Vol 1, Issue 1, August 2016
Page No. 9-13, ISSN xxxx-xxxx

ScienceVier
A new era of publication

Open Access International Journal of Applied Sciences

Phylogeny Between Wild Populations of *Oreochromis niloticus* and *Tilapia zilli* Using Randomly Amplified Polymorphic DNA (RAPD) Markers

[1]N.O. Yusuf and [2]A.T. Yisa
[1]National Biotechnology Development Agency, Abuja, Nigeria
[2]Department of Water Resources, Aquaculture and Fisheries Technology,
Federal University of Technology, Minna, Nigeria

Corresponding Author:
N.O. Yusuf
National Biotechnology Development Agency,
Abuja, Nigeria

A B S T R A C T

The phylogeny between wild *Oreochromis niloticus* (*O. niloticus*) and *Tilapia zilli* (*T. zilli*) species collected from New Bussa, Niger State was carried out using Randomly Amplified Polymorphic DNA (RAPD) markers. Four RAPD primers were used for the DNA amplification which generated a total of 55 band loci ranging from 750-7126 bp for both wild *Oreochromis niloticus* and *Tilapia zilli*. The UPGMA dendrogram divides the populations into two main clades, the first clade comprises members of *O. niloticus* and the second clade comprises members of *T. zilli*. The dendrogram revealed a distant genetic evolutionary relationship between wild *O. niloticus* and *T. zilli* which reaffirm the placing of the two species in different genera.

Key words: Phylogeny, *Oreochromis niloticus*, *Tilapia zilli*, RAPD marker

INTRODUCTION

Oreochromis niloticus and *Tilapia zilli* belong to the Family: Cichlidae, Order: perciformes, class: Actinopterygii but *Oreochromis niloticus* belongs to Genus: *Oreochromis*, Species: *niloticus* (Linnaeus, 1758) while *Tilapia zilli* belongs to Genus: Tilapia, Species: *zilli* (Gervais, 1848). Both species are native of Africa and are widely distributed across the tropical and subtropical region of Africa (Boyd, 2004). They are fresh water species and feed on varieties of food such as phytoplankton, periphyton, aquatic plants, invertebrates, benthic fauna, detritus, bacterial films (Elias, *et al.*, 2014; FAO, 2012).

Oreochromis niloticus can achieve a total length of 62 cm and weigh up to 3.65 kg and live up to approximately 9 years of age (FAO, 2012) with an average maximum length of 20 cm (Bwanika, *et al*., 2004). While *Tilapia zilli* can achieve a maximum length of 40cm and weighing 300g and live up to approximately seven (7) years of age(Fish Base, 2008).

Both species by and large lives in shallow water on the edge of lake and wide streams with adequate vegetation (Fish Base, 2008), but differs in their reproductive behavior. In *Oreochromis niloticus* the female lay her eggs on the nest built by the male, the male fertilize the eggs and after fertilization the female collect the fertilized eggs into her mouth, incubation and brooding of the fries takes place in the mouth and release the fries after the yolk sac is absorbed, hence the name mouth brooder (FAO, 2012). In *Tilapia zilli* both parents partake in the nest building using substrate, the female laid her eggs on the substrate and the male fertilize it. The eggs hatches externally, Tilapia zilli are usually refer to as substrate spawner (Fish Base, 2008).

Tilapia species, specifically *Oreochromis* and *Tilapia* has become very important in aquaculture due to their high content of protein, fast growth and palatability and are commercially rated as the second most important fish wild harvest after carp, in 2007, the wild harvest was 769,936 tonnes (metric tons) globally and is on the increase annually (FAO, 2009). It is also achieved the rank of the world's eighth most common cultured fish species, in 2007, FAO (2009), reports a commercial production of *O. niloticus* to be 2.5 million tonnes and valued of about $3.3 billion. Aquaculture production of Nile tilapia (*O. niloticus*) in 2008 reached a total of 2.3 million tonnes and ranked fifth among the most cultured fish species in the world (FAO, 2009). *T. zilli* is also commercially and ecologically important as food fish, aquarium fish, weed control and recreational fishery in its country of origin (Mehanna, 2004).

