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Genetic Differentiation Studies among Natural Populations of *Tilapia zillii*

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Abstract

The population structure of *Tilapia zillii* (Gervais 1848) from three reservoirs in Nigeria, Osun State (Opa, Osu and Igun) was determined by employing morphological and molecular (Random Amplified Polymorphic DNA) methods. For morphological studies, 25 morphometric measurements and six meristic counts were recorded on 40 individuals within each population. Principal Component Analysis (PCA) was performed on the morphometric and meristic data using the PAST software. For RAPD studies, genomic DNA was extracted from caudal fin tissue using CTAB method and five primers were used to initiate PCR amplifications. All the clusters produced by the Principal components analysis (PCA) of the morphometric and meristic parameters overlapped indicating a low level of genetic differentiation between the three populations of *T. zillii* studied. The UPGMA cluster diagram from RAPD analysis identified two major genotypic groups with inter and intra group relationships. All individuals in the first cluster were from the Osu reservoir, while individuals from Opa and Igun reservoirs constituted the second cluster. Nei's unbiased measure of genetic distances was 0.8532, 0.7321 and 0.7111 for Osu, Igun and Opa populations respectively. This revealed that Opa and Igun populations were genetically closer, while Osu populations is distant from them. The results suggest that the RAPD technique could be used to differentiate populations of *T. zillii*. However, additional methods such as microsatellite and sequence analysis can be used to maximize the efficiency of genetic differentiation studies.

Keywords: genetic distance, morphometric, PCA, RAPD, UPGMA, variation

Introduction

Tilapia zillii (Gervais 1848) commonly referred to as red belly Tilapia is an important food fish and also one of the most aqua cultured fresh water fish (Adesulu and Sydenham, 2007). It is native to Africa, endemic to Nigeria and widely distributed in Nigerian waters (Agbabiaka, 2010). According to Adesulu and Sydenham (2007) it is the fourth most cultured Tilapia fish which has broadened its base from being a subsistence oriented technology to being a component of world commerce; as a high-quality fillet product of the fish has been exported to Europe and the US. It has been cultured intensively for more than four decades; however, its genetic resources have been poorly managed (Omitogun, 2005). Understanding the genetic diversity and differentiation within the species range would be of great importance for the sustainable utilization of the species as an aqua cultural candidate, the protection of the endangered populations and for bio geographical inferences.

In developing management and conservation strategies of any species, knowledge on the biology and population structure of that species is a prerequisite (Turan *et al.,* 2006) and may be useful for studying short-term and environmentally induced variation. Knowledge of the population structure of *Cichlids* is economically important for several issues pertinent to fishery management and future development of aqua cultural strains. Genetic differentiation and level of gene flow are typically used to distinguish one population from another. Differences in morphology (morphometric and meristic study) can also be used, as they are vigorous tools for measuring discreteness of the same species and are also helpful for separating closely related genera, species and even populations within them (Cadrin, 2000).

Morphometric characters are generally being used in discriminating many fish species in several parts of the world (Gunawickrama, 2007; Murta, 2000), in Africa (Teugels, 1992; Hassanien *et al*., 2011) and also in Nigeria (Eyo 2002, 2003; Anyanwu and Ugwumba, 2003; Edema and Osagiede, 2011; Kuton and Adeniyi, 2014). However, morphological description alone has proved to be unreliable in establishing genetic relationships within and between species.

The ability to understand the genetic relationships within species at the molecular level has greatly increased through the application of Random Amplified Polymorphic DNA (RAPD) techniques (Sabir *et al.,* 2012). This technique has also been successfully exploited for stock identification and population analysis in fish (Welsh and McCelland, 1990; Williams *et al.,* 1990) as well as in differentiating individuals or breeding stocks with a given species or among different species. The advantage that RAPD technique has over other

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systems of genetic documentation includes its use of universal sets of primers, no preliminary work such as probe isolation, filter preparation, or nucleotide sequencing is necessary and relatively low cost.

RAPD markers have been used to evaluate the genetic diversity in numerous organisms and on fish populations belonging to the same family or genus (Cooper, 2000; Ali *et al.*, 2004; Usman *et al.,* 2013; Megbowon and Fashina-Bombata, 2013; Asagbra *et al*., 2014) RAPD markers can be used for species and sex identification in animals (Appannavar *et al.,* 2003; Huang *et al*., 2003).

In the present study, the genetic variation and relatedness between and among natural populations of *Tilapia zillii* from three different reservoirs in Osun State was determined using morphological and Random Amplified Polymorphic DNA (RAPD) analysis.

