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Genetic Characterization of Tilapia Fish Species in the University Fish Farm, Umudike, Niger Delta, Eastern Nigeria

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Abstract

This study was aimed at identification and characterization of tilapia species farmed in the University Fish Farm Umudike. Dichotonomous key was employed in the application of morphometric and meristic parameters in the characterization. Molecular characterization was carried out using Random Amplified Polymorphic DNA (RAPD) primer by Polymerase Chain Reaction (PCR) amplification. A total of three hundred (300) fish samples suspected to be tilapia were collected from the University Fish Farm for the study. Eleven (11) morphometric and five (5) meristic counts were studied. Deoxyribonucleic acid (DNA) was extracted from the caudal fin tissues of sixty- six (66) samples from the three hundred fish samples using the Chlorophenol Isoamyl alcohol Method. Morphometric and meristic parameters were analyzed using Principal Component Analysis (PCA) which separated all sampled tilapia species statistically into three different groups, showing specifically the parameters that accounted for this variation. The first 7 components explained 91.382 % total variability among the groups with body depth cosntributing 25.226 %, body weight 50.133 %, total length 5.969 %, standard length 3.9178 %, caudal peduncle 3.406 %, head length 3.1518 %, dorsal fin base length contributing 2.6903 % of the total variability. Cluster analysis (CA) was based on Jaccard's

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similarity coefficient. The study revealed mostly polymorphic bands from all 10 RAPD primers with a size ranged from 300 – 1000 base pairs (bp). Wide range of intra specific variation was observed within the species. Results obtained revealed that 3 distinct groups of tilapia with resemblance of *Oreochromis niloticus, Sarotherodon galilaeus* and *Hemichromis fasciatus* were present in the University fish farm. From the study, body depth and length of caudal peduncle were some of the natural markers that successfully separated all sampled species. The information obtained from this study in future could be employed in improvement programme for enhanced economic benefit of tilapia faming in Michael Okpara University of Agriculture, Umudike (MOUAU) Fish Farm.

Keywords: Genetics, Species Identification, Fish Farming, Species Improvement, Molecular Genotype and Phenotypes

INTRODUCTION

Genetic characterization was defined by Halima *et al.* (2012) as a process whereby defining features of the gene in particular species is revealed. It involves measurement of phenotypic and genotypic characters, using morphological and genetic markers. Phenotypic characters are those physical characteristics, measurable or observable traits of an organism. The phenotype, in terms of appearances or performance, reflects to some degree both the environment the organism has been exposed to and the genotype, or genetic composition of the organism itself (Greg, 2001). The origin of the term morphology is traced from the classical Greek word (morphe) meaning the study of shape or form. Morphology is concerned with the structure and arrangement of parts of an object, and how it conforms to create a whole. It is the study of shapes or forms of an organism. Morphological character according to Fabrice (2009) is a measure of both quantitative and qualitative traits while genetic markers refer to characteristics used in identification of the genotype of the individual species of fish. Genetic markers are physical characters or phenotype, for which alleles at individual loci segregate in a Mendelian manner or they are any trait which allows the identification of the genotype of an individual. They are used to study the genetics of organisms, including fish (Rossella *et al.,* 2009).

Characterization of fish plays important role in the management of fisheries resources especially in breeding programmes with the ultimate goal of getting a superior parent fish (Garcia-Rodriguez *et al.,* 2011; Ballagh *et al.,* 2012; Mulyasari, 2007; Nuryadi *et al.,* 2008).

Over 24,600 species of fish have been identified and characterized (Nelson, 1994; Fabrice, 2009). Fishes of the family Cichlidae, popularly known as 'tilapia' are among the world most important aquaculture finfish (Naylor *et al.*, 2001) contributing 28000 tonnes to over 3,000,000 tonnes from 1970 to 2010 of the world's total aquaculture production in 2005 (FAO. 2008; Fitzsimmons, 2010; Lind *et al.*, 2012). Cichlidae comprises 20 genera and 1300 scientifically described species and the taxonomic status of at least 600 species remains undecided making cichlids the third largest fish family distributed both in fresh water and in brackish water (Trewavas 1983; McKaye, 1998). According to FAO statistics as reported by Huet (1971); 16 tilapia/cichlid groups, in addition to unidentified cichlids have been cultured while Balarin and Hatton (1979) gave a quote of 23 cultured species. However, commercial tilapia culture is currently restricted to about ten species, among which are; Nile tilapia (*Oreochromis niloticus*), blue tilapia (*Oreochromis aureus*), red tilapia (*Tilapia zilli*), *Tilapia guineensis*, *Sarotherodon galilaeus*, *Oreochromis mossambicus, Sarotherodon melanotheron*. Tilapia is one of the most useful fish species for fish farming (Barlow, 2000) since they are euryphagous feeders; a feeding type that is responsible for the low cost of tilapia farming, fishing pressure on prey species, avoids concentration of toxins that accumulate at higher levels of food chain (El-Sayed, 2006), thus making tilapia the preferred "aquatic chicken" to trade. Tilapia is mainly sourced from the wild by most farmers for culture and most often not sorted before they are stocked. The implication of stocking mixed species is introgression (McAndrew, 1993). Introgression (hybridization) between closely related species has been reported that it can occur if these species share overlapping habitats or sometimes through human intervention, during captive breeding (Doiron *et al.*, 2002). Several fish species are known to show interspecific introgression and this has led to difficulty in genetic identification and characterization of closely related species (Moritz and Cicero, 2004). Generally, classification of the tilapia family is based on differences in their breeding and brooding behaviour, reproduction, development, feeding, structural characteristics and biogeography (Abumourad *et al.*, 2008; Trewavas, 1983). They spawn in an organized manner where a nest is prepared by the male and through sexual display attracts the female to release her eggs which are fertilized by the male (Thomas and Michael, 1999). In such arrangement, there is creation of territory and sexual competition among the males which will lead to large variation in reproductive success for individuals among a group. According to Fessehaye *et al.* (2009) the genetic consequence of such behaviour is reducing genetic variability in the long run, as inbreeding is likely to occur

among different generations due to differential male reproductive success. The resulting effect of inbreeding depression evident in poor growth performance is a factor amidst others that could be responsible for the low demand and profit output thus making the business of tilapia fishery unattractive (Naish *et al.*, 2013). However, observation from Lind *et al.*, 2012 shows that most aquaculture stocks in current use in the continent are genetically inferior to wild, undomesticated stocks and this is due to poor management and genetic erosion.

Systematic efforts to improve tilapia yields and to develop the sustainability of this food sector will be of critical importance. Wagner *et al.* (2004) found out that in Brazil, selection of strains that respond well to specific environmental conditions, with higher productivity and survival per unit area is an important aspect to increasing productivity in tilapia farming. Selection, using quantitative and statistical methods, can also be employed as this is responsible for increases in productivity of both traditional livestock and crop species. The correct identification of superior individuals is, therefore, important and requires knowledge about the genetic control of the traits under selection. Technology has advanced to the point that different types of biochemical and molecular markers are available to study the genetics of fish and aquatic invertebrates.

Schwartz (2008) noted the importance of genetic analyses as it will play a key role in future assessment and monitoring of fishes with commercial or conservation value because genetic analyses provide information not obtainable by other methods and the efficiency and power of genetic analyses continue to increase. Byrkjedal *et al.* (2007) emphasized that reliance on morphology can sometimes pose some problems in cases where species may have very similar morphological characters or apparent variances and are misleading. This is in line with the report of Eknath *et al.* (1993), who stated that morphological features are of limited value for identification and classification purposes and thus, DNA-based identification methods using genetic markers, offer a more analytical addition. Moreover, reproduction by random breeding in aquaculture practices may reduce the genetic diversity in domesticated strains due to the inbreeding effects of small broodstock population size. This has given rise to the fear of contamination of species of tilapia reared in farms (Macaranas *et al.,* 1986; El Serafy *et al.,* 2007); inclusive of the University Fish Farm located in Michael Okpara University of Agriculture, Umudike (MOUAU). In MOUAU Fish Farm, different species of tilapia are found in the same pond and there is possibility of free breeding among the fishes. The resulting effect of such unplanned and uncontrolled

breeding according Tave (1999) can ruin the population through loss of traditional stocks and undesirable inbreeding manifestation such as poor growth, huge disparity in sizes of fish among a batch, occurrence of deformed fish and poor production. Such uncontrolled hybridization is likely the cause of poor tilapia yield in the University fish farm (MOUAU).

Aim of the Research

It was to critically examine the genetic characterization of tilapia fish species in the University Fish Farm at Michael Okpara University of Agriculture Umudike, Niger Delta, Eastern Nigeria.

The Specific Objectives of the Research are to:

i. Classify tilapia species in Michael Okpara University of Agriculture, Umudike Fish Farm using morphometric measurement and meristic counts.

ii. Characterize tilapia species in MOUAU Fish Farm using molecular tool.

iii. Evaluate the Phylogenetic relationship between the tilapia species in MOUAU Fish Farm.

iv. Estimate the genetic distances between the tilapia species.

O'Brien (1991) reported that the most used approaches for genetic characterization were; allozyme analysis, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite typing, single nucleotide polymorphism (SNP), and expressed sequence tag (EST) markers. These markers can be classified into two groups; type I and type II markers where type I markers are related to genes of known function, and type II markers are of anonymous genome. Having noted the differences between them he stated that Allozyme markers are type I markers because they encode protein of known function while RAPD markers are classified as type II markers because RAPD bands are amplified from anonymous genomic regions via the polymerase chain reaction (PCR). Microsatellite markers are also type II markers. Type I markers are highly significant in aquaculture genetics since they serve as a bridge for comparison and transfer of genomic information. Generally, type II markers have been widely used to characterize genetic divergence intra and inter species population studies (O'Brien, 1991; Vignal *et al.,* 2002).

Stiassny and Jensen (1987) reported that Cichlids are fishes of the family Cichlidae, order Perciformes, are members of the suborder Labroidei and belong to the class Actinopterygii. Report from Froese *et al.* (2012) showed that the family is both large and diverse with at least 1,650 species that were scientifically described making it one of the largest vertebrate families regardless of the newly discovered species. The family cichlidae is the largest family in the perciformes comprising 20 genera and at least 1,650 scientifically described species, and the taxonomic status of at least 600 species remain undecided (McKaye, 1998; Froese, 2012), making cichlids the third largest fish family distributed both in fresh waters and brackish waters in tropical, subtropical and temperate region, over the world (Santiago and Laron, 2002; Idodo-Umeh, 2003; FAO, 2007). Megbowon (2011) stated that cichlids are the second most important group of wild-captured fish, after carps, with a global harvest of 769,936 tones (metric tons) in 2007 and is currently ranked as the world's eighth most common group of farmed fish species, with a commercial production of 2.5 million tonnes in 2007, corresponding to an estimated value of $3.3 billion. According to Shelton and Popma (2006), only few of the species are commercially important, and even fewer are of aquacultural significance.

Cichlids according to Streelman *et al.* (2003) cover a wide range of body sizes and exhibit a wide diversity of the body shape ranging from fairly conventional, laterally compressed and deep body shapes. The body is covered with relatively large, cycloid scales, which are not easily dislodged. The dorsal and anal fins have hard and soft rays. The pectoral and pelvic fins are large and more anterior in an advanced configuration. This character provides the fish with great control over swimming and maneuvering. Reports from Ross (2000) revealed that their fins are important for locomotion and this is why they possess red muscles designed relatively for slow but continuous movements. The number of scales, vertebrae, gill rakers and fin rays are widely used for species distinction and identification. However, the number of fin rays of the same species may vary from one strain to another and from one aquatic environment to another. Their bodies are generally characterized by vertical bars, with relatively subdued colours and with little contrast over the body colours. This provides the fish with a modest ability to change their colours, in response to stress, by controlling skin chromatophores. They have well developed sense organs, represented by prominent nares and a clearly visible lateral line. The eyes are also relatively large, providing the fish with an excellent visual capability. Species classification according to Trewavas (1982) is based on variation in dentition, bone, structure, pigmentation and

general body morphology (Fryer and Iles, 1972; Kornfield *et al.* (1979); Stiassny, 1991; 1992).