Randomly Amplified Polymorphic DNA (RAPD) markers are polymorphic DNA sequences isolated by gel electrophoresis after PCR, using one or a pair of short (8-12 base pairs (bp)) random oligonucleotideprimers (Liu and Dunham, 1998a; Liu, *et al*., 1999). The oligonucleotideprimers can scan the whole genome for perfectand sub-perfect binding sites in a PCR reaction, making RAPD to be very potent in distinguishing large numbers ofpolymorphisms. "RAPD markers are communicated and scored as dominant alleles", that is the scored of amplified DNA product is based on size and presence (Dunham, 2004).

The RAPD markers is widely used for it efficient, non -radioactive DNA fingerprinting of genotypes for the identification of genetic relationships and rapid creation of genetic linkage maps (Grattapaglia and Sederoff, 1994; Johnson *et al* .,1994; Liu and Dunham, 1998a, Liu *et al*., 1998b) and also it does not need any previous knowledge of the genetic make-up or sequence information necessary for RFLP or microsatellite analysis. The objective of this work is to reveal the phylogeny between wild *Oreochromis niloticus* and *Tilapia zilli* based on their genetic distance using RAPD markers.

MATERIALS AND METHODS

Sample collection: Samples of wild *Oreochromis niloticus* and *Tilapia zilli* (Juveniles) ten each was obtained from Kainji Lake in Nigeria with the help of the fishermen. For all the samples that was obtained for the experiment, the caudal fin was clipped, packed in micro tube filled with 90% absolute ethanol, well labeled and stored at 5-8°C in a refrigerator until used.

Isolation, purity and quantification of genomic DNA: QIAamp mini kitprotocol was used to isolate the genomic DNA from the caudal fin, edited 2012 (www.qiagen.com/handbooks), the guidelines was followed step by step with few modifications. The purity of the genomic DNA isolated was determined by measuring the optical density at 260nm to optical density at 280 nm to be 1.7-2.0 using Nano-drop spectrophotometer.

The electrophoresis was conducted to determinethe quantityof extracted genomic DNA using 1% agarose gel buffered with 0.5xTBE (500mM Tris-HC1, 60mM boric acid and 80mM EDTA) at 80 volts for 1.5hr and stained with 5 µL of ethidium bromide, thereafter, the gels image was viewed under UV light (thermo scientific, USA), snapped and stored in the computer. About 200 µL DNA which was extracted from each samples were diluted with 10µl of autoclave water and stored at -17°C for 2 days before being used for RAPD amplification. These were carried out at biotechnology center of International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

RAPD amplification and Electrophoresis: Optimization experiment was carried out following the protocol of Skoric *et al*. (2012), four RAPD primers (OPO-10, OPO-02, OPB-01 and OPT-02 were selected (Operon Technologies, USA) for the amplification. The final volumes for the amplification reactions was 10 µL, containing 3 µL of genomic DNA, 0.4 µL of 50 mM $MgCl_2$, 0.8 µLof DNTPs, 1.0 µL of the primer and 0.1 µL of Taq DNA polymerase (Fermentas Life science). The electrophoresis of the amplified products were conducted using 2% agarose gels buffered with 0.5x TBE (500 mM Tris-HC1, 60 mM boric acid and 80 mM EDTA) at 80 volts for 1.5 h and stained with 5 µL ethidium bromide, the gel picture was viewed under UV light (thermo scientific, USA), snapped and stored in the computer. The gel pictures were scored in binary form and used for the analysis of the amplified products. These were carried out at bioscience center of International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Data analysis: Amplified fragments were scored as binary data, i.e., presence as 1 and absence as 0, for homologous bands. Only data generated from reproducible bands were used for statistical analysis. Unweighed paired group method of arithmetic mean (UPGMA) dendrogram based on genetic distance was used to construct the phylogeny between both wild *Oreochromis niloticus* and *Tilapia zilli*, using power marker version 3.25 (Liu and Muse, 2006).