Materials and Methods

Collection of samples

Samples of *Tilapia zillii* were collected from Opa reservoir, Osu reservoir and Igun reservoir (all in Osun State, Nigeria) over a period of six (6) months (July-December 2013). The fishing methods employed for collecting the specimens were gillnetting and cast netting. All collected specimens were packed in polythene bags and plastic container and transported to the laboratory for further analyses. Fourty *T. zillii* specimens from each reservoir were preserved in 10% formalin (e.g. 4% formaldehyde solution) for morphometric and meristic studies and fresh *T. zillii* samples were preserved in 95% ethanol for isolation of genomic DNA. Identification was done following identification keys prepared by Paugy *et al.* (2003) and Adesulu and Sydenham (2007).

Description of study area

Opa Reservoir is located at Ife central local government area of Osun State, Nigeria. It lies between longitudes 4°30' to 4°40' East of the Greenwich and latitudes 7°27' to 7°35 ' North of the Equator.

Osu reservoir is newly impounded in 1995, located at Osu, Atakunmosa West Local government area of Osun State, Nigeria. It lies between longitude 004°38.32' E to 004°38'49.1" E and latitude 07°35.3' N and 07°35'17.9" N.

Igun reservoir is located in an abandoned gold mine area of Igun village in Atakumosa West Local Government area of Osun State, Nigeria. It extends over longitudes $4^0$30' E to $4^0$45' E and latitude $07^0$35' N to $07^0$38' N.

Osu and Igun reservoirs are closer geographically, as they belong to the same local government while Opa reservoir is distant from them. The geographical locations of the three reservoirs on the map of Osun State is shown in Fig. 1.

Morphometric and meristic studies

Twenty five measurements as in Dunz and Schliewen (2010) were recorded in 40 individuals within each population as shown in Fig. 2. Six meristic counts were also recorded as follows: number of scales on lateral line, dorsal spine, dorsal ray, gill rakers, anal fin ray and anal fin spine.

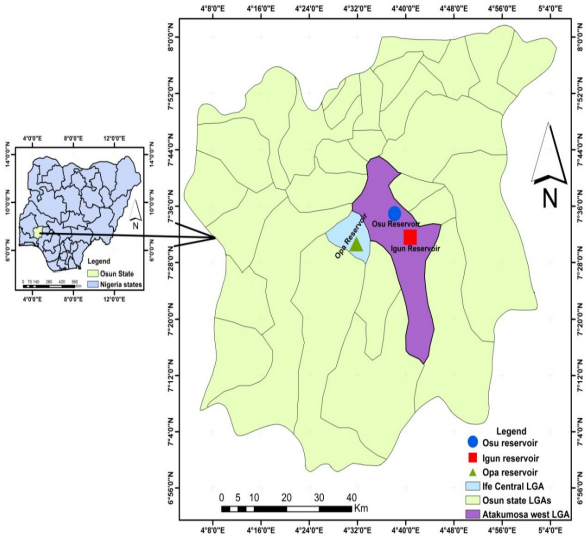

Fig. 1. Map of Osun State showing Opa, Osu and Igun reservoirs (Nigeria)

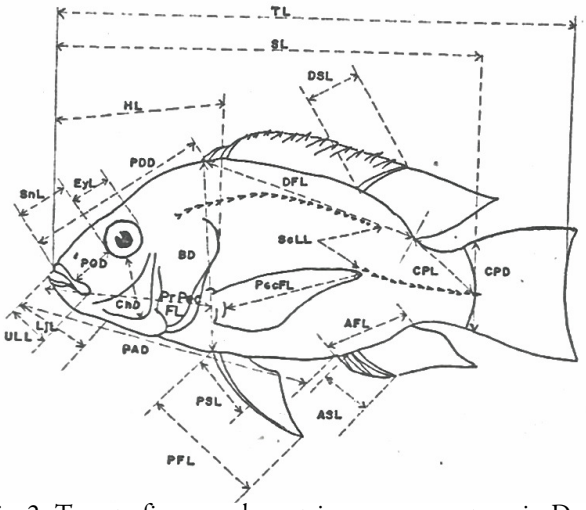

Fig. 2. Twenty five morphometric measurements as in Dunz and Schliewen (2010)

(TL=total length, SL=standard length, HL=head length, BD=body depth, SNL=snout length, ChD=cheek depth, EYL=eye length, DFL=dorsal fin length, DSL=Length of last dorsal spine. AFL=anal fin length, ASL=length of third anal spine, PFL=pelvic fin length, PDD=predorsal distance, ULL=upper lip length, LJL=lower jaw length, PrPecFL=pre-pectoral fin length, PECFL=pectoral fin length, POD=preorbital distance, CPL=caudal peduncle length, CPD=caudal peduncle depth, LJW=lower jaw width, PSL=pelvic spine length, PAD=preanal distance)

Morphometric data analysis

Measurements of each morphometric character were standardized to fish size (SL) in accordance with Reist (1985) to remove size-effect using percentage standard length as it follows: $Mn=(Mo/SL)\%$, where: Mo is the original measurement; and SL is the standard length.

