ISSN:
Print - 2277 - 0755
Online - 2315 - 7453

Journal of Agricultural Science and Environment

PHYLOGENETIC ANALYSIS ON FOUR SPECIES OF TILAPIA (*Oreochromis niloticus*, *Tilapia zilli*, *Sarotherodon galilaeus*, *Sarotherodon melanotheron*) IN NIGERIA

O.T. AGBEBI AND J. ECHEFU

Department of Aquaculture and Fisheries Management,
Federal University of Agriculture, Abeokuta
Ogun State, Nigeria. PMB 2240, Abeokuta, Nigeria

***Corresponding author:** *agbebi20@yahoo.com*

ABSTRACT

An investigation into evolutionary history of four species of Tilapia species was carried out as a taxonomy tool to relate most tilapia species found within the Nigerian waters. These species are (*Oreochromis niloticus*, *Tilapia zilli*, *Sarotherodon galilaeus, Sarotherodon melanotheron*). Blood samples from the four species of tilapia were collected and preserved on Fast Technology for Analysis (FTA) cards for DNA extraction and PCR amplification. The various nucleotide sequences of the four Tilapia species found in the Mitochondria D-loop region were copied and aligned with the use of BioEdit and Mega 6.0 softwares. Three phylogenetic trees were drawn to show the evolutionary relationship amongst the four species of tilapia. The results indicated that *Sarotherodon galilaeus and Sarotherodon melanotheron* are sister texa and share a common ancestor with *Oreochromis niloticus*. *Tilapia zilli* is an out group which is the most distantly related to the three species (*Oreochromis niloticus*, *Sarotherodon galilaeus Sarotherodon melanotheron*). *Tilapia zilli* (Israel) and *Tilapia zilli* are sister texa and share a common ancestor in Tilapia sparmanii. The study also revealed ancestry relationship among other species of fish *Cyprinus caprio* and *Clarias gariepinus* formed a clade with the three tilapia species (*Tilapia zilli*, *Tilapia* zilli (Israel) and *Tilapia sparmanii*), which share an unknown but common ancestor.

Keywords: Tilapia, Phylogenetics, Evolution, Sequences, PCR amplification, Taxonomy

INTRODUCTION

Tilapia culture and production in Nigeria is predominantly an extensive land-based (earthen ponds) system practiced at subsistence levels (Fagbenro, 2002) while commercial tilapia culture is yet to become popular and widespread (Afolabi *et al*., 2000). Its current yield is 14,388tonnes/year (Fagbenro & Adebayo, 2005). With an estimated one million hectares of coastal zone, which offer considerable potential for commercial aquaculture, the activity is a developing venture.

Tilapia culture consists of a broad spectrum of systems/practices operating through a continuum ranging from backyard household ponds to small-scale industrial systems. It contributes to food security, poverty alleviation, employment, trade and income generation (Omotosho & Fagbenro, 2005).

Phylogenetic analysis is a standard and essential tool in any molecular biologist's bioinformatics toolkit. Phylogenetic trees are mathematical structures that depict the evolutionary history of a group of organisms or genes. The aim of phylogenetic trees is to depict historical (i.e., evolutionary) relationships, and not degree of similarity (Dimmic *et al*., 2002).

There are several different methods and protocols for molecular phylogenetic analysis (Huelsenbeck *et al*., 2001; Felsenstein, 2003). This abundance of methods means that a novice user will have to make numerous decisions and choices at several different steps and levels during analysis, which may vary from one data set to another. At the most fundamental level, this estimation of phylogenetic relationships involves two decisions. The first decision is which *optimality criterion* should be used. Given a set of alternative phylogenetic trees, the optimality criterion allows the user to decide which tree explains or fits the data better. There are several different optimality criteria including, but not limited to, maximum likelihood, Bayesian inference, and parsimony(for detailed descriptions of these and other optimality criteria see Swofford *et al*., 1996; Huelsenbeck *et al*., 2001). The aim of any phylogenetic analysis is to identify which tree best estimates the true evolutionary history of the sequence data analyzed.

Therefore, this study aims to evaluate evolutionary relationship among the four species *Oreochromis niloticus, Sarotherodon galilaeus, Sarotherodon melanotheron, Tilapia zilli* of tilapia commonly found in Nigeria.