Trewavas (1983) stressed that the body of *O.niloticus*, Linnaeus 1758 is compressed; caudal peduncle depth equal to peduncle length, scales are cycloid, lateral line is interrupted, and spine and soft ray sections of dorsal fin are continuous. Dorsal spine (total) range between 16 - 17; dorsal soft rays (total) ranges from 11 - 15; anal spine is 3; anal soft rays' range between 10 -11. Caudal fin is truncated with presence of regular vertical black stripes. Pectoral, caudal and dorsal fin become reddish during spawning season as seen in plate 1 below. (Fishbase, 2004). The Dorsal spine (total) of *Sarotherodon galilaeus* range from 14 – 17 and dorsal soft rays (total) is13; anal spine is 3 while anal soft rays range from 9 - 12; head length ranges from 32.5 – 39 % of standard length (SL); body depth range from 42.5 - 56 % SL. It has 29 - 32 scales in the lateral line series with 4 - 6 faint narrow vertical bars on flank and caudal peduncle, caudal fin is slightly emarginated and its body colour is silvery or brassy yellow (Yashouv, 1958; Fishbase, 2004).

Pullin (1983), the dorsal spine (total) of *Hemichromis fasciatus* is XIV and dorsal soft ray (total) ranges between 11 – 12, anal spine (total) is III and anal ray (total) ranges between 9 –10. It has 5 dark spots along each side with the first black spot on the operculum and the last on the caudal fin base. Dorsal and upper part of the caudal fin is red and operculum is also characterized with red spot.

It has been observed that the body shape characteristics of some species like *O. niloticus* and *O. mossambicus* are of great resemblance and according to De Silva and Ranasinghe (1989), these two species are known to exhibit introgressive hybridization and as such, populations of the two species have mixed characteristics. According to Amarasinghe and De Silva (1996) differentiation using conventional morphometric characteristic and phenotypic variations is therefore problematic. Hence, it is of importance to employ a more effective method for morphological differentiation of all cichlid species such as Truss network (Strauss and Bookstein, 1982).

Strauss and Bookstein (1982) recommended the Truss network systems constructed with the help of landmark points as being a powerful tool for fish identification. Truss measurements are powerful tool for the analysis of shape, and generally are designed to cover all, or most, of the animal's body. According to Dwivedi and Dubey (2013), the Truss network is very important and effective method for analyzing the morphology of a

fish. It has better data collection and uses different methods for data analysis when compared to traditional morphometric method. Thus it is able to differentiate between phenotypic stocks due to the constructed landmarks that cover the entire fish body with no loss of information. Report showed that it is very sensitive to the slightest change during measurement (Lim, 2008).

Dhanya *et al.* (2004) showed from his results that the simplest method of identification and characterization are measurement of morphological parameters. The studies of morphometric and meristic characters of a fish give substantial information with regard to exact identification key of the species. Hussain (2007) demonstrated the usefulness of morphology in classification of species and defining shape and size differences among the *Channa* species. Yakubu and Okunsebor (2013) studied the relationship between two closely related fish groups using stepwise discriminant analysis and results showed that some morphometric variables (standard length, dorsal fin length and caudal fin length) that distinctively separated 98% of the samples into two groups at mature age. A study was also carried out by Bagherian and Rahman (2009) where truss network was used to morphologically separate two groups of cyprinid, *Chalcalburnus chalcoides*. Results provided a basis for stock structure, which may be useful for determination of variation from environmental factors. More recently, Torres *et al.*, (2010) also used measurements to identify variations between fish populations (Murta, 2002; Tzeng, 2004; Cheng *et al.*, 2005; Pinheiro *et al.*, 2005; Buj *et al.*, 2008).

El-Zaeem (2011) studied the differences in phenotypes based on morphometric parameters and meristic counts between *Mugil cephalus* and *Liza ramada*, and found significant differences in most of the measurements but non-significant differences in most of meristic counts. Olufeagba *et al.* (2016) investigated the morphological variations of wild and cultured *Oreochromis niloticus* from two locations revealed significant higher values in seventeen morphometric parameters out of twenty-three and in four of five meristic counts with clear overlaps, of the cultured specimen. Sabry *et al.* (2006) revealed that morphometric measurements had striking similarities and overlapping data among tilapia species. thus, making them in-separable whereas meristic characters reveal that tilapia species could be differentiated into four species (*Oreochromis niloticus, O. auraeus, Sarotherodon galilaeus* and *Tilapia zillii*) using lateral line scales and into three species using dorsal and anal fin ray count (Confusion between *O. niloticus* and *O. aureus*).

El–Serafy *et al.* (2007) recorded morphometric data showing obvious similarities and overlapping among *Tilapia* spp., making it impossible to differentiate those species on basis of morphometric measurement; while meristic characteristic identified four major groups (*O. niloticus, O. aureus, S. galilaeus* and *T. zillii*) and that the lateral line scales varied significantly among all *Tilapia* spp. while the number of dorsal fin rays and anal fins rays differed significantly between *S. galilaeus* and *T. zillii.*

Genetics present the knowledge of the gene variations within and between species. Genetic differences between species are much larger than between populations within a species. This means that very small sample sizes can be used (3 to 5 individuals). Even when two species are almost indistinguishable morphologically, they are likely to be easily distinguished genetically (Okumus and Ciftci, 2003). Most of the molecular markers have been used in inter- and intraspecific variations. According to Liu and Cordes (2004) genetic diversity may be characterized by different techniques; observation of inherited genetic traits, through direct view of the chromosomes carrying the genes under a microscope, and by studying the genetic attributes carried on the chromosomes by applying molecular techniques. Different molecular techniques have been discovered and are employed for identifying differences within and between species in taxonomic challenges and to detect genetic introgression in species. The detection of genetic differentiation would imply that the source groups comprise different stocks according to Carvalho and Hauser (1994) and Greig *et al.* (2005) who reported that the choice of molecular technique was dependent on the questions that were addressed and that molecular techniques differed in the way they sample within the genome and in the type of data generated.

The report of Conkle (1981) revealed that allozymes have been the most important type of genetic marker in forestry and have been used in many species for many different applications. According to Adams *et al.* (1994) they are allelic forms of enzymes that can be distinguished by a procedure called electrophoresis. The more general term for allozymes is isozymes, and refers to any variant form of an enzyme, whereas allozyme implies a genetic basis for the variant form.

Saad *et al.* (2012) used ISSR as tool for species identification and classification for four Tilapia species (*O. niloticus, O. aureus, T. zilli and S. galilaeus*). Results revealed that percentages of polymorphism ranged from 0% to 67% within *S. galilaeus*, while polymorphism values ranged from 0% to 100% in the other studied fish species. The

highest genetic dissimilarity value was observed between *O. aureus* and *T.zilli*. On the other hand, the lowest dissimilarity value was observed between *O.niloticus* and *O. aureus*. Antunes *et al.* (2010) examined the phylogenetic relationships among 6 Brycon species using mitochondrial and nuclear molecular markers. Phylogenetic tree obtained from the data divided the species into two clades: one comprised only *B. cf. pesu*, and the second comprised the remaining *Brycon* species and they concluded that ISSR primers can be used for the identification of species-specific bands in fish, such as *Brycon* spp.

Ahmed *et al.* (2004) applied RAPD to assay polymorphism among 3 genera and between 2 species using 14 random 10-mer and six 20-mer primers and reported that sixteen of 20 primers (84.21 %) produced polymorphic bands among the 3 genera and the total number of bands generated per primer varied from 3 to 15. Results revealed that there were great variations among the three genera of tilapia fish. These data also demonstrated that RAPD markers can be useful for systematic investigation at the species level in tilapia.

Bardakei and Skibinski (1994) also used RAPD analysis to differentiate among three species of the tilapia genus *Oreochromis* and 4 subspecies of *O. niloticus*. Results revealed differences in band patterns for different species as well as provided evidence to the usefulness of RAPD for systemic investigations at the level of species and subspecies.

Avise *et al.* (1988) stated that molecular genetic data can provide information on the relationship among existent populations, and can also reveal information on present evolutionary history such as past population size. The assemblage of fish species from a different source or population is the reason for determining their genetic similarities. Thus Barman *et al.* (2003) stated the usefulness of RAPD-PCR in estimating the genetic variability and degree of similarity among fish species. According to El-Zaeem *et al.* (2012), the amount of variation in phenotype and genotype using morphometric measurements, meristic counts, RAPD and fingerprinting among different wild and cultured Nile tilapia (*O. niloticus*) populations studied revealed significant differences ($P \leq 0.05$) in most of morphometric measurements and meristic counts among the different wild and cultured Nile tilapia populations tested. They also reported inter population genetic similarity among the different populations. El-Alfy *et al.* (2009) in a study on genetic similarities and differences between species using RAPD markers reported that *Oreochromis aureus* is closest to *Oreochromis niloticus* and farthest from *Tilapia zilli*. Nxomani *et al.* (1999) used RAPD

analysis to study colour forms in *Tilapia guinasana,* and results showed that colour forms of *Tilapia guinasana* are genetically distinct.

Usman *et al.* (2013) used RAPD to characterize two populations of cichlids species. Depending on the similarity coefficient through the use of RAPD primers (OPC 04, 05, 10, OPR 02 and OPI 05), the similarity between *T. guineensis and S. melanotheron* indicated a high probability of hybridization between the very closely related species. Sabry *et al.* (2015) used RAPD, ISSR and Rep-PCR markers to characterize seven popular Saudi fish species. Data of the three types of genetic markers were combined for phylogenetic analysis and the resulting dendrogram produced two large lineages with around 59 % genetic similarity. Danish *et al.* (2012) studied to identify genetic relationship and diversity of *Clarias betrachus* L. populations collected from hatchery and wild stocks through random amplified polymorphic DNA (RAPD). From the bands, percentage polymorphism ranged from 56.4 to 59.6% and similarity within the population from wild varied from 0.40 to 0.83 with a mean ± SE of 0.57 ± 0.08, with two major clusters were formed, which indicated that the genotypes belonging to same clusters were genetically similar and those belonging to different clusters were dissimilar. Yusuf and Ali (2007) used RAPD to identify eight whiting fish species and to genetically characterize and evaluate their genetic relationships and diversity. Results revealed that their similarity coefficient ranged from 0.676 - 0.836 and cluster analysis showed that two stations were classified from the dendrogram.

MATERIALS AND METHODS

Study Location

The study was conducted in the University Fish Farm, Michael Okpara University of Agriculture Umudike (MOUAU), Abia State, Nigeria and the experiment lasted for a period of 3 months. Michael Okpara University of Agriculture (MOUAU) fish farm is one of the numerous fish farms in Nigeria where fish and fishery products are produced. The farm is located within the University premises, and one of the sources of fishes into the farm is Anya River (a tributary to Qua Ibom River and empty into the Niger River). This study is aimed at characterizing tilapia fish species in the University Fish Farm, Umudike.

Collection of Samples

A total of 300 samples of different tilapia species were collected for the entire study from both concrete and earthen pond in the farm. Sixty-six samples were preserved and taken to the laboratory for the molecular analysis.

Identification of Tilapia Species in Mouau Fish Farm

Dichotomous key and local reference collection were employed to identify tilapia species found in MOUAU Fish Farm following careful evaluation of the morphology, morphological features of the available tilapia species according to Trewavas *et al.* (1983) and FishBase: Froese and Pauly (2012).

Morphometric Measurements

A total of eleven (11) morphometric characters were measured and all characters were measured to the nearest 0.1cm using a measuring board, measuring tape and vernier caliper as applicable as seen in Plate 5. Measurements were taken as described by Uka and Sikoki (2016) and they include:

Body weight (BW): A measure of the total weight of life fish.

Total length (TL): The distance from the tip of the snout to the tip of the caudal fin.