RESULTS AND DISCUSSION

RAPD amplification products: The amplicons generated using 4 operon primers (OPO-02, OPO-10, OPB-01, OPT-02) to reveal the genetic structure of wild populations of *Oreochromis niloticus* and *Tilapia zilli* are shown in Plate 1-4.

The four operon primer used generated different bands at different loci in the two species, a total of 55 band loci were generated for both species, wild *Oreochromis niloticus* and *Tilapia zilli* having 34 band loci and 21 band loci ranging from 750-7126 bp, respectively as show in Table 1.

Phylogeny: The phylogeny between wild *Oreochromis niloticus* and *Tilapia zilli* was constructed using Unweighed Paired Group Method of Arithmetic Mean (UPGMA) dendrogram, based on Nei's genetic distance (Fig. 1). The UPGMA dendrogram separate the two populations into two main clades. The first main clade comprises seven members of wild *Oreochromis niloticus* and the second main clade is divided into two sub clades, the first sub clade comprises eight wild *Tilapia zilli* and second clade comprises of three wild *Oreochromis niloticus* and two wild *Tilapia zilli*.

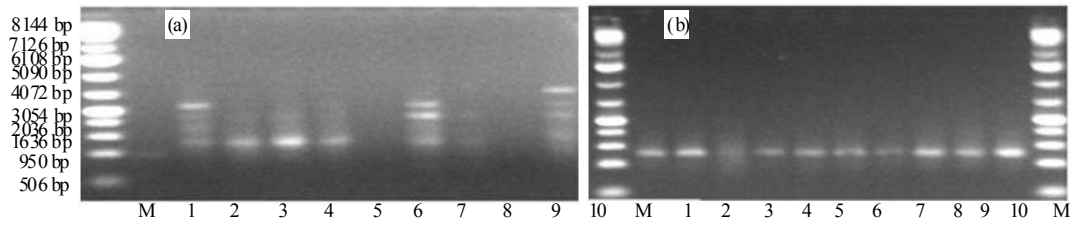

Plate 1: Primer OPO-02 Primer OPO-02 *Oreochromis niloticus* wild *Tilapia zilli* wild

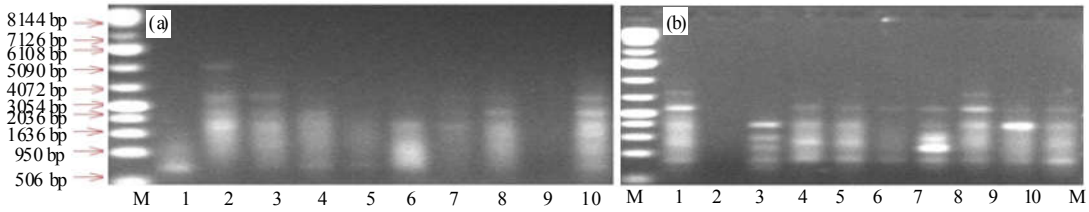

Plate 2: Primer OPO-10 Primer OPO-10 bp *Oreochromis niloticus* wild *Tilapia zilli* wild

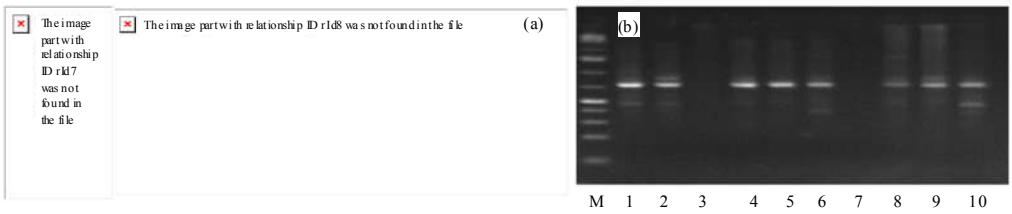

Plate 3: Primer OPB-01 Primer OPB-01 *Oreochromis niloticus* wild *Tilapia zilli* wild

