

Full Length Research Paper

Molecular characterisation of two cichlids populations (*Tilapia guineensis* and *Sarotherodon melanotheron*) from different water bodies in Lagos State, Nigeria

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This study used random amplified polymerase DNA technique (RAPD) to characterize two populations of cichlids species *Tilapia guineensis* and *Sarotherodon melanotheron* from selected two water bodies in Lagos using molecular approach. Ten samples of each *S. melanotheron* and *T. guineensis* with standard length value ranging between 8.4 and 17.2 cm and their weight ranging between 26.1 to 133 g were collected from Lagos Lagoon, Badagry, Nigerian Institute for Oceanography and Marine Research and Akinsateru fish farm, respectively. The DNA of the fish samples were extracted using salt-out method. RAPD analysis was carried out using RAPD primers (OPC 04, 05, 10, OPR 02 and OPI 05). Depending on the similarity coefficient through the used primers, the similarity between *T. guineensis* and *S. melanotheron* from the wild was 78%; between *T. guineensis* and *S. melanotheron* from the culture was 80%; and 100% of both wild and culture of samples; 82 and 88% of *T. guineensis* and *S. melanotheron* from wild and culture, respectively. The description of this similarity coefficient is not simple, especially when more than one character is involved in the same cluster. The values of the genetic distances obtained were utilized to generate a distance matrix to construct a dendrogram which linked the studied species DNA for possible variation in their genetic makeup. This generated banding pattern populations of *T. guineensis* and *S. melanotheron* from the wild and cultured environment have similar DNA profile, which indicated that there were similarities among the fish species from these environments which can lead to high probability of hybridization between the very closed species to improve the genetic characters; also, high quality and quantity of DNA can be gotten in using salt-out method for extraction and pure strain of the samples can be gotten from wild and culture.

Key words: Molecular characterization, DNA, RAPD primers, PCR, tilapia, water bodies.

INTRODUCTION

Nigeria is endowed with a lot of resources including fisheries resources that contribute immensely to the

nutritional needs, economic growth and development of the nation. Fish provides essential amino acids like lysine

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and methionine which are limited in other animals. It is also rich in vitamins A and D as well as lipids which help to reduce thickness of blood thereby allowing it to flow easily in the body (Akande, 1990). Tilapia is a large genus in cichlid family - Cichlidae, which according to Nelson (2006) is the third largest fish in the world and one of the most diverse fish species. The species is chosen because of its relatively short generation time of about six months and its suitability for investigation of the application of genetics in aquaculture from conservation of genetic resources to breeding programmes (Eknath et al., 1993) and breed in captivity and they are enjoying wide acceptance as food fish because of their high palatability and history of use from inland fisheries. Information about the genetic diversity of wild fish populations and culture population is essential for effective management. However, genetic diversity has been measured indirectly and inferentially through controlled breeding and performances studies or by classical systematic analysis of phenotypic traits.

Furthermore, another important prerequisite for effective management of population of fishes population is understanding of their taxonomic identity, this has been approached using morphometric and meristics analysis (Kuton and Kusemiju, 2010; Pante et al., 1988); allozyme electrophoretic analysis (Macaranas et al., 1986; Lee and Kocher, 1996; Ahmed et al., 2004); serum protein analysis (Avtalion et al., 1976); immunology and agglutination assays, mitochondria DNA restriction analysis (Seyoum, 1989; Seyoum and Kornfield, 1992); karyotype analysis (Crosetti et al., 1988); DNA fingerprinting (Harris et al., 1991; El-Zaeem et al., 2006) and DNA microsatellite analysis (Lee and Kocher, 1998; McConnell et al., 2000; Bo-young Lee et al., 2005).

The widespread and mixing of two or more tilapia species in ponds and in natural water bodies makes the identification of tilapia to be difficult by mere traditional method. There is also need to address the taxonomic problems and also to identify the distinctness of both natural and aquaculture populations and inadequate expression of genetic strains. Therefore, this study is aimed to ascertain inter and intra-specific genetic heterogeneity among the wild and cultured both species (*Tilapia guineensis* and *Sarotherodon melanotheron*) and examine phylogenetic relationship between the species.