The morphometric measurements were then transformed to common logarithms because linearity and normality are usually more closely approximated by logarithms than by original variables (Hair *et al*., 1998). Size-corrected data were analysed by multivariate method. Morphometric and meristic characters were analysed separately, since these variables are different both statistically (the former are continuous while the latter are discrete) and biologically (the latter are fixed

early in development, while the former are more susceptible to the environment) (Allendorf *et al.*, 1987). Principal component analysis (PCA) on morphometric and meristic data was performed using the software, PAST (Hammer *et al.*, 2001). To find out the morphometric factors that can discriminate among the three populations, Principal Component Analysis (PCA) was used in which factor loadings based on eigens values were used to determine the morphometric factors.

RAPD/PCR

Total genomic DNA was extracted from caudal fin tissue (1 cm^2) following standard CTAB method (Saghai *et al.*, 1984). DNA concentration of all the samples was measured on spectrophotometer at 260 nm and 280 nm and the genomic purity were determined. The quality of DNA was also detected by agarose gel electrophoresis. Electrophoresis was done at 80 V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source. The DNA extracts were subjected to PCR amplification with primers purchased from Operon Technologies, USA. Ten different primers were tested on fish samples and five, exhibiting the highest quality banding patterns and sufficient variability, were selected for population analysis. The primers identities are presented in Table 1. Gel products were visualized, photographed and subsequently analysed for polymorphism. The RAPD profile generated by each set of primer was transformed into numerical values, where the presence of a band was scored as 1 and absence of a band was scored as 0. The binary values were transferred into NTSYS software for analysis using UPGMA method (Rolf *et al.*, 2000). The index of similarity (Band Sharing Frequency) between individuals was calculated according to Nei and Li (1979) formula: $F=2NXY/(NX+NY)$, where NXY is the number of bands shared in common between individuals X and Y, NX is the total numbers of bands scored for individuals X and NY is the total numbers of bands scored for individuals Y. Thus, F reflects the proportion of bands shared between two individuals and ranges from 0 (no common bands) to 1 (all bands identical). The genetic distance (d) was calculated as: $d=1-F$ (Nei and Li, 1979).

Results

The Principal Component Analysis (PCA) diagram of the 25 morphometric measurements of *T. zillii* from the three reservoirs of study is shown in Fig. 3, while the PCA diagram for the six meristic counts is shown in Fig. 5. All the clusters produced by the Principal Components Analysis (PCA) of the morphometric measurements overlapped. The character most responsible for variation among the three studied populations of *T. zillii* was lower jaw width (loading 0.6981) as shown in Fig. 4. The PCA diagram of the six meristic counts showed no specific pattern of differentiation.

RAPD results

All five primers employed in this study produced good RAPD fragments with varying bands. The bands ranged in molecular size from approximately 200 to 4500 bp. No

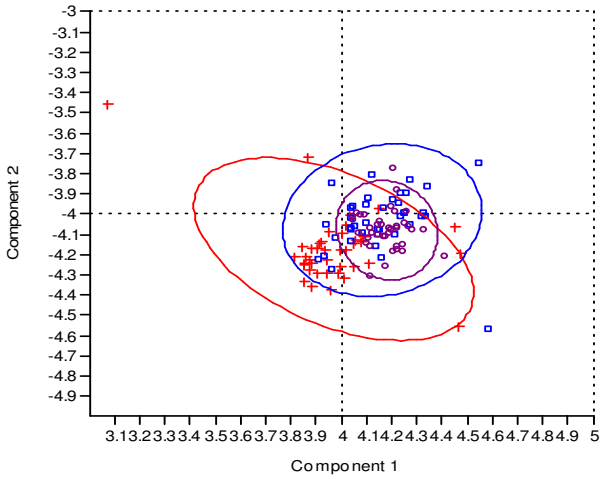

Fig. 3. Principal components analysis based on 25 morphometric measurements of *T. zillii* showing overlap of characters between populations from Opa Reservoir (red), Igun Reservoir (purple) and Osu Reservoir (blue)