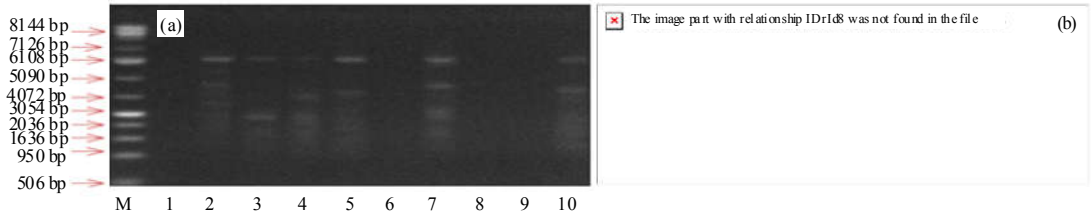

Plate 4: Primer OPT-02 Primer OPT-02 *Oreochromis niloticus* wild *Tilapia zilli* wild

Table 1:

	Oreochromis niloticus	*Tilapia zilli*		
Primers	Ta (°C)	TBL	TBL	Band size range (bp)
OPO-02	38	7	1	5090-950
OPO-10	38	7	9	5090-750
OPB-01	38	9	5	7126-203
OPT-02	38	11	6	6150-1636
Total		34	21	

Ta: Annealing temperature, TBL: Total bands loci, bp: Base pair

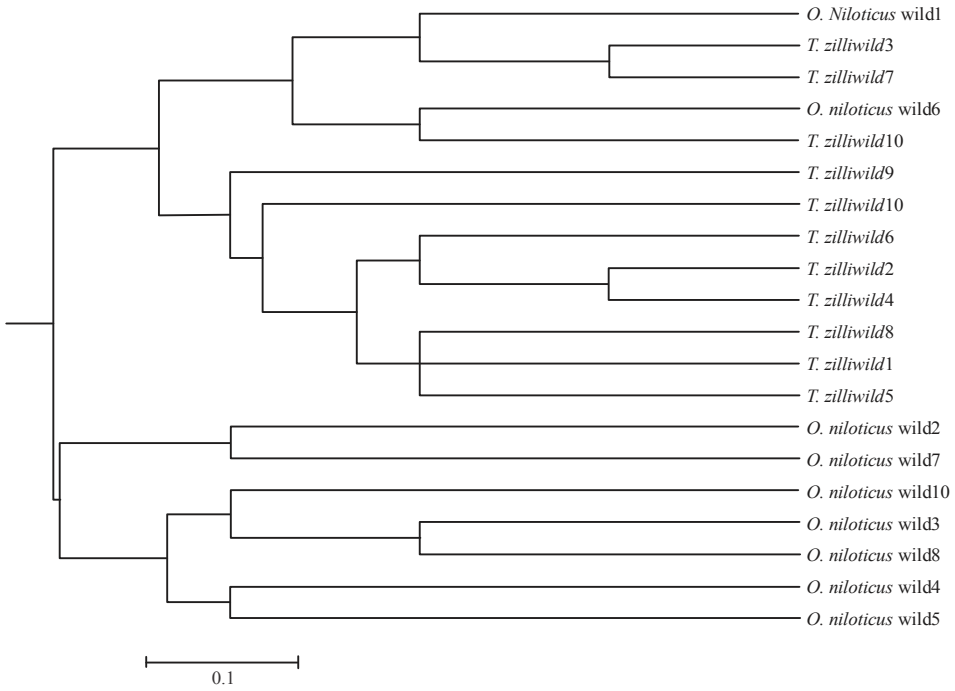

Fig. 1: Phylogeny between both wild *Oreochromis niloticus* and *Tilapia zilli*

The Unweighted paired group method with arithmetic mean (UPGMA) dendrogram based on Nei's (1972), genetic distance was used to construct the phylogeny between wild *Oreochromis niloticus* and *Tilapia zilli* (Fig. 1). The UPGMA dendrogram divides the populations into two main clades. The first clade comprise of seven members of *Oreochromisniloticus* and the second clade was subdivided into two, the first of sub clade comprising of eight members of *Tilapia zilli* and second sub clade comprising of two of the three members of *Oreochromis niloticus* and two member of *Tilapia zilli*. The two main clades indicates that wild *O. niloticus* and *T. zilli* are evolutionarily distant apart, the three member of *O. niloticus* that were found in the second sub clade might have under gone mutation and still retaining the ancestral gene that link them with *T. zilli*, thus, making them to be distant away from the rest of the members and closer to *T. zilli*. The result of this research is in agreement with report by Abdel-Kader, *et al*., (2013), who carried out genetic diversity among three wild species of Tilapia in Egypt detected by Random Amplified Polymorphic DNA Marker, reported that, *Tilapia zilli* shows highest degree of genetic variation compared to *Oreochromis niloticus* and *oreochromis aureus* and that the *Oreochromis* species are closer genetically than *Tilapia zill*.