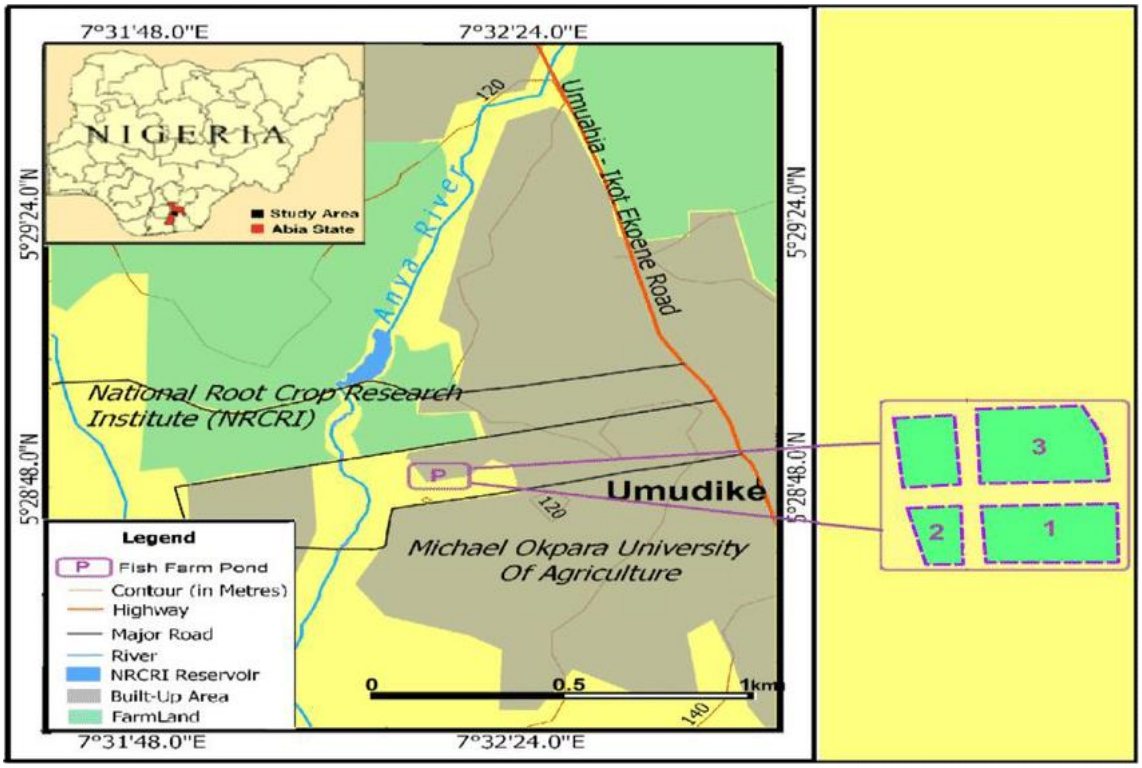

Figure 1: Map showing experimental Location (MOUAU Fish Farm)

Standard length (SL): The distance from the tip of the snout to the origin of the caudal fin.

Body depth (BD): The vertical distance from the anterior part of the dorsal fin and ventral part of the body.

Head length (HL): The straight line distance from the tip of the snout to the opercula

Length of dorsal fin base (LDFB): The distance from the (first dorsal spine) origin of the dorsal fin to the (last dorsal ray) end of the dorsal fin.

Length of caudal fin base (LCFB): The distance from the first caudal spin to the last to the last caudal fin.

Length of anal fin base (LAFB): The distance from the beginning of the anal fin to end of the anal fin.

Length of pectoral fin (LPF): The distance between base of pectoral fin and its tip.

Length of pelvic fin (LPF): The distance between the base of pelvic fin and its tip.

Caudal peduncle depth (CPD): It is the minimum depth of the caudal peduncle.

Meristic Counts

Five (5) meristic variables were described and they include:

Dorsal fin count: The number of soft fine rays and hard spine present in the dorsal fin.

Anal fin count: The number of soft fine rays and hard spine present in the anal fin.

Pectoral fin count: The number of soft fine rays and hard spine present in the pectoral fin

Pelvic fin count: The number of soft fine rays and hard spine present in the posterior part of the pelvic fin.

Caudal fin count: The number of soft fine ray present in caudal fin.

In addition, Morphometric measurements of various body parts of tilapia:

Total length- TL

Standard length- SL

Body depth- BD

Head length- HL

Dorsal fin base- DFB

Anal fin base- AFB

Caudal peduncle - CP

Caudal peduncle depth- CPD

Intra Specific Characterization of Tilapia Species Using Molecular Tools

Collection of Fish Sample for Deoxyribonucleic Acid (DNA) Analysis

Identified tilapia species as stated above were collected from the University Fish Farm. Fin samples were clipped with a forcep and cut with a pair of scissors from the caudal fin of each fish. The scissors and forceps were rinsed with ethanol to prevent contamination and immediately, fin samples were preserved in separate eppendorf tubes containing 95 % ethanol. The eppendorf tubes containing fin samples were taken to the Laboratory in Fisheries Department, Michael Okpara University of Agriculture Umudike and stored at -18 °C.

Isolation of genomic deoxyribonucleic acid (DNA)

The fin samples were taken out from ethanol and dried on tissue paper. For each sample, approximately 30 mg of fin tissues was cut into small pieces using a pair of scissors. These small pieces were placed in 1.5 ml micro - centrifuge tube. 230 µl extraction buffer was poured into tube and the sample was ground manually with tissue homogenizer stick. 230 µl of extraction buffer and 20µl of proteinase K, enzyme were added. Then the homogenates were incubated overnight at 37 °C in hot water bath for digestion.

For purification, equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed by vortexing for 30 seconds. Then the solution was centrifuged for 3 minutes at 13,000 rpm to allow precipitation of cell debris. The organic layer was then removed from the lower portion and the extraction was repeated with equal volume (460 µl) of chloroform: isoamyl alcohol (24:1) followed by further vortexing for another 20 seconds.

After centrifugation at 13,000 rpm for 3 minutes, the solution showed two separate layers; upper layer contained DNA and lower layer contained protein debris. The upper layer was taken to a new eppendorf tube and DNA was precipitated first using 0.6 volumes (240 µl) of isopropanol.

Gentle finger tapping, the DNA was observed as cotton-like structure. DNA was pelleted by centrifugation at 13000 rpm for 15 minutes. After removing the solution completely, pelleted DNA was air-dried and re-suspended in 180 µl Tris ethylenediaminetetraacetic acid (T- EDTA) buffer. To each tube 20 µl of 3M sodium acetate and 500 µl of absolute ethanol were added and the DNA was re-precipitated by centrifugation at 13,000 rpm for 15 minutes.

After removing the liquid completely, the pelleted DNA was washed with 0.5 ml of 70 % ethanol and centrifuged at 13,000 rpm for 10 minutes. The ethanol was then discarded and the pellet was air-dried for about an hour. Finally, the DNA pellets were resuspended in 50 µl T- EDTA buffer and the tube containing the DNA samples was stored in freezer at -18 °C.

The extraction method described above is the Chlorophenol Isoamyalcohol protocol by Sambrook and Russell (2001) with slight modification in the digestion stage.

Confirmation of The Presence of Genomic Deoxyribonucleic Acid (DNA)

All DNA samples were tested qualitatively (presence of RNA or degradation of DNA) on 1 % agarose gel.

Preparation of Agarose Gel

For preparing 1 % agarose gel, 1.5 g agarose powder was taken in 500 ml Erlenmeyer flask containing 150 ml of electrophoretic buffer (1 × TBE buffer). The flask was heated in a microwave with occasional swirling until complete disappearance of agarose particles. The flask was then kept in a microwave at 50 °C. When the flask was cool enough to hold with bare hand, 2 µl ethidium bromide (10 mg/µl) was added to the solution and mixed well by gentle shaking in order to make the DNA visible in ultraviolet light. Then the gel was poured on to the gel tray placed on a bench and the appropriate comb was inserted and bubbles were removed carefully. When the gel became completely solidified, the comb was removed gently and the gel was used for electrophoresis. Five microlitre (5 µl) of loading dye was added to the 2 µl DNA and mixed well using micropipette. The samples were loaded carefully in the gel. Deoxyribonucleic acid (DNA) ladder was loaded on either side of the gel. The gel was placed on gel chamber carefully with 1× TBE buffer and the buffer

level above the gel. Electrophoresis was performed at 100 V for 45 minutes. After electrophoresis, the gel was viewed with UV transilluminator and pictures were taken.

Polymerase Chain Reaction Amplification and Electrophoresis

Deoxyribonucleic acid (DNA) samples extracted from fish samples were amplified using ten RAPD primers (OPE-1, OPA-04, OPA-05, OPA-7, OPA-9, OPA-10, OPA-11, OPB-8, OPC-4, OPC-10 and OPD-14). A total volume of 20 µl of the PCR master mix which consisted of (master mix with 12.5 mM MgCl), 17.15 µl autoclaved water, 0.5 µl dNTP (0.2 mM; nucleotides), 0.1µl of Taq polymerase, 0.25 µl of primers (25 p/mol) and 2 µl of DNA (10 ng) was run on PCR Machine (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 seconds, annealing at 35 °C for 1min and extension at 72 °C for 2 minutes; a final extension at 72 °C for 10 min was carried out. The samples were cooled at 40 °C. After completing the PCR, 2.5 µl loading dye was added to each PCR tube and mixed well and Agarose gel. Electrophoresis was performed using a 1.5 % agarose gel, at 70 V for 1.5 hrs in a 1 x TBE buffer. Deoxyribonucleic acid **(**DNA) bands were observed under UV transilluminitor and photographed by a Gel Doc with digital camera.

Evaluation of Phylogenetic Relationship Between Tilapia Species

A measure of how closely related or far apart an individual or a specie is from another and this was determined from the data extrapolated from PCR results obtained.

Estimation of Genetic Distance Between Tilapia Species

Genetic similarities and differences were determined between and within species so as to know their genetic distance calculated from results of molecular analysis.

Statistical Analysis

Identification and Classification of Tilapia Species

Principal Component Analysis (PCA) and cluster analysis (CA) were employed based on Jaccard's similarity coefficient (Garcia-Ville and Puigbo, 2002) to correctly identify and characterize tilapia species in the farm.

Molecular Characterization of Tilapia Species

Genetic variability among the species were accessed by determining the number of alleles per locus (P %), according to Ayala and Valentine (1979). Each individual was scored for the presence or absence of amplified products. Accordingly, relative molecular weight of each fragment was measured and scored manually and by Gel Analyzer Ver. 3, program software (2007).

Estimation of Phylogenetic Relationships

Phylogenetic relationship was determined from dendrogram from cluster analysis of both morphological and molecular data generated using unweighted pair-group method (UPGMA) according to Sneath and Sokal (1973).

Estimation of Genetic Distance Using Genetic Similarity Analysis

Hierarchical cluster analysis was performed using quantitative and genetic variables and dendrogram was constructed based on Euclidean distance between species using unweighted pair-group method to group the species into their morphological and genetic similarities.

RESULTS AND DISCUSSION

Identification of Tilapia Species Using Morphological and Morphometric Parameters

In the present study, 300 samples of tilapia species were successfully separated into different groups based on physical observation on their morphological features using the dichotonomous key. For shape variation, three different groups were observed indicating that all sampled species can be identified using the dichotonomous key based on body shape character. Two groups had distinct shape that separated them whereas 3 groups showed great overlap in shape morphology and were pooled together as the same species (Plates 6a, 6b and 6c).

Caudal fin shape, pattern and colour were another parameter used for further separation of these species. Group1 had round caudal fin with continuous vertical dark stripes, group 2 had truncated and long caudal fin while group 3 had round caudal fin with 5 spots on each side. (Plate 6a, 6b and 6c). From the field observation, tilapia species were successfully separated into three distinct groups using the dichotonomous key for species identification

based on their body morphology and caudal fin shape (Swain and Foote, 1999; Robinson and Wilson, 1995; Pimm *et al.,* 2006 and Cope *et al.,* 2012).

Plate 6a: Pictorial identification of *Sarotherodon galilaeus*

Source: FishBase, 2004

Plate 6b: Pictorial identification of *Hemichromis fasciatus*

Source: FishBase, 2004

Plate 6c: Pictorial identification of *Oreochromis niloticus*

Source: FishBase, 2004

For further separation using meristic characters, tilapia species with dorsal fin xvi - xviii, 12 - 15; anal fin iii, 8 - 11, with rounded caudal fin mixed with black stripe, were pooled together into 2 groups (1and 4). Species with dorsal fin xvi - xvii, 12 - 14; anal fin 10 and with pinkish truncated caudal fin were pooled together into group 2. Species with dorsal fin xiv, 11 - 12; anal fin iii, 9 - 11 with five spots were pooled together into group 3. The above data showed that species were separated into two distinct groups with a huge overlap in the third group. Most of the morphometric characteristics of the fishes in the present study reveal overlap in the mean values recorded.