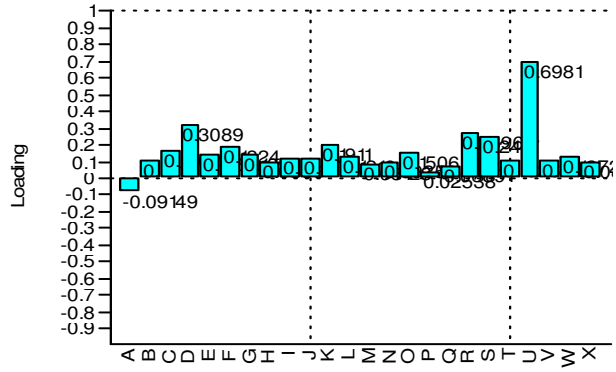

Fig. 4. Respective *T. zillii* morphometric characters and their loadings on PC1 of the principal components analysis showing lower jaw width as the character most responsible for variation among the studied populations of *T. zillii*

Key: A=total length (TL), B=head length (HL), C=body depth (BD), D=snout length (SNL), E=cheek depth (CHD), F=eye length (EYL), G=dorsal fin length (DFL), H=anal fin length (AFL), I=length of last dorsal spine (LDS), J=length of third anal spine (LTAS), K=pelvic fin length (PFL), L=predorsal distance (PDD), M=upper lip length (ULL), N=lower jaw length (LJL), O=lower lip width (LLW),P=lower lip length (LLL) ,Q=pectoral fin length (PECFL),R=preorbital distance (POD), S=caudal peduncle length (CPL), T=caudal peduncle depth (CPD),U=lower jaw width (LJW), V=pelvic spine length (PSL),W=preanal distance (PAD) and X=distance lower jaw to pelvic fin (PELD).

artifacts were observed and the number of fragments amplified per primer varied. A total of 320 individual bands were obtained from the five primers, of which 243 bands were polymorphic and 77 bands were monomorphic (Table 2). Primer OPAE-05 (250bp-2500bp) produced the highest number of fragments among the primers used, with an average of 141 bands, while primer OPAE-04 produced the lowest number of fragments with an average of 18 bands. Result of the phenogram analysis for polymorphism in each primer is shown in Table 3. The total number of RAPD bands produced in Opa, Osu and Igun populations were 69,

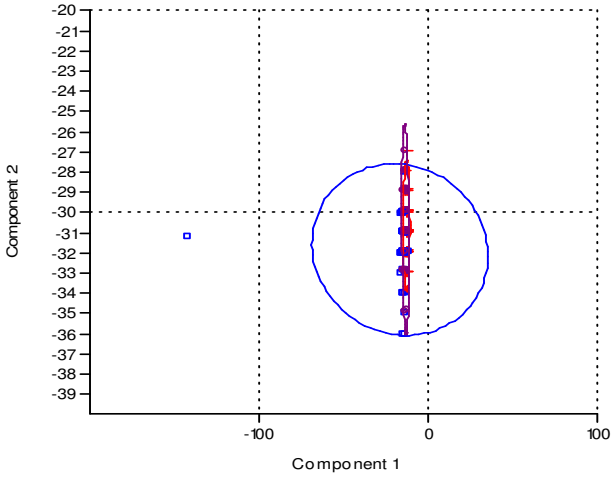

Fig. 5. Principal components analysis based on six meristic counts of *T. zillii* from Opa Reservoir: (red) Igun Reservoir: (purple) and Osu Reservoir (blue) showing homogeneity of characters

157 and 94 respectively (Table 3). There was no band that was population specific.

The values of pair-wise comparisons of Nei's (1978) unbiased genetic distance (*d*) between populations, computed from combined data for the five primers ranged from 0.71 to 0.85 (Table 4). The Opa reservoir was the most genetically distinct population, which was segregated from the Osu population with the *d* value of 0.85 and from the Igun reservoir population with the value of 0.73. The Opa and the Igun populations were separated from each other with the lowest genetic distance (d=0.71). The index of similarity (F) values showed that Opa and Igun are more similar, with a value of 0.29 compared to Osu and Igun (F=0.27) and Osu and Opa (F=0.15). The band-sharing based similarity indices were higher for within-river samples than for all between river sample comparisons. This implies that the *T. zillii* population of one river was more homogenous than the combined group of the three populations. Dendrogram showing the cluster analysis of the individuals' genotype is presented in Fig. 6. The UPGMA cluster diagram identified two major genotypic groups with inter and intra group relationships. All specimens in the first cluster were from the Osu reservoir, while specimens from Opa and Osu reservoirs constituted the second cluster. However, all the groups had varied inter-relationships showing a highly heterogeneous population.

Discussion

Significant morphological heterogeneity was evident among the three population samples of *Tilapia zillii* although the level of differentiation between them was small as evidenced by wide overlap of the morphometric data. This is consistent with the report of Carvalho (1993) that if localized populations inhabit similar environments, they may fail to display great heterogeneity in phenotypic or genetic traits.

