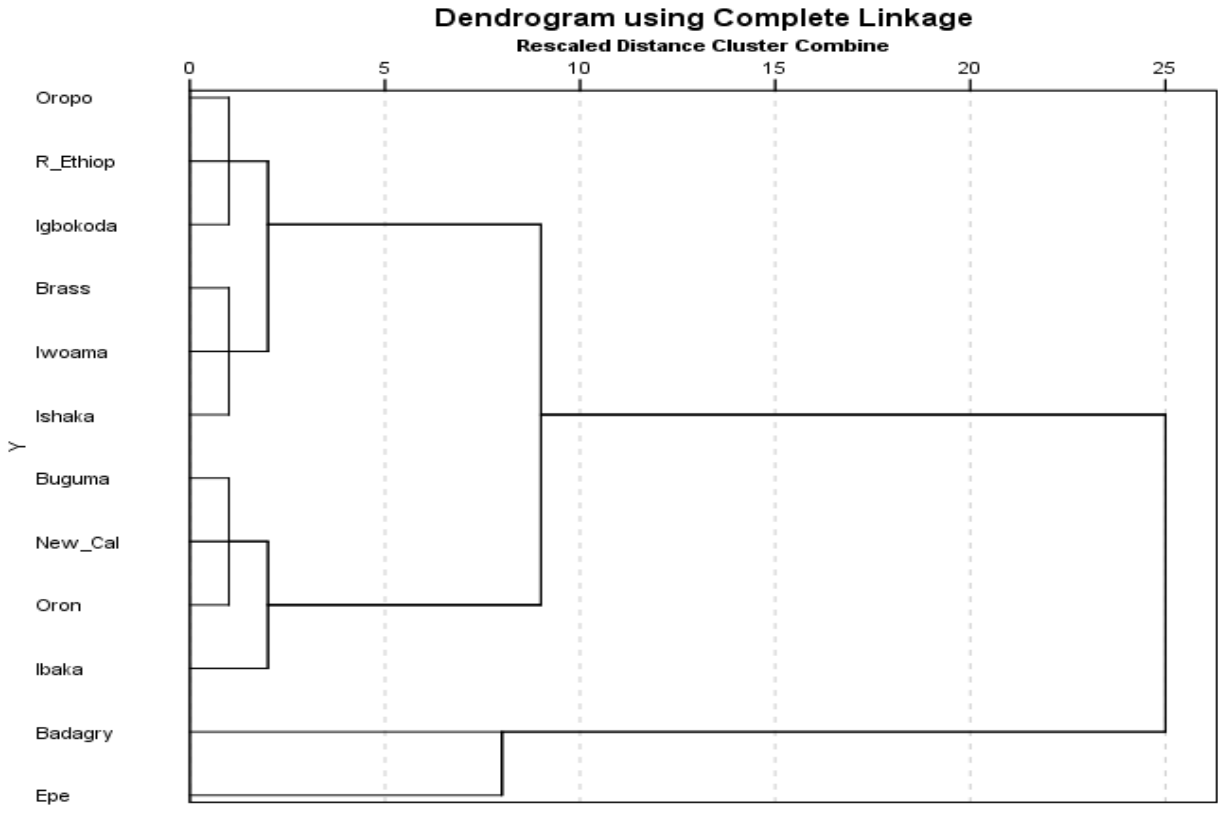

Figure 4. Dendrogram based on geographical location using longitudinal and latitudinal location of the populations.

(that is, Buguma, Badagry and Brass) should be considered as sources of fish for improvement programmes.

Inbreeding coefficient (Fis) is expressed as a deficiency in heterozygotes, the theoretical value ranges from -1 to +1, where by positive values indicate heterozygote deficiency possibly due to inbreeding (Boris Brinez et al., 2011). In the present study, all the loci showed positive

inbreeding coefficient (Fis) except two loci suggesting that inbreeding leading to reduced number of heterozygotes exists in *T. guineensis*. Deleterious consequences of inbreeding which include loss of diversity, fitness amd extinction had been discussed in other studies (Antunes et al., 2006).

Clustering based on the genetic distance gave four major clusters indicating some level of genetic variability between the studied populations. However, genetic clustering due to microsatellite analysis did not agree with clustering based on geographical location. Therefore, proximity may not be a significant factor favoring gene flow between these populations. It is therefore likely that *T. guineensis* migrate through long distance during breeding season.

Conclusion

Despite some evidence of inbreeding and low biodiversity among *T. guineensis* populations, there is still some genetic variability in some of the studied populations. Buguma, Badagry and Brass still contain sufficient genetic diversity that can be exploited for breeding and conservation programmes to improve economic and nutritional qualities of *T. guineensis* in Nigeria.

Conflict of interests

The authors have not declare any conflict of interest.

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