Data obtained from this study showed the result of analysis of the morphometric features and their mean values expressed in percentage of total length (TL) (Table 4.1). The weight (Body mean weight) recorded 44.5 %, body depth 32.0 %, head length 27.0 %, caudal peduncle 12.0 %, total length 16.01 cm, standard length 82.0 % etc and these values are in line with records obtained from Fish base (2004). From the above report, group 1 and 4 portray characteristic features of tilapia species of the genus *Oreochromis,* group 2 can be classified under the genus *Sarotherodon* and group 3, *Hemichromis* (Zharif *et al.*, 2016; Olufeagba *et al.*, 2016; Sabry *et al.,* 2007).

Table 1: Parameters and Mean Values Of Tilapia Species In MOUAU Fish Farm

Parameters (cm)	Group 1	Group 2	Group 3	Group 4
Body weight	44.5	21.6	19.0	81.7
Body depth	32.0	37.0	29.0	30.0
Total length	160.1	102.9	84.0	231.9
Standard length	82.0	3.0	2.0	81.0
Head length	27.0	26.3	11.0	28.0
Caudal peduncle length	12.0	11.0	9.0	11.0
Dorsal fin length	43.0	76.0	41.0	44.0
Dorsal fin base	16.0	13.0	12.0	19.0
Caudal fin length	16.0	18.0	14.0	18.0
Caudal fin base	12.0	10.0	8.0	12.0
Anal fin length	16.0	16.0	14.0	20.0
Anal fin base	13.0	11.0	10.0	15.0
Length of pectoral fin	27.0	28.0	24.0	25.0
Base of pectoral fin	6.0	6.0	23.0	9.0

Length of pelvic fin	18.0	18.0	9.0	21.0
Base of pelvic fin	5.0	3.0	2.0	4.0
Dorsal fin spine	4.01	16.8	9.0	69.0
Dorsal fin ray	79.0	12.1	46.0	52.0
Anal fin spine	18.0	29.0	12.0	13.0
Anal fin ray	5.0	87.0	36.0	42.0
Pectoral fin ray	18.0	12.6	51.0	55.0
Pelvic fin spine	6.0	0.09	36.0	4.0
Pelvic fin ray	31.0	0.48	19.0	21.0

The body depth (BD) of all identified groups (genera) in this study varied slightly from one another with tilapia species of the genera *Sarotherodon* having a wider body depth of 37.0% when compared to 32.0% recorded by *Oreochromis* and 20.0% of *Hemichromis* (Sabry *et al.*, 2006). All identified groups vary from one another in percentage mean values of some morphomeristic characters in relation to total length: head length, body depth, dorsal fin length, anal fin base, pelvic fin base, dorsal fin spines and ray, caudal fin spines and ray, anal fin spines, with emphasis on meristic characters since meristic counts are independent of body size and there is no change in meristic counts with increase in body length (Saroniya *et al.,* 2013; Talwar and Jhingran, 1992; Muhammad *et al.*, 2002 and Turan *et al.*, 2006).

Identification of tilapia species is so complicated by the extensive intraspecific variations of the morphometric measurements used for quick species identification. The differences observed from this report could be attributed to management strategy, environment and genetic factors (Alberston *et al.,* 1999).

Identification of Tilapia Species Using Principal Component Analysis (PCA)

In order to describe the morphological characteristics of some identified Tilapia (*O. niloticus, S. galilaeus, H. fasciatus*) species based on similarities and differences, principal component analysis was used to explain the percentage variance obtained by each dependent variable using the Eigen value. The larger the Eigen value, the more the percentage variance in the dependent variable (morphomeristic characters). The variations among them were obtained from the following characters: body weight, body depth, total length, standard length, head length, dorsal fin base length and count, caudal peduncle,

caudal fin base length and count, pelvic and pectoral fin base length and fin counts. The PCA results represented in scatter and screen plots are as shown in Figures 1 and 2. The summary of the morphometric characters is also presented in Table 2.

The PCA of some tilapia species were performed on mean values of 26 parameters aiming to identify similarities and differences among them. The first seven principal components explained 91.4 % variability among the species. Body depth contributed 21.9 %, body weight- 44.1 %, total length- 9.3 %, standard length- 5.3 %, caudal peduncle- 4.0 %, head length- 3.5 %, dorsal fin base length- 3.0 % of the total variability. This indicates that the PCA was largely successful in separating the tilapia species used in this experiment, considering the fact that one of the aims of PCA is to reduce the many variables to a small number of derived variables called components which eventually summarized the given information (Hatcher and Stepanski, 1994). Variables such as head length, caudal peduncle, and dorsal fin base length regardless of its contribution have been reported by other researchers (Yakubu and Okunsebor, 2011; Ola- Oladime *et al.,* 2016) and are similar to that obtained from this study.

The scatter plot shows the position of fish species samples in the space of the first two PCs (Figure 2). Scores were arranged in three areas as there was clear separation between the species and this pointed out the differences in morphological parameters. From the characteristic's features displayed among the species, the upper part of the plot was populated with species from the genus *Sarotherodon*. Species from the genus.

Table 2: Principal Component Analysis for Morphomeristic Characters on Identified Tilapia Species

Components	Eigenvalue	Variance (%)
1	9.7553	44.121
2	4.8344	21.865
3	2.0597	9.3154
4	1.1811	5.3418
5	0.8744	3.9546
6	0.7842	3.5469
7	0.6616	2.9921
8	0.5411	2.4473
9	0.4461	2.0178

10	0.3259	1.4741
11	0.2200	0.9951
12	0.1104	0.4993
13	0.0823	0.3723
14	0.0714	0.3231
15	0.0456	0.2063
16	0.0361	0.1635
17	0.0306	0.1385
18	0.0186	0.0843
19	0.0140	0.0631
20	0.0065	0.0294
21	0.0054	0.0246
22	0.0037	0.0166
23	0.0017	0.0078
24	1.1E-31	4.1E-31
25	7.8E-35	3.5E-34
26	2.3E-35	1.0E-34

Oreochromis were located along the same (PC1) axis. This relationship could be attributed to origin while the distribution along the PC2 axis could be related to similar morphology characters. The PCA result in this study is related to the work carried out by vida *et al.* (2012).

Results of morphometric parameters obtained from scattered plot illustrated cluster formations of some sampled *T. species* along negative and positive values of geographical X and Y axis (Fig. 1). Clusters revealed that all the species on the positive side of the Y axis are close together except for one or two which is a bit distant from the others. The implication may be that they share similar morphometric characteristic features indicating that they may be the same species or that there exists a strong genetic tie between them. Such close cluster may also indicate that the species may be from the same geographical location. This agrees with the report of Saber *et al.* (2014) who stated that moderate segregation confirmed by PCA graphs for all samples in morphometric evaluation reveals that such populations were distinct from each other.

Some morphological differences between populations are often quite difficult to explain may be in course as there were one or two *species* of the genus *Oreochromis* that were seen clustered distantly from the others. However, from Fig.2 below, the combined cluster observed among species of the genus *Sarotherodon* and *Oreochromis* may be as a result of over time environmental factors, effect of geographical location where fishes were sourced from to the farm, or genetic factors; this is somehow related to the observations of (Turan *et al.*, 2005 and Poulet *et al.,* 2004). Also, results from this study agreed with the observations of (Yakubu and Okunsebor, 2011) that the discriminating variables obtained in their studies might not be exhaustive as the inclusion of other variables might produce similar morphological traits.

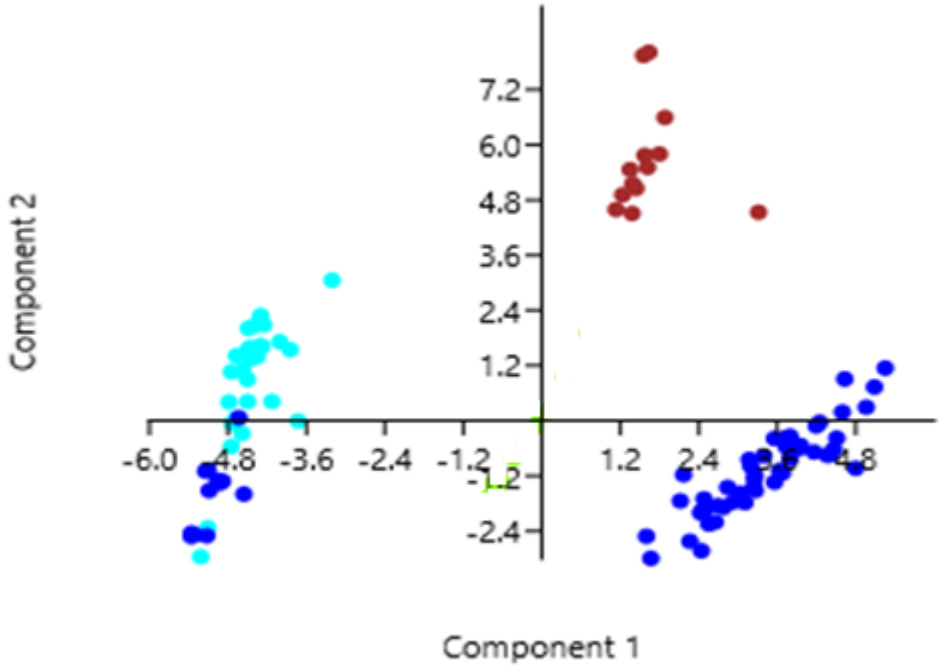

Figure 2: Scatter diagram based on PCA of significant morphometric variables of three identified tilapia species. Red colour° = *H. fasciatus,* Blue and light blue°° = *O. niloticus.,* Green= *S. galilaeus spp.*

Results from the scattered plot presented in Fig. 2 revealed another cluster with heavy cluster among *O. niloticus* species as there was no infiltration of any other different species of tilapia. The heavy cluster suggests that all the sampled fishes in the cluster may be from the same geographical location or may share same morphological trait. In general, from

Fig. 2, there was no clear cluster for the *S. galilaeus* as this may be pointing / suggesting to it, undergoing through a morphological transformation towards the trait of *O. niloticus* or there may be a possible strong ancestral linkage between *O. niloticus* and *S. galilaeus* or could be possibly a hybrid. The report of Fernando and Amarasinghe (2011) stated that hybrid forms may possess mixed characteristics of two species, making it difficult to differentiate using simple morphometric data.

Identification of Tilapia Species Using Cluster Analysis for Morphometric Characters

Figure 3 illustrates a dendrogram representation of cluster analysis for the morphometric characters of tilapia species in MOUAU fish farm. The hierarchical cluster analysis was performed using the group method, Euclidean distances, on the species to identify whether the individual's morphology could be separated. The hierarchical cluster was separated into three main groups. Group 1 represents *Sarotherodon galilaeus,* while group 2 and 4 is *Oreochromis niloticus and* group 3, *Hemichromis fasciatus*. However, groups 2 and 4 revealed sub cluster within *Oreochromis niloticus*. Results of the hierarchical cluster analysis show that groups 2 and 4 are closely related with different sub cluster which formed a separate cluster in the dendrogram (Fig. 3 and Fig. 4). It can clearly be seen that both groups may share same morphometric characters hence the similarity.

From the dendrogram presentation, the first four species of *S. galilaeus* do not share same close morphometric character with the rest of *Sarotherodon* specie*s* identified. Rather, there was a distant connection between them and the first set of *Oreochromis* species. The distant connection observed among the first few species of the genus *galilaeus* may suggest a possible hybrid formation and this observation is in line with the report of (Fernando and Amarasinghe, 2011). Also, the close distant linkage between groups 2 and 4 may affirm that they are possibly of the same species. Therefore, the difference may be as a result of 'physiological processes' which the fishes were undergoing at the time of sampling. This is in line with the report of (Shubha and Reddy, 2011). From the dendrogram obtained during this study based on morphometric measurements and meristic counts (Fig. 3), 4 clusters was seen and on close observation, groups 1 and 1V fell almost within the same phylogenetic distance, showing that samples of these clusters could be of same species and may share same morphological features. This result was verified on comparison to that

obtained from RAPD (Fig. 5 and 6) to actually ascertain the number of groups and species available in the University Fish Farm.