The Principal Component Analyses (PCA) diagram of the six meristic counts of *T. zillii* from the three reservoirs of study showed homogeneity of characters (Fig. 3). The differentiation pattern shown by the meristic characters is not informative, as all the samples from the three reservoirs clustered together. Usually, morphometric characters reveal much greater differences among groups than the meristic. This reduced importance of the meristic differences is stressed by the lack of validation by the Principal Component Analysis. Morphometric characteristics showed considerably greater discriminatory power to distinguish the *T. zillii* populations than did the meristic characters.

Several authors have reported evidence of genetically determined morphometric and meristic characters: Carscadden and Leggett (1975), Ihssen *et al.* (1981b), Murta (2000). However, in several situations the patterns of differentiation shown by meristic characters have been considered worse than morphometrics for these purposes (Misra and Carscadden, 1987; Murta, 2000). The number of dorsal fin rays, dorsal fin spine, anal fin rays and anal fin spine from the three locations were constant, indicating identity in parental stock. The fairly constant values of fin rays observed in the three populations agree with the findings of Holden and Reed (1972) and Reed *et al.* (1967) that fin rays of the tribe *Tilapiini* do not vary much.

The morphometric results are insignificant to establish the genetic structure of the population which often leads to taxonomic uncertainty. This is consistent with the report of Daniel (1997), Ponnian and Gopalakrishnana (2000) and Garg *et al.* (2009b). RAPD method uncovered 30 polymorphic loci from five primers selected for population analysis. The result showed that RAPD primers were polymorphic and were able to detect private allele in the studied populations. This is consistent with the fact that the RAPD technique, being able to screen more easily a larger part of the nuclear genome than some other genetic markers (such as allozyme), may assess higher levels of genetic variation. Therefore, the maintenance of genetic variability is very important for the conservation of a species (Barroso *et al.,* 2005). The UPGMA cluster diagram of individual's genotype from the three populations showed that *T. zillii* population from Opa and Igun reservoirs are more similar genetically than to those of the Osu reservoir population.

Nei's (1978) unbiased genetic distance values also showed that Opa and Igun reservoir populations were closer genetically, while Osu reservoir populations was distant from them. The geographically closest populations (Igun and Osu as shown in Fig. 1) were not the most genetically similar as we had predicted. This is consistent with the findings of Szitenberg *et al.* (2012) in Israel. They reported that the population structure of *T. zillii* did not match the geography of the Israeli water-basins, based either on molecular or morphological data collected. In addition to geographical distance, other factors influencing the genetic relatedness of populations include anthropogenic and environmental factors (Wright,

Table 1. List of the used primers and their sequences

S/N	Primer name	Sequence	Fragment size
1	OPAE 05	CCTGTCAGTG	250bp-2500bp
2	OPAF 09	CCCCTCAGAA	200bp-4500bp
3	OPAF 07	GGAAAGCGTC	250bp-3500bp
4	OPAD 09	TCGCTTCTCC	200bp-2500bp
5	OPAE 04	CCAGCACTTC	200bp-2500bp

Table 2. Total bands scored by the primers and their percentage polymorphism

Primers	Total bands	Monomorphic bands	Polymorphic bands	% polymorphism
OPAE 05	141	35	106	75
OPAF 09	52	13	39	75
OPAF 07	63	14	49	78
OPAD 09	46	6	40	88
OPAE 04	18	9	9	50
TOTAL	320	77	243	76

Table 3. Total bands scored in each population

Population	Total bands scored
Opa	69
Osu	157
Igun	94
Combined population	320

Table 4. Pair wise comparison of Nei's genetic identity of the three populations of *T. zillii* studied with genetic distance (below diagonal) and index of similarity (above diagonal)

Population	Opa	Osu	Igun
Opa	****	0.15	0.29
Osu	0.85	****	0.27
Igun	0.71	0.73	****