MATERIALS AND METHODS

Study sites

The fish culture at Akin Sateru Farms at Ajah and Nigerian Institute for Oceanography and Marine Research Fish Farm at Badore, both in Lagos State, Nigeria while fish sampling (wild) was at Lagos lagoon at Oworo and Badagry both along the coast of Nigeria, West Africa. However, the samples of cultured were also taken from the farm named earlier. The sizes of the samples ranged between 8.4

to 17.2 cm in standard length and between 26.1 to 133 g in weight (include their standard deviation 26.1 ± 36.66 to 133 ± 21.7). The samples were transported in ice chest to the laboratory at 20°C to Nigerian Institute for Oceanography and Marine Research Biotechnology laboratory. DNA was isolated from caudal fin clip using salt -out method according to Lopera-Barrero et al. (2008). Purity of the DNA was assessed by determining the value of absorbance of the DNA at 260 and 280 with value ranged between 1.63 to 2.10. DNA samples extracted from fish samples were amplified using five RAPD primers (OPC 04, 05, 10, OPR 02 and OPI 05, Operon Technologies Inc. USA). A total volume of 20 µl of the PCR ingredients which consists of 2 µl Solis Biodyne (SBD) 5x fire pol (master mix with 12.5 mM MgCl), 17.15 µl autoclaved water, 0.5 µl dNTP (0.2 mM; nucleotides), 0.1 µl of Taq polymerase, 0.25 µl of primers (25 p/mol) and 2 µl of DNA (10 ng) was run on Thermocycler. Initial denaturation of the PCR analysis was at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 350°C for 60 s and extension at 720°C for 2 min; a final extension at 72°C for 10 min was carried out.

The samples were cooled at 40°C. Horizontal electrophoresis was performed using a 1.5% agarose gel, at 70 V for 1.5 h in a 1 x TBE buffer. The gel was marked with ethidium bromide and the pictures taken on top of a UV transilluminator. The RAPD fragments were counted at different loci for the five primers and inputted into a software NTSYS to generate a dendrogram.

RESULTS

Purity of the DNA was carried out spectrophotometrically by measuring absorbance at 260 and 280 nm using TE buffer as blank which ranges between 1.63 to 2.10. RAPD analysis was performed on 80 genomic DNA samples of fishes from different water bodies using five 10 bp-oligo-nucleotide random primers (Table 1), which shows different numbers of stable amplification and polymorphism bands.