Molecular Characterization of Identified Tilapia Species

A total of 66 different randomly amplified DNA fragments from specimens of species of the family Cichlidae suspected to be *O. niloticus, S. galilaeus, and H. fasciatus* were detected consistently with all 10 RAPD primers in the sample populations. The RAPD primers were selected and used in this study for quantity and reproducibility capacity of fragments amplified. The size of amplified fragments per primer varied from 300 base pair (bp) to 1000 bp. However, all the RAPD fragments were polymorphic with 3 - 5 alleles among all the populations. The 10 RAPD fragments amplified were found to be shared among individuals of all the populations. Only the polymorphic bright bands with clear amplification were analyzed and the faint bands were not scored for presence or absence. In this population, a total of 17 bands were absent in different primers from the 66 sample populations. The numbers of fragments and polymorphic bands recorded with each primer are given in Table 3.

The use of RAPD primer OPE 1 on sample population shows clear bands of polymorphism of 47 samples population with 2 - 5 alleles fragment at 300 bp, 400 bp, 450 bp, 500 bp and 600 bp and 700 bp. The RAPD OPE1 did not amplify any (Plate: 7).

Possibly ancestral linkage between the sampled species may be the reason why most of the clear bands in Plate 7 fall between the same base pairs and almost having equal number of genes on the same loci. This agrees with the report of (Antunes *et al.*, 2010). Also, the less visible bands for samples 47 and 48 of group 2 (*O. niloticus*) and of samples 49 – 56 which are of the same species may indicate that probably, there exist a level of genetic variation among them, this trend agrees with (Antunes *et al.*, 2010) where they stated that less phylogenetic relationship found among Brycon species, may be associated with geographical distribution as well as adaptation patterns for individual species.

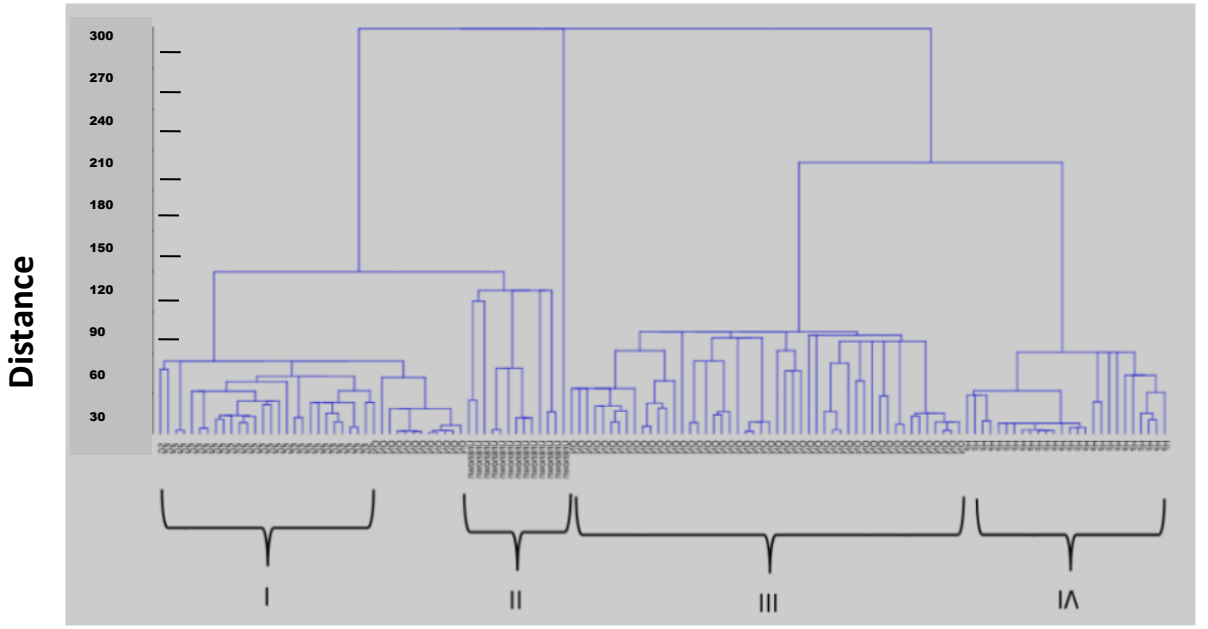

Figure 3: Dendrogram from hierarchical clustering of some identified fish species from MOUAU fish farm, based on their morphological characterization showing distance. (Group 1 & 1V = *O. niloticus*, Group III = *S. galilaeus*, Group I1 = *H. fasciatus*).

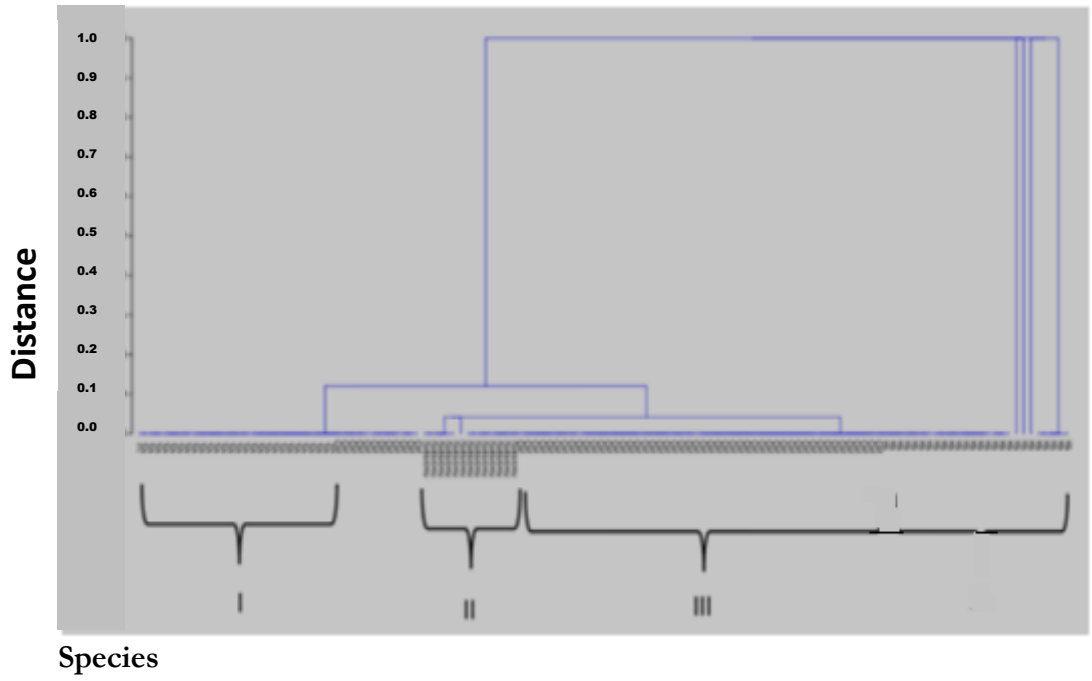

Figure 4: Dendrogram from hierarchical clustering of species group 1, 2, 3 from the MOUAU, based on their morphological characterization showing their relationship Group 1= *O. niloticus*, Group II = *H. fasciatus* and Group III = *S. galilaeus*.

The results of RAPD primer OPC - 2 indicates that polymorphism was observed among the 61 samples populations and the length of the bands ranges from 300 bp – 800 bp. These bands were between 1 - 4 alleles per sample in the population. Thus, 5 samples did not show fragment: Plate 8. Clearer band observed in OPC - 2 gel photos further clarify the view that some primers are more suitable for genetic evaluation than others. From OPC - 2, similar band quality were seen around samples 52 – 54 which are *O. niloticus* (group 4). This may indicate that they may not just be of the same species but that they share some genetic response to environmental changes, geographical location and other adaptation properties.

There are 66 sample population but 16 samples did not show amplification with the RAPD primers OPA - 4. This primer indicates polymorphism on 50 of the sampled populations with a fragment size range from 300 bp to 800 bp. The numbers of alleles observed were 2 - 5 among the four tentative group sample population (Plate 9). The gel electrophoresis of PCR product with RAPD primer OPA - 5 showed 43 samples amplified and revealed polymorphism. The band ranges from 250 bp – 1000 bp having 2 - 4 alleles per sample and the fragment bands were not detected in 23 sampled populations (Plate 10). When the RAPD primers OPA-07 was used on the fish samples, the PCR product separated on agarose gel electrophoresis are polymorphic. A total of 19 samples did not show bands fragment in RAPD primer 07. While 47 fish samples have 1- 6 numbers of alleles observed and 6 alleles per sample were detected. However, the polymorphism shows the bands fragment ranged from 300 bp to 1000 bp (Plate 11).