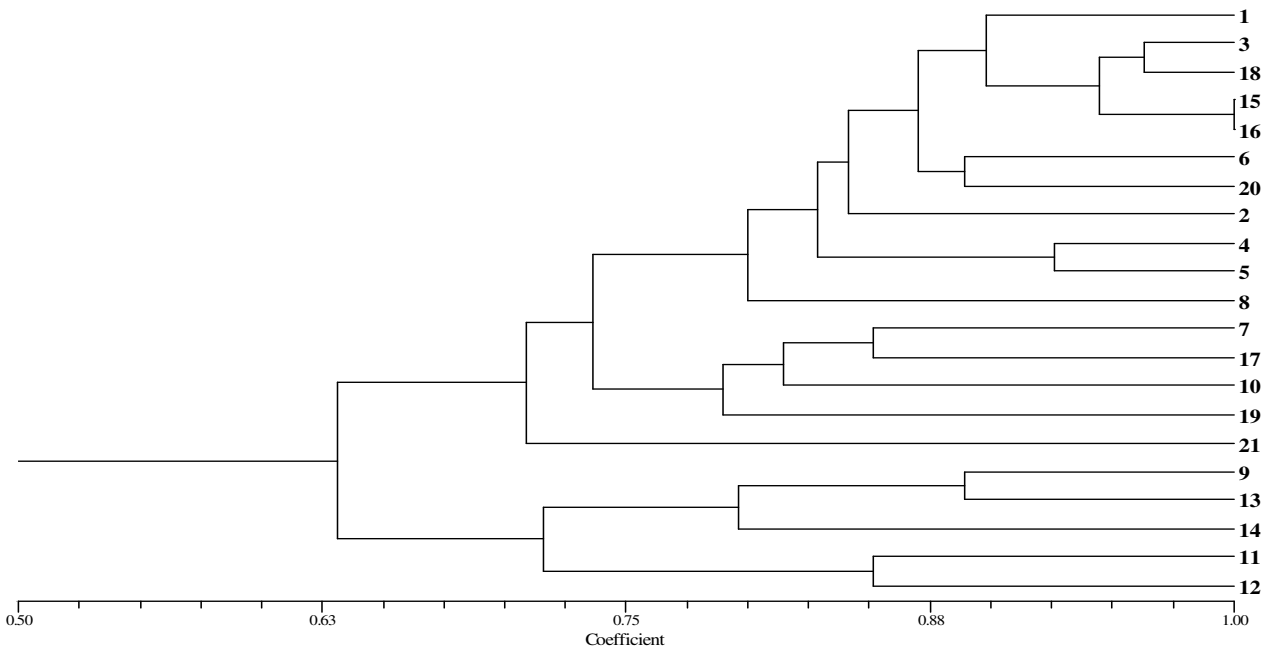

Fig. 6. UPGMA dendrogram, summarizing the data on differentiation between *Tilapia zillii* populations, according to RAPD analysis. Samples 1-7 is from Opa Reservoir, samples 8-14 is from Osu Reservoir and samples 15-21 is from Igun Reservoir

1943). Extrinsic factors, such as habitat heterogeneity, climatic and geological events also shape the large-scale population differentiation pattern.

The environmental conditions of Igun and Opa reservoirs are similar, as both populations have suffered severe bottleneck events due to heavy pollution for the past few decades. Reports show the presence of high concentration of heavy metals in Igun reservoir as a result of the mining activities going on around it (Aderinto, 2013) and studies also revealed that Opa reservoir is heavily polluted in terms of heavy metal contamination (Adesakin, 2013). Assessment of heavy metals contamination in Osu

reservoir has shown that the concentration of heavy metals in the reservoir are within permissible limits (Obayemi, 2013).

Genetic drift and natural selection are two main evolutionary mechanisms that cause population differentiation (Hufford and Mazer, 2003). Natural selection by ecological factors leads to development of ecological adaptation or ecotypes. However, there is need to determine whether the observed population differentiation pattern in this study resulted from any natural selection.

Conclusions

The present investigation contributes to the knowledge on morphological and genetic variation of *T. zillii* specie. The comparative results from both morphological and genetic analysis revealed a reasonable degree of variation in populations of *T. zillii* from Opa, Osu and Igun reservoirs in Osun state. The findings in this study would therefore have wide application in utilization and management of genetic resources of *T. zillii* species. Further studies involving large number of samples and primers need to be conducted to get more precise information about the genetic structure of the three reservoir stocks of *T. zillii*. In future, additional methods such as microsatellite and sequence analysis can be used to maximize the efficiency of genetic differentiation studies.

References

Ahmed KA, Muniandy S, Ismail IS (2007). Role of N-(carboxymethyl) lysine in the development of ischemic heart disease in type 2 diabetes mellitus. Journal of Clinical Biochemistry and Nutrition 41(2):97-105.

Aderinto TM (2013). Assessment of heavy metals in an abandoned gold mine reservoir, Igun Reservoir, Ilesha, Osun State, Nigeria. BScThesis. Obafemi Awolowo University.

Adesakin TA (2014). Effect of discharges from re-channeled rivers and municipal run off on the water quality of Opa Reservoir, Ile-Ife, Southwest, Nigeria. MSc Thesis. Obafemi Awolowo University.