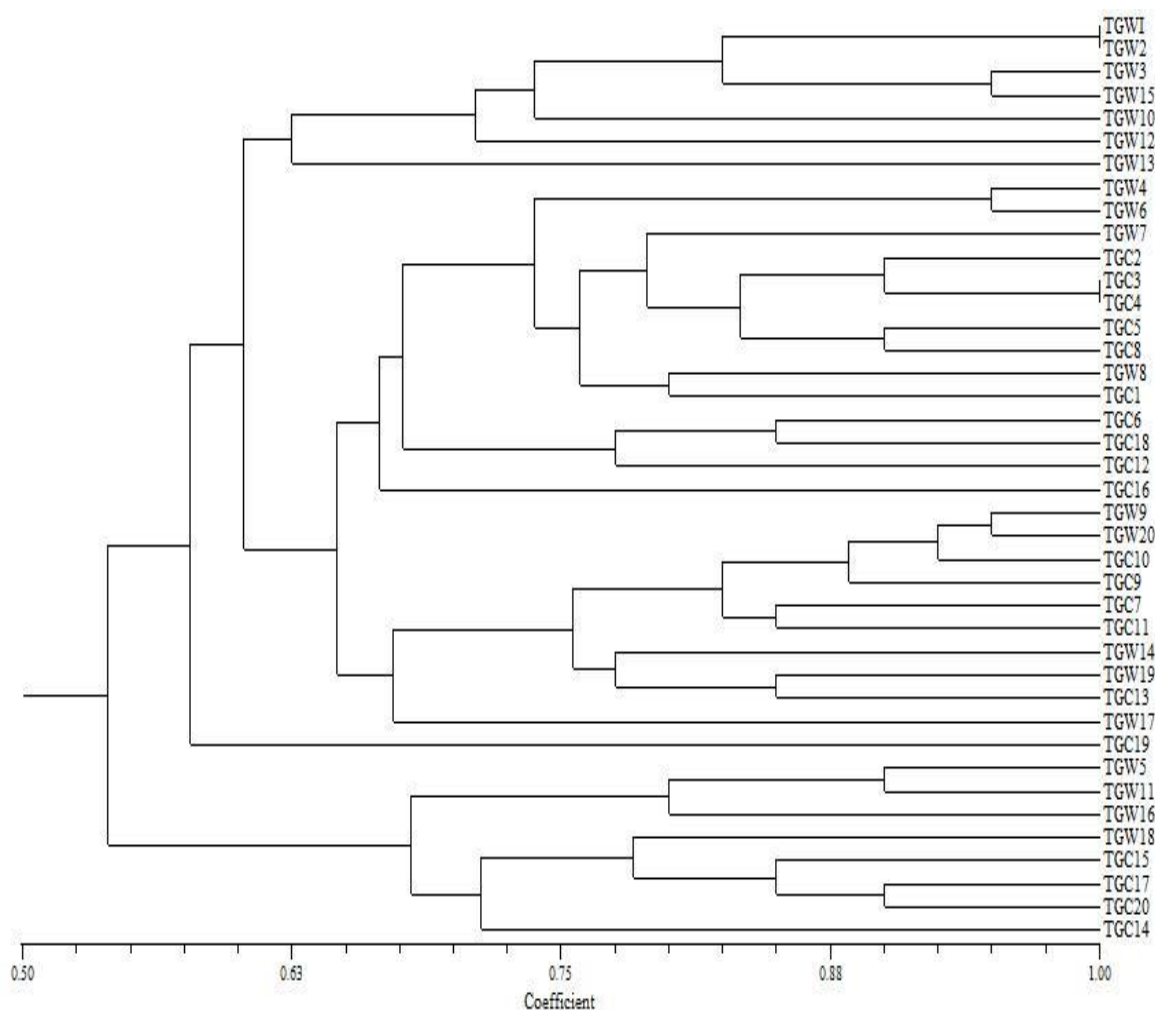
In Figure 1, four major clusters was formed which consists of minor clusters at various degree of co-efficient phylogenetic analysis using NTSYS software produced a dendrogram. At co-efficient 1 (100%), a cluster was formed between samples wild (TGWI, TGW2) and culture (TGC3, TGC4) indicating a very high level of genetic similarity. The 1st cluster consist of TGW5, TGW17, TGW16, TGW8, TGC1 TGC17, TGC20 and TGC14; 2nd cluster are TC19, TGW17, TGC13, TGW19, TGW19, TGW14, TGC11, TGC7, TGC9, TGW20, TGW9 and TGC14; 3rd cluster are TGC16, TGC12, TGC18, TGC6, TGC1, TGC8, TGC5, TGC4, TGC3, TGC2 and TGW17; while 4th cluster are TGW6, TGW13, TGW12, TGW11, TGW15, TGW3, TGW2 and TGW1. From Figure 2, five major clusters were formed which consists of minor clusters at degree of co-efficient phylogenetics analysis. SW6, SW7, SW13 and SW14 which are wild samples from both sites form a cluster at co-efficient 1(100%) indicating a very high level of genetic similarity. 1st cluster (SC19, SC13, SC15, SC14 and SC20), 2nd cluster (SC12, SC16, SC8, SC7, SW10 and SW8), 3rd cluster (SC2, SC5, SC4, SC3, SW19, SC11 and SW18),

Table 1. Sequences of primers, GC% and number of bands.