MATERIALS AND METHODS

The four species of tilapia were gotten from three different locations; The Federal University of Agriculture Abeokuta (FUNAAB) Reservoir Ogun state, Ministry of Agriculture Odeda farm institute (Eweje Odeda) and IFSERAR (Institute of Food Security, Environmental Resources and Agricultural Research) FUNAAB. The Tilapia species were identified morphologically during harvest and selection from the water bodies. Nile tilapia has distinctive, regular, vertical stripes extending as far down the body as the bottom edge of the caudal fin, with variable coloration. *Sarotherodon malanotheron* was identified by its low numbers of vertebrae (26–29, usually 27–28), 12–19 lower gill rakers, 14–16 dorsal spines. The Tilapia zilli was identified by marks on the dorsal fins retained from juvenile to adult, two horizontal stripes overlay by 8-9 crossbars and black blotch on upper edge of operculum. Blood samples from the four species of tilapia were collected on Fast Technology for Analysis (FTA) cards for DNA extraction and PCR amplification.

DNA Extraction

1mm disk of the blood sample was punctured from the FTA® classic cards and put in a 1.5ml eppendorf tube. 50µL of ddH_20 was added and vortex three times and left to rest for 10 minutes. The spent water was removed as much as possible. Then, 100µL ddH_20 was added so as to submerge the disks. The tube with the disks was then transferred to a heating block and heated at 99^0C for 15mins. The samples were vortex and briefly centrifuged. The extracts were then pipetted and put in a new tube; the

preparation has 60-150µL of DNA.

PCR Amplification

The PCR amplification reaction consist of 10x PCR Buffer, 50µM dNTPs, H_2O Nuclease free, 250µM $MgCl_2$, 10µM of primer forward, 10µM of primer forward, and10µL Surf Hot Tag. Amplification was performed in a Thermocycler (Agilent Surecycler 8800, Applied Biosystem, Foster City, USA) programmed as follows: an initial denaturation at 96^{0}C for 15mins, followed by 40 cycles each consisting of 45secs denaturing at 56.9^{0}C for *Sarotherodon melanotheron*, 60^{o}C for *Tilapia zilli*, 62.7^{o}C for *Sarotherodon galilaeus* and *Oreochromis niloticus*, 90secs primer annealing at 72^{0}C, 7mins extension at 72^{0}C and then a final 8mins extension at 12^{0}C.

PCR was carried out using different primer for the four species:

Tilapia zilli: Czilli fwd 5' GGATTTTAACCCTTACCCC 3'

Czilli Reverse 3' AGTAAAGTCAGGACCAAGCC 5'

Oreochromis niloticus: Fish-comum-D-loop Fwd 5' GGATTYTAACCCYTRCCCC 3'

Czilli Rev 3' AGTAAAGTCAGGACCAAGCC 5'

Sarotherodon melanotheron: Fish – D-loop2-fwd 5' RCCCCTAACTCCCAAAGC 3'

Fish-D-loop2 Rev 3'TAAAGTCAGGACCAAGC 5'

Sarotherodon galiIaeus: Fish-comum-D-loop Fwd 5' GGATTYTAACCCYTRCCCC 3'

Czilli-rev 3'AGTAAGTCAGGACCAAGCC 5'

SEQUENCING OF THE mt DNA

The sequencing was carried out in10µl comprising approximately 250µM of $MgCl_2$ 50µM of dNTP, H_20 Nuclease free, 10 µM of primer forward, 10 µM of primer reverse and 10 µM of surf Hot Tag initial denaturation at 96^{0}C for 1min, followed by 30 cycle of denaturing at 96^{0}C for 10seconds, annealing at 50^{0}C for 6secs and extension at 60^{0}C for 4minutes and then a final 8mins extension at 12^{0}C for 10minutes.

SEQUENCE ALLIGNMENT

Bioedit® and Mega® 6.0 software was used for the multiple DNA sequences alignment.

The process of DNA extraction to sequencing were carried out in STAB Vida Laboratory, at Madan Parque, Portugal.