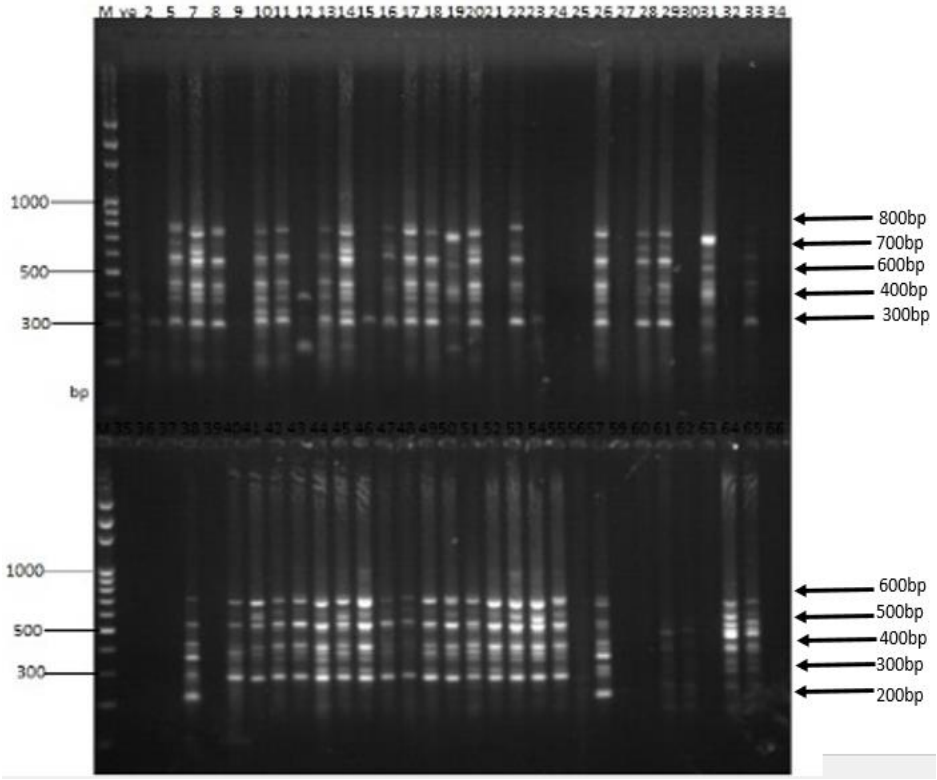

Plate 7: The gel electrophoresis of RAPD primer OPE-1

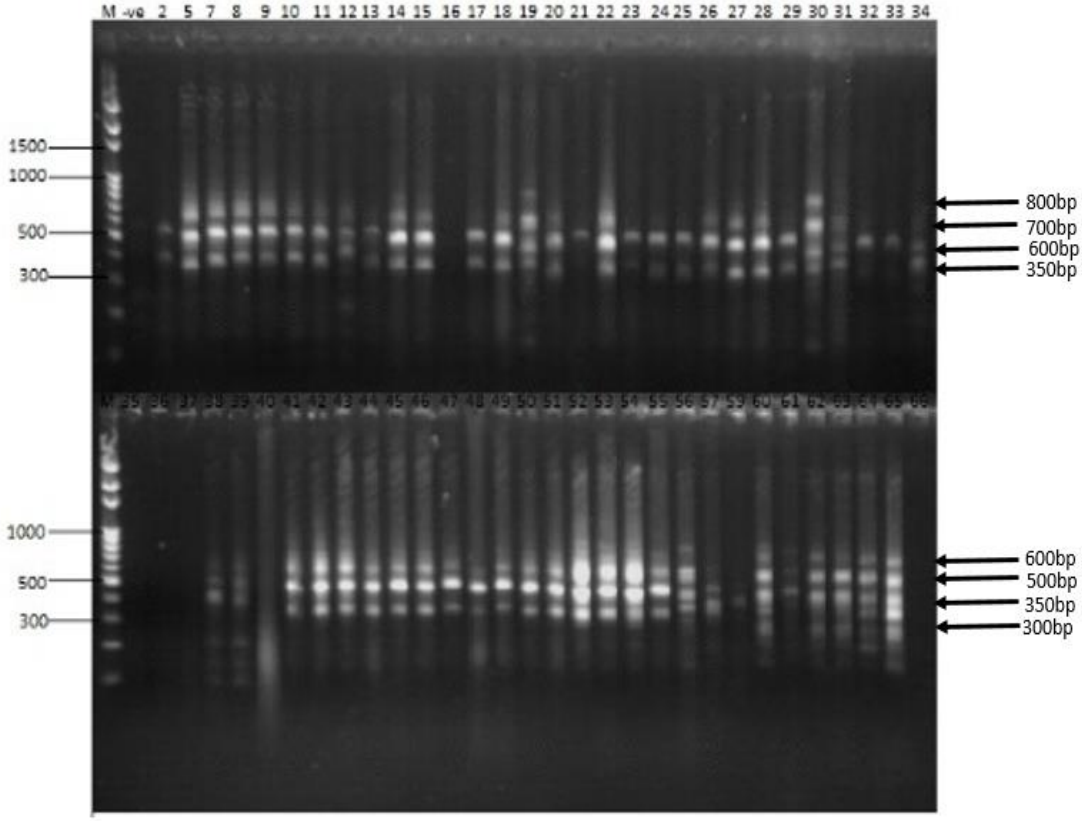

Plate 8: The gel electrophoresis of RAPD primer OPC-2

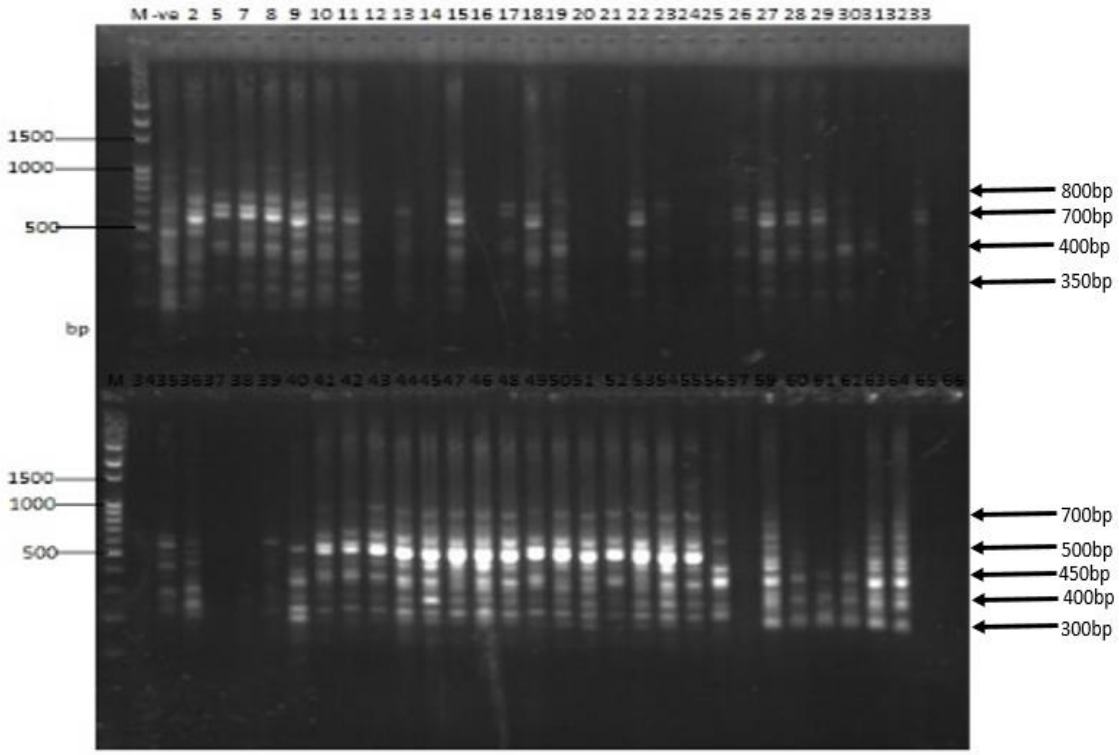

Plate 9: The gel electrophoresis of RAPD primer OPA- 4

A total of 54 amplified DNA fragments were detected consistently with the RAPD primer OPB -8 of the fish samples. The size of the fragments ranged from 250 bp to 800 bp while 12 samples did revealed bands and these bands were polymorphic in this population having 2 - 4 alleles (Plate 12). The RADP primers used on the sample population resulted in the generation of a total of 42 samples with reproducible fragment bands, while 24 did not amplify. The RAPD primer OPA-9 has the highest number of samples that did not amplify in the population. However, the highest number of 7 alleles was observed with this primer and the bands ranged from 300 bp – 800 bp among the sampled species (Plate 13). RADP primers OPA-10 amplified 44 samples while 22, detected no bands fragments even though the primer was not specific and not separated properly, but shows fragment size ranging from 200 bp – 700 bp which is polymorphic. All amplified samples with visible bands showed polymorphic with non-monomorphic band. Such high polymorphism observed affirms to the fact that polymorphism is common in nature and the sampled fishes have great biodiversity, genetic variation and adaptation (Leimar, 2005).

The RAPD primer OPA - 11 used on the samples were found to produce good quality band patterns. The primers produced multi-band patterns of 57 samples among the population ranging from 2 - 4 per samples, revealed polymorphism between them and compare to other primers used in the same sample in the experiment. The size of fragment varied at 200 bp to 700 bp (Plate 15). There were 4 out of 66 samples using the RAPD primer OPD - 14 which showed no amplification of any bands while the 52 samples that were subjected to this primer revealed polymorphism with 2 - 5 alleles per sample. The fragment size within the population ranges from 300 bp – 800 bp (Plate 16).

Table 3: Primer sequences of RAPD markers used in this study

S/N	Original Name	Current Symbol	Sequence
1	RAPD-OPE-1	P1	CCCAAGGTCC
2	RAPD-OPA-4	P2	AATCGGGCTG
3	RAPD-OPA-5	P3	AGGGGTCTTG
4	RAPD-OPA-7	P4	GAAACGGGTG
5	RAPD-OPA-9	P5	GGGTAACGCC
6	RAPD-OPA-10	P6	GTGATCGCAG
7	RAPD-OPA-11	P7	CAATCGCCGT
8	RAPD-OPB-8	P8	CCGCATCTAC
9	RAPD-OPC-2	P9	TCTCTGGGTG
10	RAPD-OPD-14	P10	CTTCCCCAAG