Adesulu EA, Sydenham DHJ (2007). The freshwater fishes and fisheries of Nigeria. Macmillan Nigerian Publishers Ltd. Lagos, Ibadan.

Agbabiaka LA (2010). The current Ichtyofauna of River Otamiri, South-Eastern Nigeria. International Journal of Tropical Agriculture and Food Systems 4(1):7-9.

Agnese J, Gourene B, Owino J, Pouyaud L, Aman R (1999). Genetic characterization of a pure relict population of *Oreochromis esculentus*, an endangered *Tilapia*. Journal of Fish Biology 54:1119-1123.

Ali BA, Huang TH, QinDa N, Wang XM (2004). A review of random amplified polymorphic DNA (RAPD) markers in fish research. Review in Fish Biology and Fisheries 14:443-453

Allendorf FW, Ryman N, Utter, FM (1987). Genetics and fishery management. In: Ryman N, Utter F (Eds). Population genetics and fishery management. Washington Sea Grant Program. University of Washington, Seattle pp 1-9.

Anyanwu AO, Ugwumba OA (2003). Studies on the morphometric, meristic and electrophoresis patterns of *Pseudotolithus* species. The Zoologist 2(1):70-77.

Appannavar MM, Govindaiah MG, Ramesha KP (2003). Genetic distance study among deoni breed of cattle using random amplified DNA markers. Asian-Australian Journal of Animal Science 16(3):315-319.

Asagbra MC, Adebayo AS, Ugwumba OA, Ugwumba AAA, Anumudu CI (2014). Genetic characterization of fin fish species from the Warri River at Ubeji, Niger Delta, Nigeria. African Journal of Biotechnology 13(27):2689-2695.

Barroso RM, Hilsdorf AWS, Moreira HLM, Cabello PH, Traub-Cseko Y (2005). Genetic diversity of wild and cultured populations of *Brycon opalinus* (Cuvier, 1819) (Characiforme, Characidae, Bryconiae) using microsatellites. Aquaculture 247:51-65.

Cadrin SX (2000). Advances in morphometric identification of fishery stocks. Reviews in Fish Biology and Fisheries 2:91-112.

Carscadden JE, Leggett WC (1975). Meristic differences in spawning populations of American shad, *Alosa sapidissima*: evidence for homing to tributaries in the St John River, New Brunswick. Journal of the Fisheries Research Board of Canada 32:653-660.

Carvalho GR (1993). Evolutionary aspects of fish distribution: genetic variability and adaptation. Journal of Fish Biology 43:53-73.

Cooper ML (2000). Random amplified polymorphic DNA analysis of southern brown bandicoot (*Isoodon obesulus*) populations in Western Australia reveals genetic differentiation related to environmental variables. Molecular Ecology 9:469-479.

Daniel RJ (1997). Taxonomic uncertainties and conservation of the Western Ghats. Current Science 73:169-170.

Dunz A, Schliewen UK (2010). Description of a new species of *Tilapia* (Smith, 1840) (Teleostei:Cichlidae) from Ghana. Zootaxa 2548:1-21.

Edema CU, Osagiede HI (2011). Morphological and trophic status of three Polynemid fishes from the Warri River, Nigeria. Nigerian Journal of Agriculture, Food and Environment 7(1):53-57.

Eyo JE (2002). Conspecific discrimination in ratio morphometric characters among members of the *Pisces* genus: *Clarias scopoli*, 1777. The Zoologist 1(2):23-34.

Eyo JE (2003). Congeneric discrimination of morphometric characters among members of the Pisces genus: *Clarias* (Clariidae) in Anambra River, Nigeria. The Zoologist 2(1):1-17.

Garg RK, Sairkar P, Silawat N, Vijay N, Batav N, Mehrotra NN (2009b). Genetic diversity between two populations of *Heteropneustes fossilis* (Blech) using RAPD profile. International Journal of Zoology Research 4:171-177.

Gunawickrama KBS (2007). Morphological heterogeneity in some estuarine populations of the catfish *Arius jella* (Ariidae) in Sri Lanka. Ceylon Journal of Science 36(2):101-108.

Hair JF Jr, Anderson RE, Tatham RL, Black WC (1998). Multivariate data analysis. 5th edition, Prentice-Hall, Upper Saddle River, USA.

Hammer O, Harper DAT, Ryan PD (2001). PAST: Paleontological statistics software package for education and data analysis. Paleontologia Electronica 4(1):50-56.

Hassanien HA, Kamel EA, Salem MA, Dorgham AS (2011). Multivariate analysis of morphometric parameters in wild and cultured Nile Tilapia, *Oreochromis niloticus*. Journal of the Arabian Aquaculture Society 6 (2):424-440.