EVOLUTIONARY RELATIONSHIP

A multiple sequence alignment was carried out on the nucleotide sequences of the species(*Oreochromis niloticus*, *Tilapia zilli*, *Sarotherodon galilaeus*, *Sarotherodon melanotheron*) with the other sequences retrieved from FASTA using (Bioedit® and Mega®6.0) was used to draw the dendogram to show the evolutionary relationship amongst the species.

RESULTS

Evolutionary relationships among the nucleotide sequences of four species of Tilapia

The evolutionary history was inferred using the Neighbor-Joining method [Saitou and Nei, 1987). The optimal tree with the sum of branch length = 9.41641598 is shown (Figure 1) next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 40 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 731 positions in the final dataset. Evolutionary analyses were conducted with Mega® 6.0.

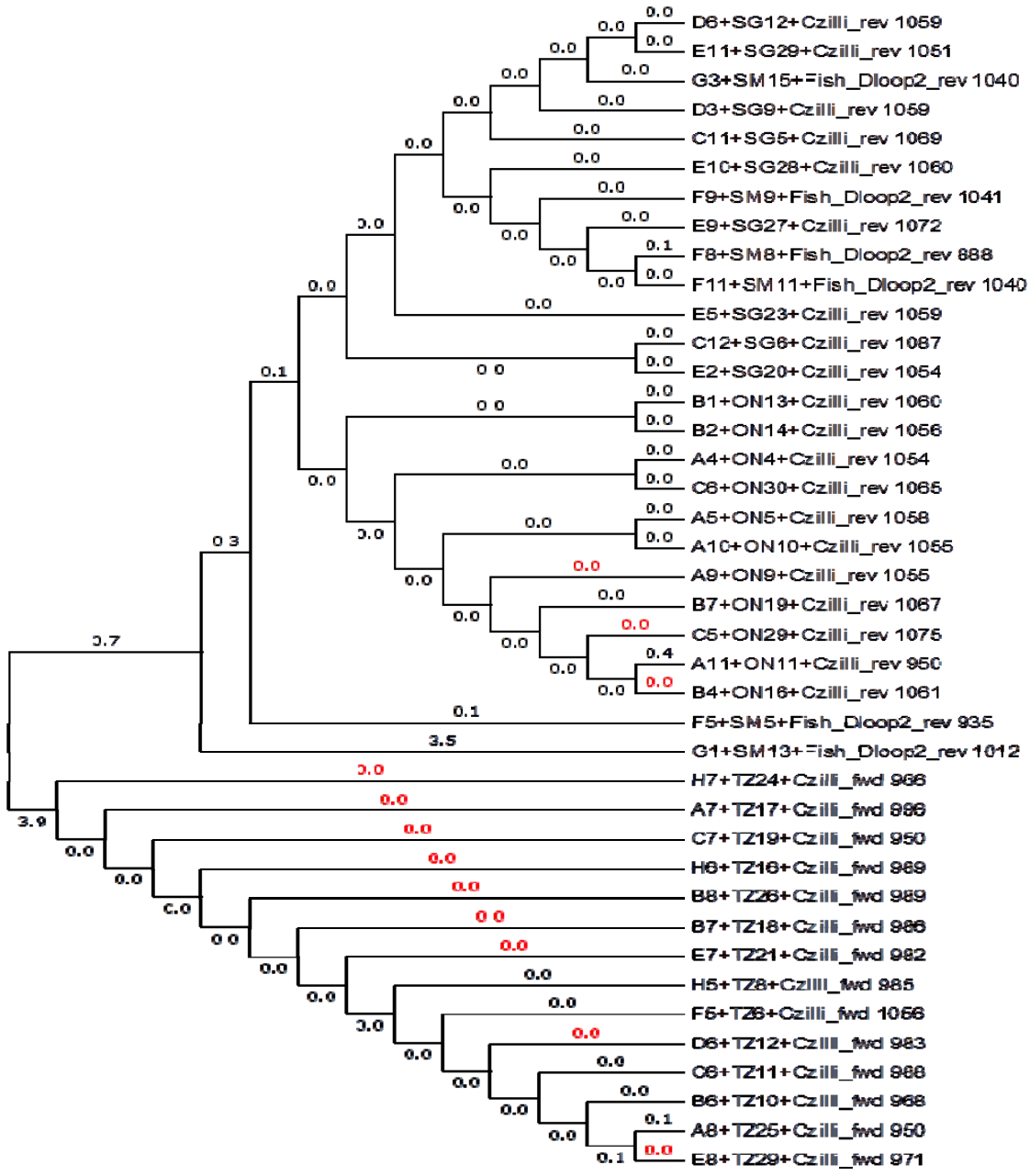

Figure 1: Phylogenetic tree of nucleotide sequence of four tilapia species
ON = *Oreochromis niloticus*, **SG** = *Sarotherodon galilaeus*, **SM**=*Sarotherodon melanotheron*, **TZ** = *Tilapia zilli*

4.2 Evolutionary relationship among four species of Tilapia

The evolutionary history was inferred using the Neighbor-Joining method [Figure 1]. The optimal tree with the sum of branch length = 1.98119157 is shown (Figure 2). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 4 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 839 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

Evolutionary relationship amongst the four Tilapia species, mammals and other fish species.

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 8.66212555 is shown (Figure 3). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 124 positions in the final dataset from the evolutionary analyses conducted.