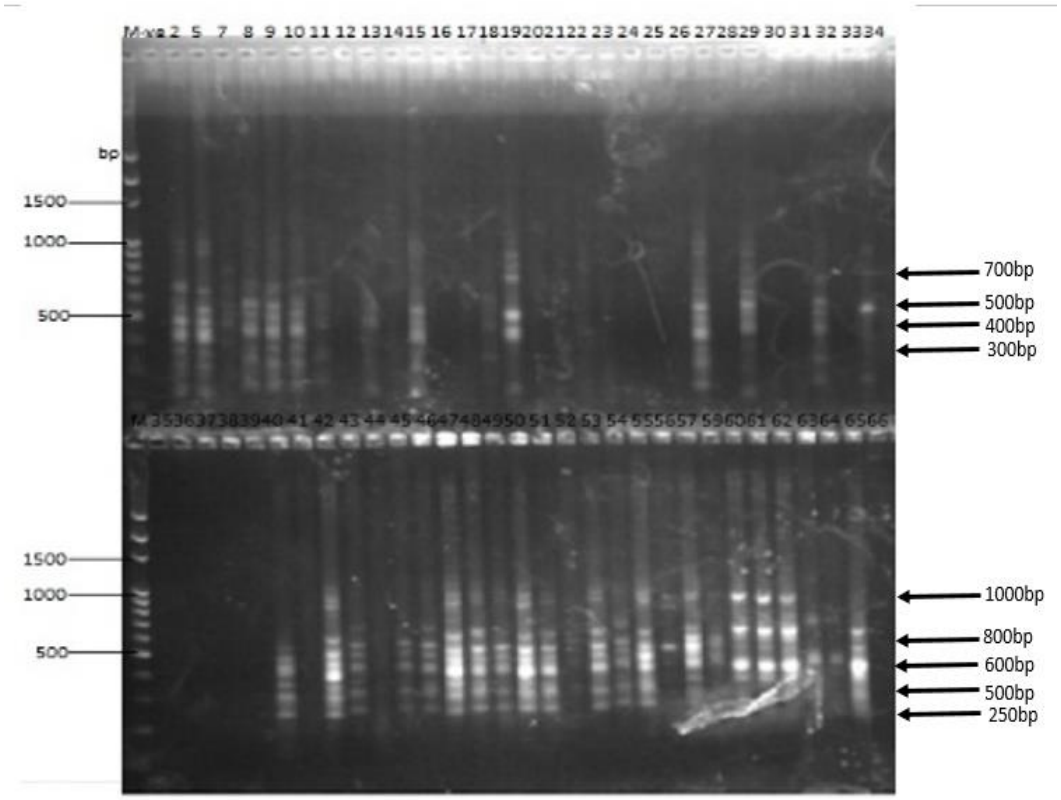

Plate 10: The gel electrophoresis of RAPD primer OPA - 5

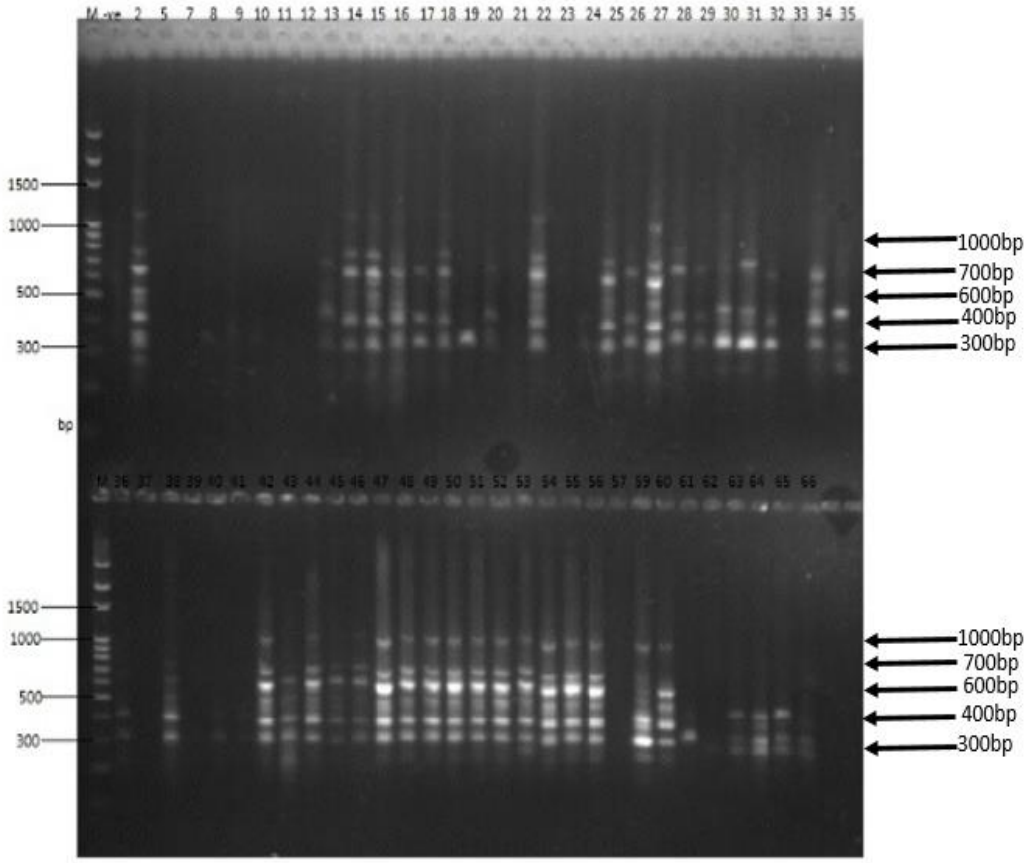

Plate 11: The gel electrophoresis of RAPD primer OPA – 7

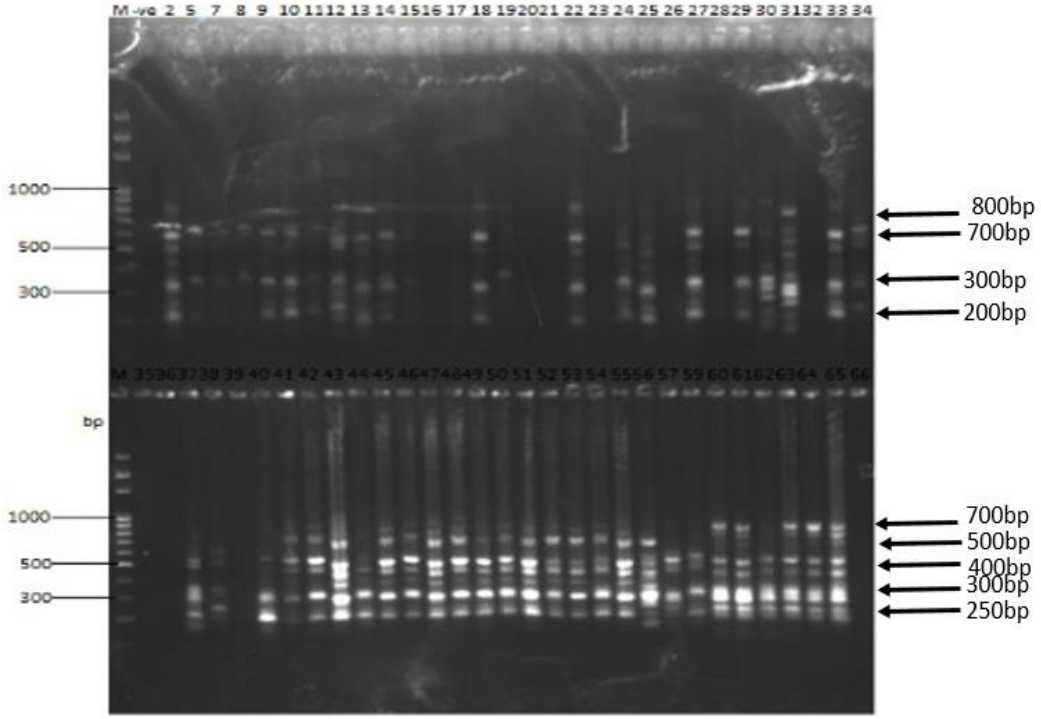

Plate 12: The gel electrophoresis of RAPD primer OPB - 8

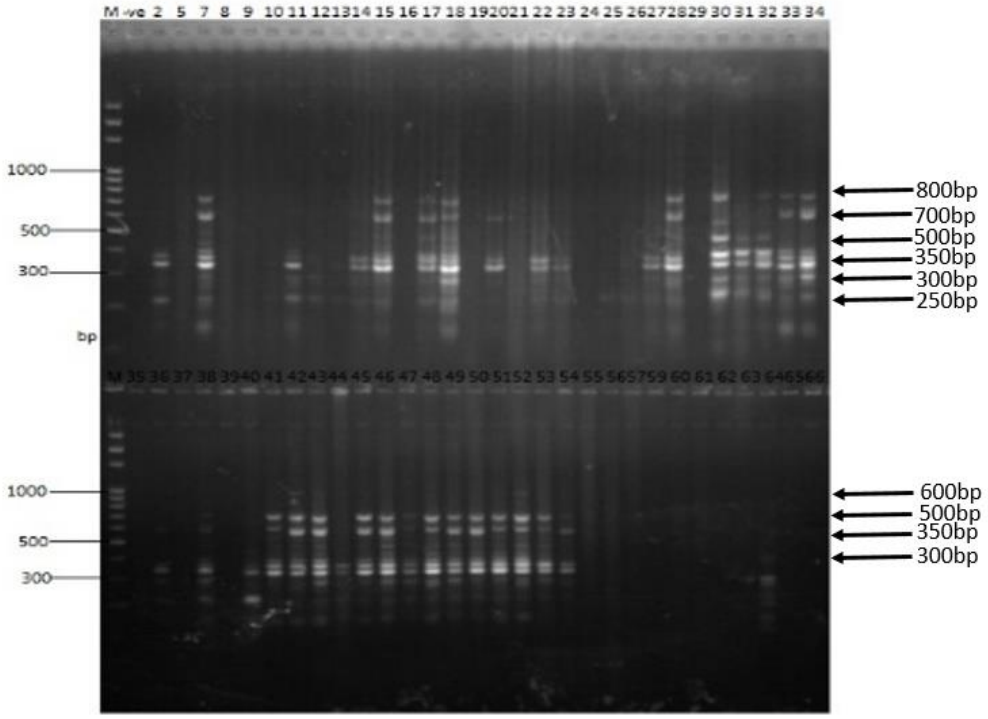

Plate13: The gel electrophoresis of RAPD primer OPA - 9.