Hold M, Reed W (1972). West African fresh water fish, 1st ed. West African Nature Handbooks, Longmans.

Huang MC Y-M, Horng H-L, Huang Y-L Sin, Chen M-J (2003). RAPD fingerprinting for the species identification of animals. Asian-Australasian Journal of Animal Science 16(10):1406-1410.

Hufford KM, Mazer SJ (2003). Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology and Evolution 18:147-155.

Ihssen PE, Booke HE, Casselman JM, Glade MC, Payne NR, Utter FM (1981a). Stock identification: materials and methods. Canadian Journal of Fisheries and Aquatic Sciences 38:1838-1855.

Kuton MP, Adeniyi BT (2014). Morphological variations of *Tilapia guineensis* (Bleeker 1862) and *Sarotherodon melanotheron* (Ruppell 1852) (Pisces: Cichlidea) from Badagry and Lagos lagoon, South-West, Nigeria. Journal of Fisheries and Livestock Production 2(112):2332-2608.

Megbowon I, Fashina-Bombata HA (2013). Molecular characterization of an unidentified Cichlid, commonlly called, 'Wesafu', Using Rapd Markers. IOSR Journal of Environmental Science, Toxicology and Food Technology 5(2):22-26.

Misra RK, Carscadden JE (1987). A multivariate analysis of morphometrics to detect differences in populations of capelin (*Mallotus villosus*). Journal du Conseil International pour l'Exploration de la Mer 43:99-106.

Murta AG (2000). Morphological variation of horse mackerel *(Trachurus trachurus)* in the Iberian and North African Atlantic: implications for stock identification. ICES Journal of Marine Science 57(4):1240-1248.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of National Academy of Sciences USA 74:5267-5273.

Nei M (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Obayemi OE (2013). Preliminary study of heavy metal concentration in the gills, fillet and liver of *Parachanna obscura* (Gunther, 1861) in Osu Reservoir in Atakunmosa West Local Government, Osun State, Nigeria. BSc Thesis. Obafemi Awolowo University.

Omitogun OG (2005). A case study on applications of DNA level polymorphism in *Tilapia* genetic studies and stock management. International Livestock Research Institute CD ROM.

Paugy D, Leveque C, Teugels GG (2003). The fresh and brackish water fishes of West Africa, Volume I and II. IRD Editions. Publications scientifiques. Du museum.

Ponnian AG, Gopalakishnan A (2000). Cultivable, ornamental, sport and food fishes endemic to peninsula India with special reference to Western Ghats. In: Ponnian AG, Gopalakishnan A (Eds). Endemic fish diversity of Western Ghats NBFGR-NATP Publication, National Bureau of Fish Genetic Resources, Lucknow, India.

Reed W, Burchard J, Hopson AJ, Jenness J, Yaro I (1967). Fish and fisheries of northern Nigeria. Gaskiyya Corporation, Zaria.

Reist JD (1985). An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Canadian Journal of Zoology 63:1429-1439.

Rolf J (2000). NTSYS pc Version 2.0.Exeter software, Setauket, New York.

Sabir JSM, Mohamed HZM, El-Hanafy AA, Mohamed MA (2012). Genetic similarity among four breeds of goat in Saudi Arabia detected. Animal Genetics 24(6):14-22.

Saghai Maroof MA, Solima KM, Jorgenson RA, Allard RW (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proceedings of the National Academy of Sciences USA 81:8014-8018.

Szitenberg A, Goren M, Huchon D (2012). Mitochondrial and morphological variation of *Tilapia zillii* in Israel. BMC Research Notes 5(1):172.

Teugels GG (1982). Premiliminary results of a morphological study of five African species of the subgenus Clarias (Pisces; Clariidae). Journal of Natural History 16(3):439-464.

Turan C, Oral M, Ozturk B, Duzgunes E (2006). Morphometric and meristic variation between stocks of bluefish (*Pomatomus saltatrix*) in the Black, Marmara, Aegean and northeastern Mediterranean Seas. Fish Research 79:139-147.

Usman BA, Agbebi OT, Bankole MO, Oguntade OR, Popoola MO (2013). Molecular characterisation of two cichlids populations (*Tilapia guineensis* and *Sarotherodon melanotheron)* from different water bodies in Lagos State, Nigeria. International Journal for Biotechnology and Molecular Biology Research 4(5):71-77.

Welsh J, McCelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18:7213-7218.

Williams JGK, Kubelik AR, Livak J, Rafalski JA, Tingey SV (1990). DNA polymorphism amplified by arbitrary primers is useful as genetic markers. Nucleic Acids Research 18:6531-6535.

Wright S (1943). Isolation by distance. Genetics 28:114-138.