Primer number	Sequence (5' - 3')	GC content (%)	Total bands	
			<i>Tilapia guineensis</i>	<i>Sarotherodon melanotheron</i>
OPC 04	5' CCGCATCTAC 3'	60	101	123
OPC 10	5' TGTCTGGGTG 3'	60	0	0
OPC 05	5' GATGACCGCC 3'	70	122	139
OPI 05	5' TGTTCACGG 3'	60	58	60
OPR 02	5' CACAGCTGCC 3'	50	107	140

Tilapia guineensis from the Lagos Lagoon (TGW1-TGW10). *Tilapia guineensis* from Badagry Lagoon (TGW11-TGW20). *Tilapia guineensis* from Akinsateru farm (TGC1-TGC10). *Tilapia guineensis* from Nigerian Institute for Oceanography and marine Research Farm (TGC11-TGC20).

Sarotherodon melanotheron from Lagos Lagoon (SW1-SW11). *Sarotherodon melanotheron* from Badagry lagoon (SW12-SW20). *Sarotherodon melanotheron* from Akinsateru farm (SC1-SC10). *Sarotherodon melanotheron* from Nigerian Institute for Oceanography and marine Research Farm (SC11-SC20).

**Figure 1.** Dendrogram depicting the degree of relationship between *Tilapia guineensis* from wild and culture.

4th cluster (SW14, SW16, SW15, SW12, SW11, SW14, SW13, SW9, SW7, SW16 and SW15); and 5th cluster (SC16, SC17, SC18 and SW1), respectively.

Figure 3 dendrogram showed depicting the degree of relationship between *Tilapia guineensis* and *Sarotherodon melanotheron* from the culture from both