The RAPDs have also achieve significant attention especially in populace hereditary qualities (Liu and Rank, 1996), species and subspecies distinguishing proof (Bardakci and Skibinski, 1994), phylogenetic, identification of linkage gathering, chromosome and genome mapping, investigation of interspecific gene flow and cross breed speciation and examination of blended genome tests (Hadrys *et al*., 1992), rearing investigation is a great hotspot for single-locus genetic fingerprints (Brown and Epifanio, 2003).

The RAPD examination has also been utilized to assess hereditary differences for species, subspecies and populace or stock recognizable proof in tilapia (Bardakci and Skibinski, 1994), brown trout and Atlantic salmon (Elo *et al*., 1997), largemouth bass (Williams *et al*., 1998), Naish *et al*. (1995), observed that, RAPD investigation is helpful in spotting differences within and among species of *O. niloticus*. The UPGMA dendrogram revealed a distant evolutionary relationship between wild *Oreochromis niloticus* and *Tilapia zilli*.

CONCLUSION

In conclusion, the dendrogram revealed a distant genetic evolutionary relationship between wild *O. niloticus* and *T. zilli* which reaffirm the placing of the two species in different genera.

REFERENCES

Abdel-Kader, H.A.M., Z.G. Abdel-Hamid and K.F. Mahrous (2013).Genetic diversity among three species of Tilapia in Egypt detected by random amplified polymorphic DNA marker. Journal of Applied Biological Sciences, 7: 57-64.

Bardakci, F. and D.O.F. Skibinski (1994). Application of the RAPD technique in tilapia fish: Species and subspecies identification. Heredity, 73: 117-123.

Boyd, E.C. (2004). Farm-Level Issues in Aquaculture Certification: Tilapia. Report commissioned by WWF-US, Auburn University, Alabama 36831, 1-29. Retrieved on March 15, 2014 from www.fisheries.tamu.edu

Brown, B. and J. Epifanio, (2003).Nuclear DNA. In E.M. Hallerman (Ed.), Population genetics:principles and applications for fisheries scientists. American Fisheries Society.Bethesda, Maryland, 101-123.

Bwanika, G.N., B. Makanga, Y. Kizito, L.J. Chapman and J. Balirwa (2004). Observations on the biology of Nile tilapia, *Oreochromis niloticus*, Lnnaeus (1758), in two Ugandan Crater lakes. African Journal of Ecology, 42: 93-101.