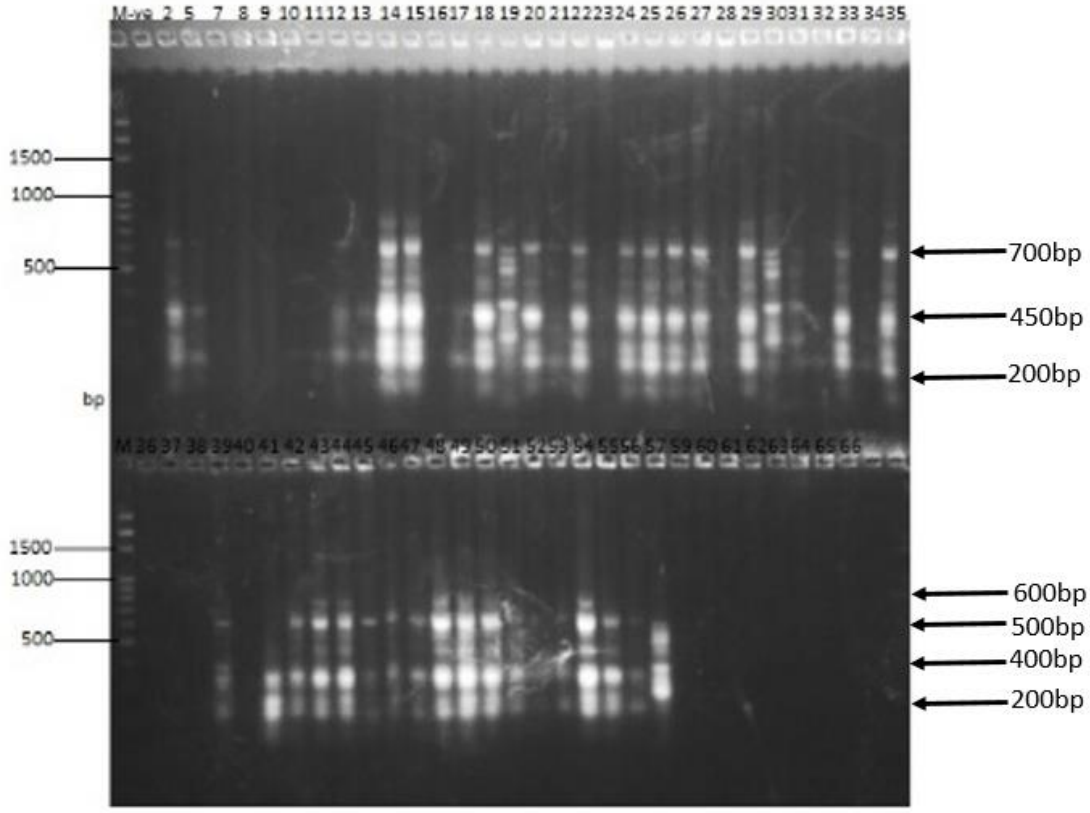

Plate 14: The gel electrophoresis of RAPD primer OPA - 10

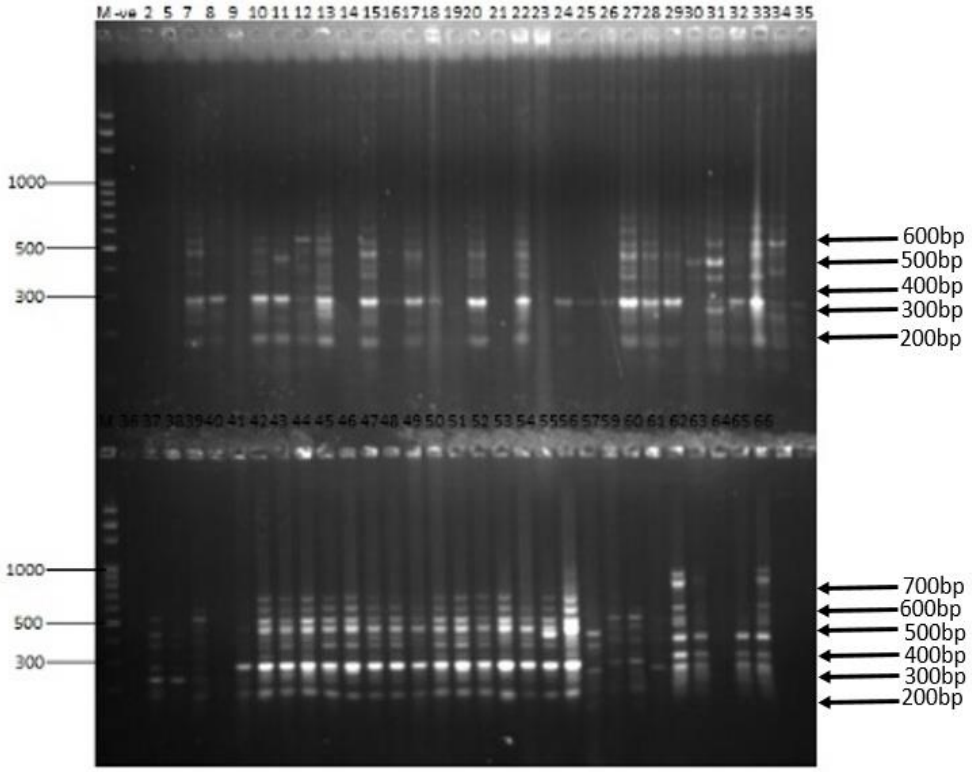

Plate 15: The gel electrophoresis of RAPD primer OPA - 11

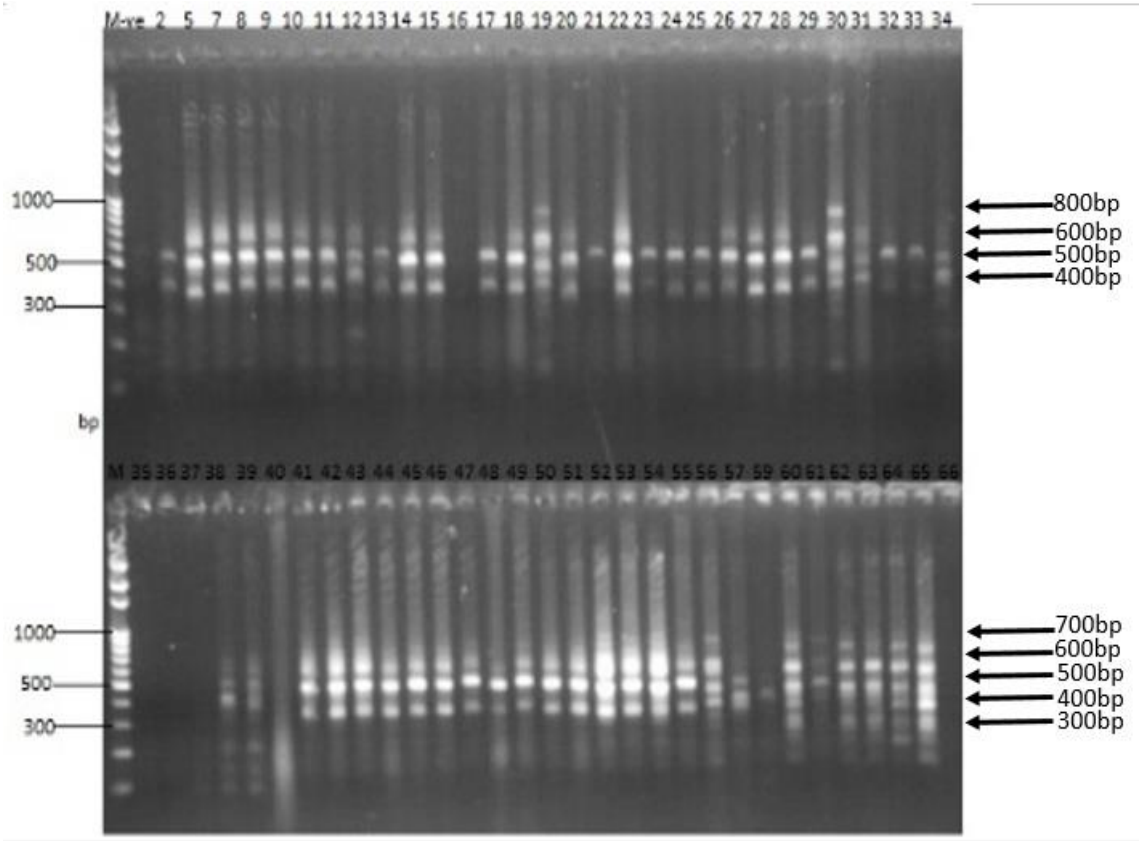

Plate 16: The gel electrophoresis of RAPD primer OPD-14.

The proficiency of RAPD markers used in this study to detect genetic variations among tilapia fish species confirms to the statement of Rashed *et al.* (2011) and Hassan *et al.* (2014) who concluded that RAPD is best suitable genetic marker to identify lines or strains, define stock diversity, monitor interbreeding, diagnose simple inherited trait and to improve stock. They found out that *Tilapia zilli* species was distantly related from both *O. aureus* and *O. niloticus* species. It should also be noted that from these studies, there exist close relationship between group 2 and 4 (*O. niloticus*) whereas huge relationship difference was genetically observed among *O. niloticus* and *S. galilaeus* in all the plates.

Phylogenetic Relationship and Genetic Distance

The RAPD dendrogram constructed from genetic distances showed that the dendrogram is divided into three major clusters of fish species of *S. galilaeus, O. niloticus and H. fasciatus*. Group 1 consists of two sub clusters of *S. galilaeus and O. niloticus,* group 3 included *O. niloticus* while group 4 clusters consists *H. fasciatus* (Fig. 5). According to the similarity

matrix for fish species from different locations based on RAPD analyses, the results showed that species of *O. niloticus* had highest genetic similarity. Within species of *S. galilaeus*, according to RAPD, there observed also a good harmony with the polymorphic. On the other hand, *S. galilaeus* species exhibited high genetic similarity with *O. niloticus* when compared to that of *H. fasciatus*). The study provides evidence that RAPD markers could be used for genetic differentiation of closely related species.

Figure 5 illustrate molecular characterization of sampled tilapia species using dendrogram. Similar observations as noticed in Fig. 4, were also observed in Fig. 5 such similarites as that which were noticed between *S. galilaeus* and *O. niloticus* in the molecular data, goes a long way to validate the observation of the morphological data.

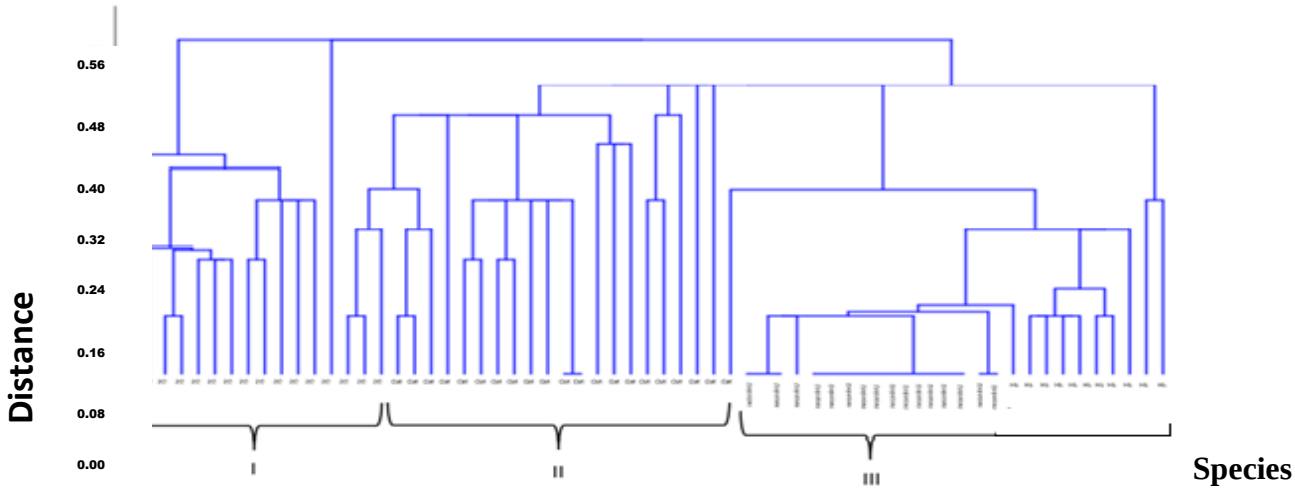

Figure 5: A Dendrogram construction of RAPD primers showing the genetic distance among representative fish population derived from UPGMA (Group 1 = *O. niloticus,* Group II = *S. galilaeus*, Group III = *H. fasciatus*)

This agrees with the report of Sabry *et al.* (2015), who stated that from their studies, molecular evaluation is effective in detecting similarities between fish species and provide a potencial tool for studying the intra specific genetic similarities and establishment of genetic relationship. Same idea was also used by Rashed *et al.* (2011) and Hassan *et al.* (2014).

The molecular dendrogram result further established a stronger relationship between all the species of the genus *Oreochromis*. Similarities between species may be due to persistent ancestral traits, while differences possibly reflect varying selective forces associated with

ecological and mating signal divergence (Ptacek, 2000; Panhius *et al.,* 2001; Schlutter, 2001). These relationships as stated earlier may be as a result of environmental changes, hybrid formation or speciation and physiological sex driven changes among the sampled species.

Figure 6 illustrates the genetic relationship between the sampled tilapia species. Generally, genetic variation among fresh water fish species is low due to natural limitations such as chances of mating with other populations (Azrita, 2014). This statement further illustrates reasons why most of the sampled species from the molecular dendrogram result share common genetic similarities. This could be seen from the close relationship between *O. niloticus* and *H. fasciatus*.

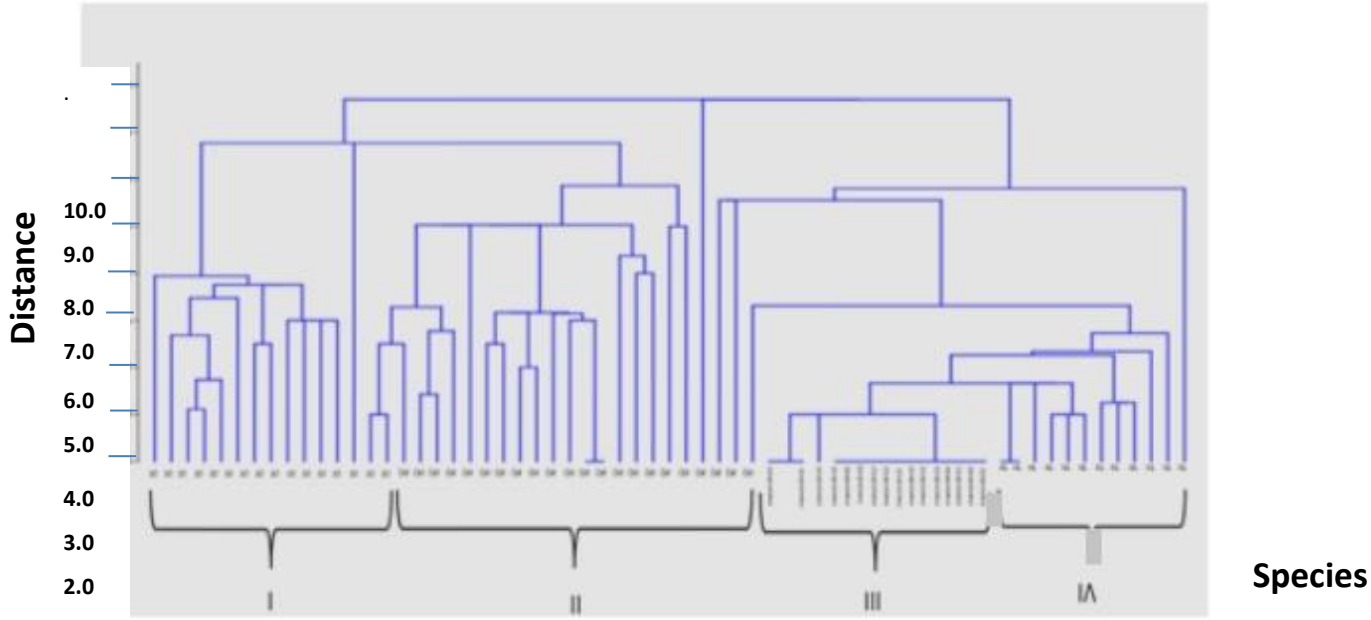

Figure 6: A Dendrogram construction of RAPD primers showing the genetic relationships amongrepresentative fish species from MOUAU fish farm, derived from UPGMA method obtained with Jaccard's similarity coefficient. (Group 1 = *O.niloticus*, Group 11= *S.galilaeus*, Group III = *H. fasciatus*).

CONCLUSION

The research work suggests that morphometric parameters such as body depth and lengths of caudal peduncle among others are identified natural markers that can be employed in identification of cichlid family of the genus *Oreochromis, Sarotherodon* and *Hemichromis*. Rapid

amplified polymorphic deoxyribonucleic acid (RAPD) Primers OPA -11,OPC-02 and OPD-14 were the most producible primers among the ten RAPD primers used in this study, since the number of alleles per sample were 1-6 while the lower alleles produced by RAPD primers OPA- 04 and OPB- 08. On the other hand, the primer 11, 02 and 14 produced the most shared markers among the four proposed groups of tilapia fish species ascertaining that there were actually three groups. The effectiveness of RAPD primer in detecting polymorphism among different fish varieties, their applicability in population studies, and the establishment of genetic relationships among various fish populations have been investigated in this study. A high degree of polymorphism was observed in this study, suggesting a high degree of genetic variability between all species (*S. galilaeus, O. niloticus* and *H. fasciatus*).

The result obtained has proved to be useful in identifying, characterization and discrimination of tilapia fish species. Therefore, it was observed in this study that either morphological or genetic analysis based on RAPD -PCR can be used to discriminate fish species with the same results up to the intraspecific level. However, it is essential to optimize RAPD amplification condition and ascertain the reproducibility of RAPD markers for individual species prior to applying RAPD to any genetic analysis. By using ten different primers, RAPD fragments showed a reasonable degree of genetic similarity as well as variation within and between the species.

Recommendations

The genetic data collected during this work will be useful in the future in improvement programme for enhanced economic benefit of tilapia species faming in Michael Okpara University of Agriculture, Umudike (MOUAU) fish farm.

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