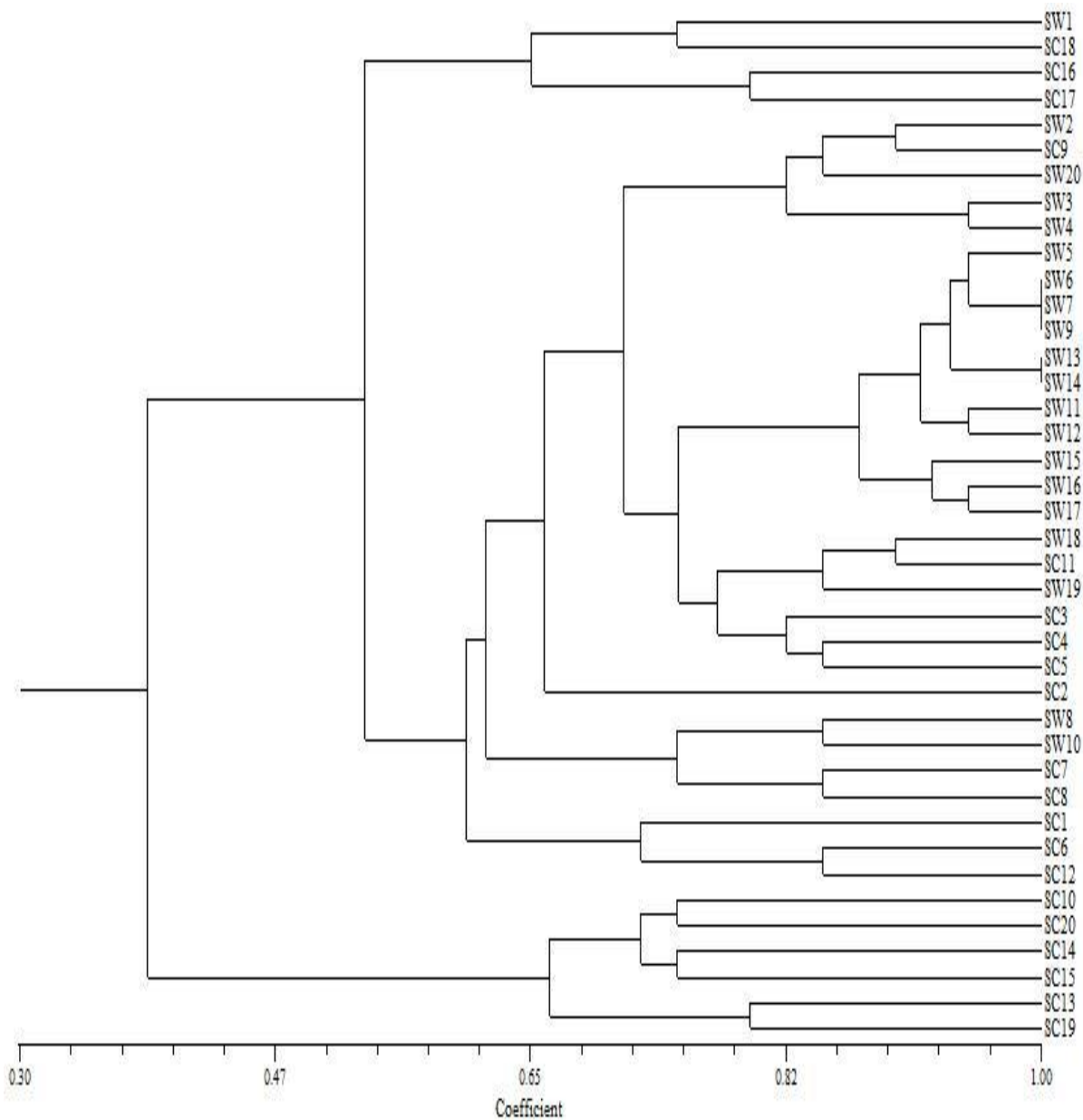


Figure 2. Dendrogram depicting the degree of relationship between *Sarotherodon melanotheron* from wild and culture.

sites which made up three major clusters; TGC3 and TGC4 are 100% similar. 1st cluster consists of SC19, SC13, SC15, SC20, TGC 14, SC8, SC7, SC18, SC10, SC10, SC14, G20, TGC17, TG12, TGC18 and TGC6, 2nd cluster are SC17, SC9, TG16, SC5, SC4, SC4, SC3, SC2, SC12, SC6, SC11, SC1 and TGC. The 3rd cluster includes TC13, TG11, TG10, TGC9, TGC7, TGC8, TGC5, TGC3 and TGC4.

Figure 4 also has four major clusters. SW6 and SW7, TGW1 and TGW2, SW13 and SW114 were similar at coefficient of 1. 1st cluster (TGW18, TGW16, TGW11, TGW5, TW6 and TW4), 2nd cluster (SW8, TGW19,

TGW14, TGW17, SW17, SW16, SW15, SW15, SW12, SW14, SW13, SW9, SW7, SW6 and SW5), 3rd cluster (TGW8, SW19, SW18, TGW10, TGW20, TGW9, TGW15, TGW3, TGW2, TGW1) and 4th cluster (SW14, SW3, SW2, TGW13, TGW13, SW13 and SW14).

DISCUSSION

Random amplified polymorphic DNA (RAPD) bands in this study were always stable (that is strong, faint and sharp, except OPC 10 that had no amplification) bands