Dunham, R.A. (2004). Aquaculture and fisheries biotechnology. Genetic approaches. 875 Massachusetts Avenue 7th Floor Cambridge, MA 02139 USA, CABI, 1-19.

Elias, D., K. Negesse, S. Solomon and B. Kassaye (2014). Food and feeding habits of the red-belly tilapia (*Tilapia zillii* Gervais, 1848) (Pisces: Cichlidae) in Lake Ziway, Ethiopia. Agriculture, Forestry and Fisheries, 3: 17-3. DOI: 10.11648/j.aff. 20140301.14

Elo, K., S. Ivanoff, A. Jukka, J. Vuorinen and J. Piironen (1997). Inheritance of RAPD markers and detection of interspecific hybridization with brown trout and Atlantic salmon. Aquaculture, 152: 55-56.

Fish Base, (2008). *Tilapia mariae,* spotted tilapia: summary. Retrieved on September 14, 2014 from www.issg.org/database/species/ecology.asp

Food and Agriculture Organization of the United Nations (FAO).(2012). Cultured Aquatic Species Information Programme. *Oreochromis niloticus*. Retrieved on February 02, 2015 from http://www.fao.org

Food and Agriculture Organization of the United Nations (FAO).(2009). FAO year book.Fishery and aquaculture statistics. Retrieved on November 08, 2014 from http://www.fao.org/fishery/publications/yearbooks/en

Grattapaglia, D. and R. Sederoff (1994). Genetic linkage maps of Eucalyptus grandis and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. *Genetics,* 137: 1121-1137.

Hadrys, H., M. Balick and B. Schierwater (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular ecology*, 1: 55-63.

Johnson, S.L., C. Midson, E.W. Ballinger and J.H. Postlethwait (1994). Identification of RAPD primers that reveal extensive polymorphisms between laboratory strains of zebrafish. Genomics, 19: 152-156.

Liu, R. and G.H. Rank (1996).Use of RAPD analysis to estimate population genetic parameters in the alfalfa leaf-cutting bee, Megachilerotundata, Genome, 39: 655-663.

Liu, Z.J. and R.A. Dunham (1998a).Genetic Linkage and QTL Mapping of Ictalurid Catfish. Alabama Agricultural Experiment Station, Circular Bulletin, 321, 1-19. Retrieved on December 14, 2014 from www.intl-pag.org

Liu, Z., P. Li, B.J. Argue and R.A. Dunham (1998b). Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and their F1, F2 and backcross hybrids. Animal Genetics 29: 58-62.

Liu, Z.J., P. Li, B.J. Argue and R.A. Dunham (1999). Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish. Aquaculture, 174: 59-68.

Liu, K. and S.V. Muse (2006). Power Marker: an integrated analysis environment for genetic marker analysis software.

Mehanna, S.F. (2004). Population dynamics of two cichlids, *Oreochromis aureus* and *Tilapia zillii*, from Wadi El-Raiyan Lakes. Egyptian journal of Agricultural and Marine Science. 9: 9-16.

Naish, K.A., M. Warren, F. Bardakci, D.O.F. Skibinski, G.R. Carvalho and G.C. Mair (1995).Multilocus DNA fingerprinting and RAPD reveal similar genetic relationships between strains of *Oreochromis niloticus* (Pisces: Cichlidae). Molecular Ecology, 4, 271-274.

Skoric, M., B. Siler, T. Banjanak, J. Zivkovic, S. Dmitrovic, D. Misic and D. Grubisic (2012).The reproducibility of RAPD profiles: Effects of PCR components on RAPD analysis of four *Centaurium* species. Archives of Biological Science, Belgrade, 64: 191-199.

Williams, P., P.A. Young, V.H. Wheeler, P.K. Coryell, H. Gary and G.H. Thorgaard (1998). A Detailed Linkage Map of Rainbow Trout Produced Using Doubled Haploids. Genetics, 148: 839-850.