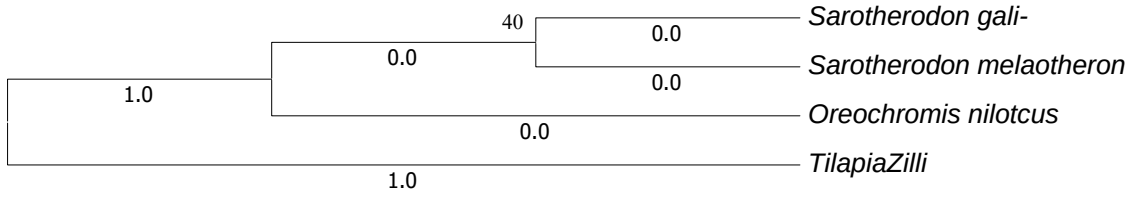

Figure 2: Phylogenetic tree of four tilapia species

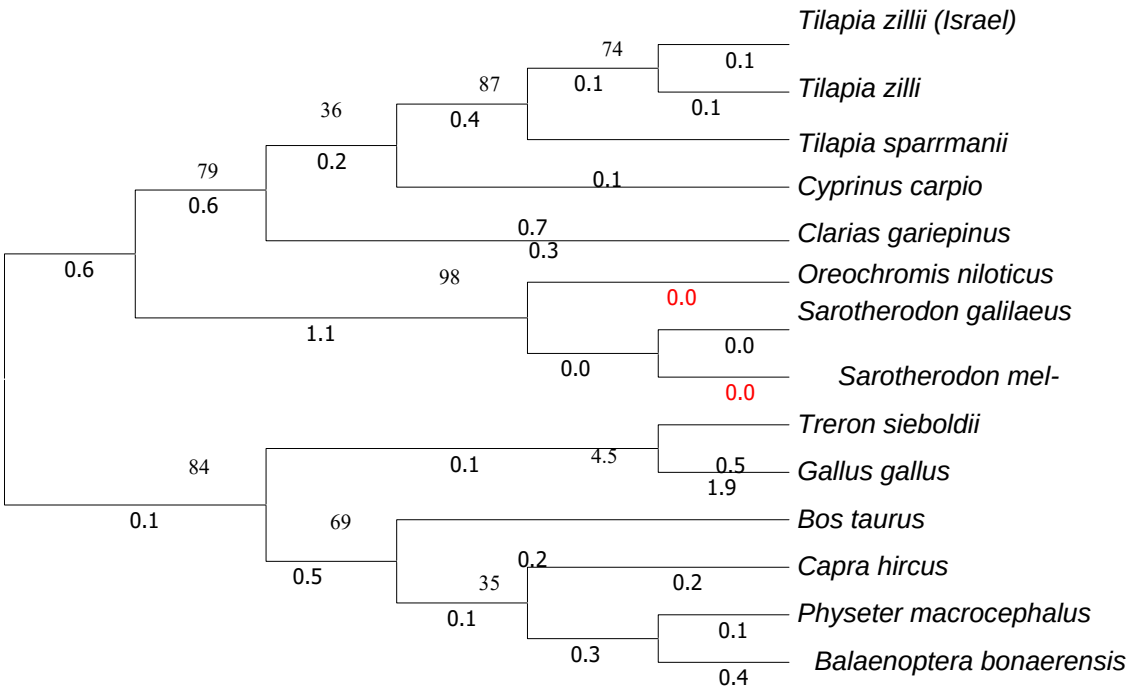

Figure 3: Phylogenetic tree of four tilapia species, mammal and other fish spe-

DISCUSSION

It can be observed that *Sarotherodon galilaeus* and *Sarotherodon melanotheron* from the phylogenetic tree are sister taxa which means that they have a lot of evolutionary history in common, are characteristic mouth brooders and have a common ancestor that is unique to them which is *Oreochromis niloticus* while *Tilapia zilli* is the most distantly related of the four species and can be regarded as out group which the other species evolved from. This backs up the systematics by Trewavas, 1942 and supported by Klett and Meyer, 2002 who stated that Tilapia is not a monophyletic group. Out of the three genera, the genus *Oreochromis* is of great economic importance in global fisheries and aquaculture (Bostock *et al*., 2010) with the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) ranking first within the genus (Josupeit, 2010; Agnèse *et al*., 1997). This species is also ranked 5th among the most cultured species in the world after grass carp (*Ctenopharyngodon idella*), Silver carp (*Hypophthalmichthys molitrix*), Common carp

Tilapia zilli (Israel) and *Tilapia zilli* are sister texa and share a common ancestor in Tilapia sparmanii. The two other species of fish *Cyprinus caprio* and *Clarias gariepinus* formed a clade with the three tilapia species (*Tilapia zilli*, *Tilapia* zilli (Israel) and *Tilapia sparmanii*), which share an unknown but common ancestor.

The other three Tilapia species, *Sarotheron galilaeus, Sarotherodon melanotheron,* and *Oreochromis niloticus* was observed to be clustered **together** as *Sarotheron galilaeus* and *Sarotherodon melanotheron* form sister taxa and *Oreochromis niloticus* as a common ancestors to both. The same conclusion was reached by McAn-