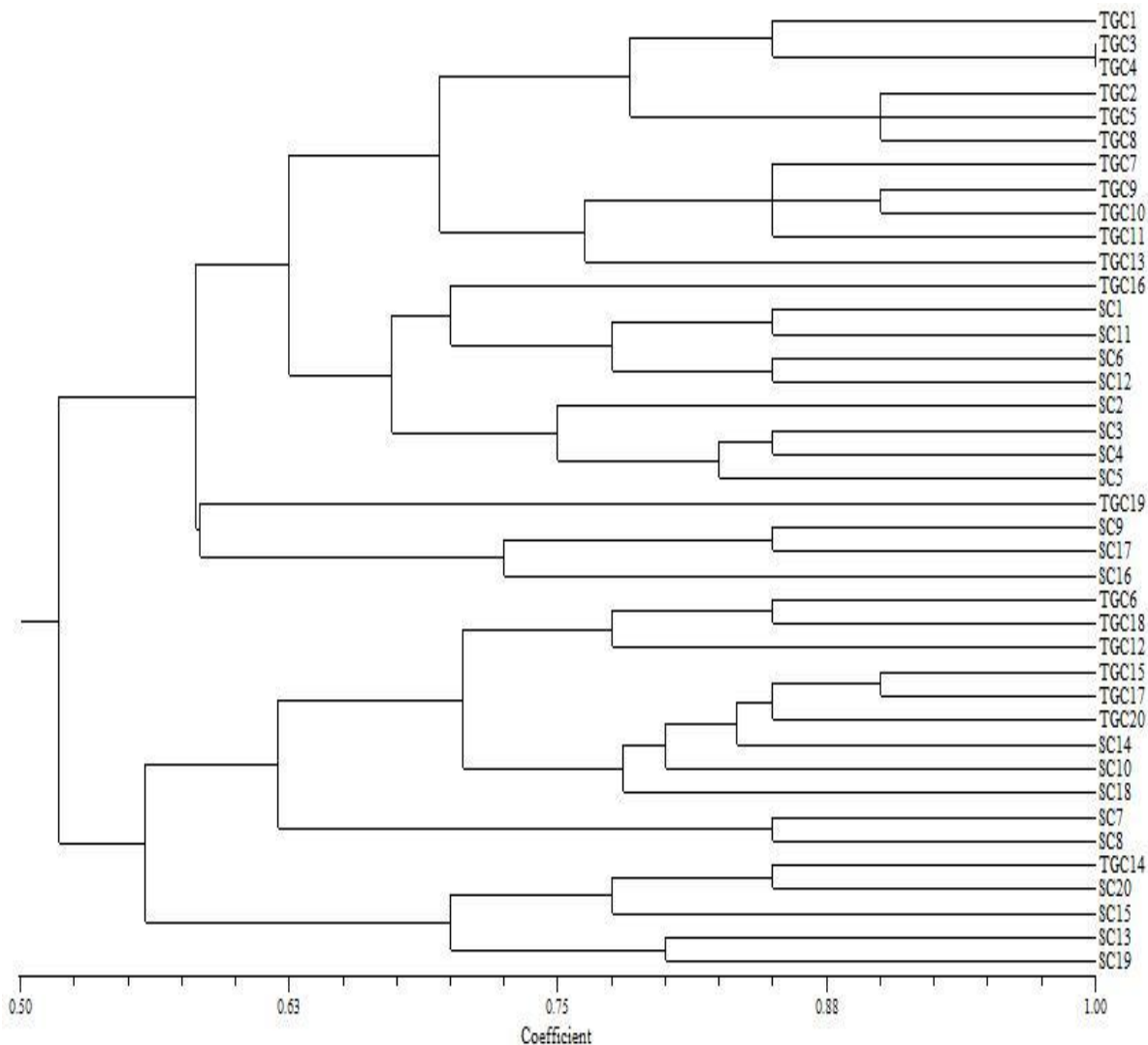


Figure 3. Dendrogram depicting the degree of relationship between *Tilapia guineensis* and *Sarotherodon melanotheron* from culture.

generated with each primer because one or more copies of DNA may exist per genome or may be attributed to the varying of the annealing process between the primer and the DNA. However, the five random primers in RAPD-PCR analysis generated strong amplification profile with distinct bands (Plates 1 to 30) and some mixed bands. The problem of mixed bands shows the well known sensitivity of PCRs (Bielawski et al., 1995). These results are in agreement with Bardkci and Skibinski (1994) who stated that, patterns of similarities and differences between populations showed broad agreement across primers and the overall similarity level varied between primers. Thus, primer choosing is very important for this technique. Samples from wild water body are found to have a similarity coefficient of 100% from Figure 1 (TGW1 and TGW2) and Figure 2 (SW6, SW7, SW13 and SW12) showed that pure strain *T. guineensis* and *S.*

melanotheron. However, the cultured samples are close to that of the wild samples (Kuton and Kusemiju, 2010). The similarity between *T. guineensis* and *S. melanotheron* from the wild was 78%; between *T. guineensis* and *S. melanotheron* from the culture was 80%; and 100% of both wild and culture of samples; 82 and 88% of *T. guineensis* and *S. melanotheron* from wild and culture, respectively.

The dendrogram (Figures 3 and 4) indicates the relationship among the species which are of family Cichlidae. These findings indicated high probability of hybridization between the very closed species to improve the genetic characters; also high quality and quantity of DNA can be gotten in using of salt-out method for extraction and pure strain of the samples can be gotten from wild and culture in spite of the close morphology of body shape they all share. It can be concluded also that,

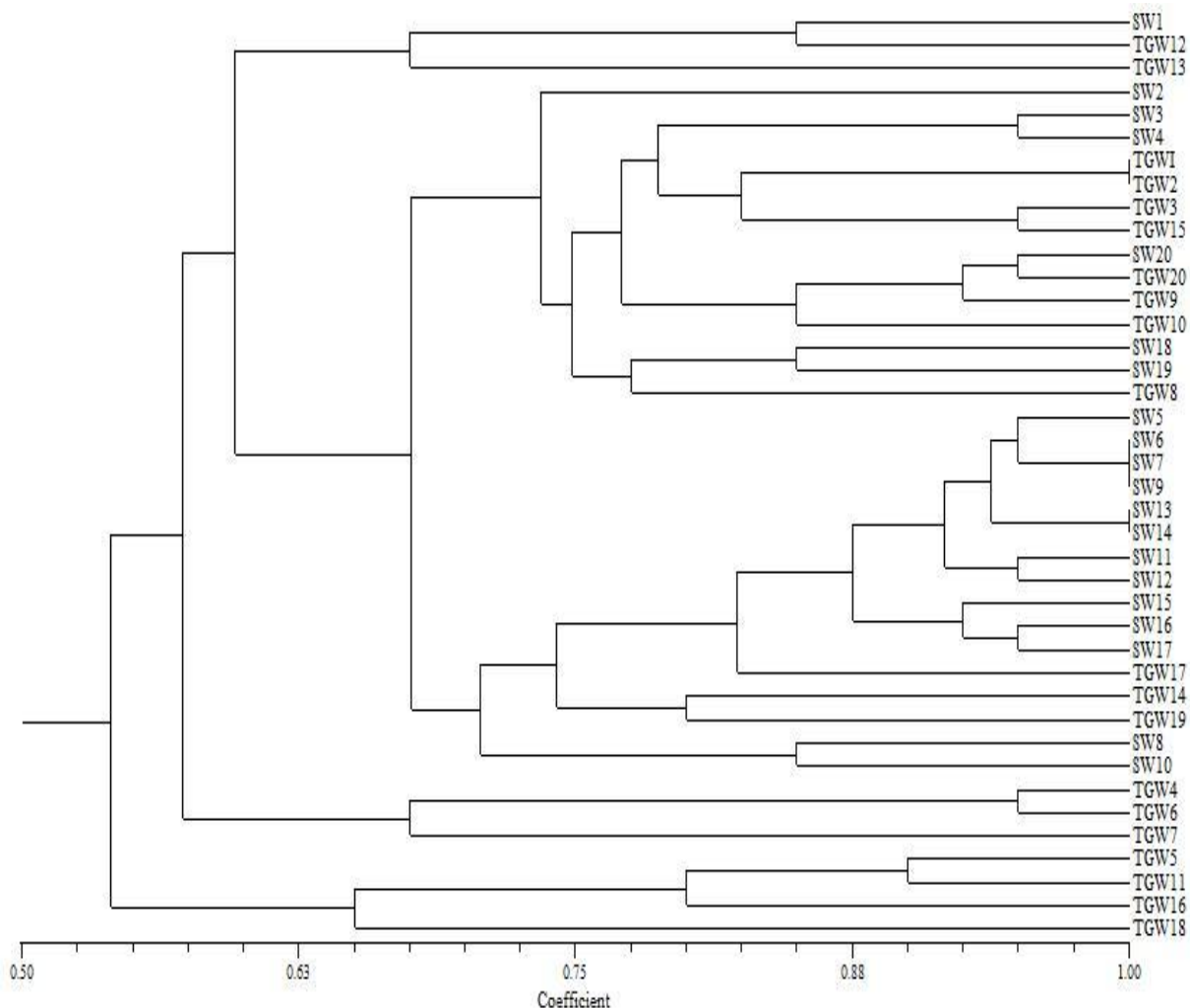


Figure 4. Dendrogram depicting the degree of relationship between *Tilapia guineensis* and *Sarotherodon melanotheron* from wild.

RAPD-PCR could prove to be a useful tool for estimating the genetic variability and degree of similarity among fish species.

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