drew and Majumdar (1983) who proposed a close relationship of the mouth brooding *Oreochromis* and *Sarotherodon* genera as distinct from the substrate spawning *Tilapia* genus.The three tilapia species are related to other Tilapia fishes through their distant relatives which are *Cyprinus carpio* and *Clarias gariepinus*. In general, *Tilapia zilli, Tilapia zilli (Israel)* and *Tilapia sparrmanii* are descendants of *Sarotheron galileus, Sarotherodon melanotheron,* and *Sarotheron galileus* (Ansah and Frimpong, 2015)..

The two poultry species *Treron sieboldii* and *Gallus gallus* were sister taxa. The two species of whales *Physeter macrocephalus* and *Blaenoptera bonaerensis* were also found to be sister taxa formed cluster with *Bos taurus and Carpa hircus* (Lowe et al., 2000). Conclusively, it can be reported that other species include avians and mammals are outgroup and distantly related to the four Tilapia.

CONCLUSION

Phylogenetic trees are mathematical structures that depict the evolutionary history of a group of organisms or genes. The aim of phylogenetic trees is to depict historical (i.e., evolutionary) relationships, and not degree of similarity.

In conclusion, it was observed that *Sarotherodon galilaeus* and *Sarotherodon melanotheron* have a common ancestor originated from *Oreochromis niloticus*. It was further revealed that *Tilapia zilli* (Isreal), *Tilapia zilli*, *Tilapia spermanii*, *Cyprinus carpio* share a common ancestor with *Clarias gariepinus*.

RECOMMENDATION

This study revealed the evolutionary relationship amongst four tilapia species using phylogenetic analysis, as a taxonomy tool to relate most tilapia species found within the Nigerian waters. The create a broad understanding of the evolutionary relationship among the mostly farmed tilapia species in Nigeria. Further research is recommended on the ancestory relationship of other Tilapia species in order to relate the evolution of these four Tilapia species.

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(Manuscript received: 21st December, 2015; accepted: 7th June, 2016)