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Comparison of genetic diversity of farmed *Oreochromis niloticus* and wild unidentified tilapia (Wesafu) using microsatellite markers

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Abstract. *Ukenye EA, Megbowon I. 2023. Comparison of genetic diversity of farmed* Oreochromis niloticus *and wild unidentified tilapia (Wesafu) using microsatellite markers. Biodiversitas 24: 2953-2957.* Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) and unidentified tilapia (Wesafu) are cichlid species of important nutritional and economic value with good aquaculture potential. Assessing and comparing the genetic diversity of these cichlid species will assist in information development for conservation and management strategies to improve their overall productivity and sustainable aquaculture production. Using twenty fish specimens each from farmed *O. niloticus* and ecotype, Wesafu from the wild respectively, we examined the genetic diversity between these two cichlid species. Eight microsatellite loci were utilized to molecularly characterize and compare these two cichlid species from the wild and farm based on their genetic variation levels. Genetic diversity was investigated from isolated, amplified and resolved DNA of the two cichlid species obtained from the wild and farm. The microsatellite markers analysis revealed that ecotype, Wesafu from the wild exhibited higher genetic diversity than farmed *O. niloticus* as evidenced by the effective number of alleles (1.756), Shannon information index (0.596), observed and expected heterozygosity values (0.682 and 0.400). All genetic diversity indices were observed to have declined in farmed populations due to inbreeding. However, farmed *O. niloticus* recorded more polymorphism (87%) than ecotype, Wesafu from the wild (75%). Low genetic differentiation was found between the farmed and wild cichlid species according to the fixation index (-0.628) while Principal Coordinate Analysis (PCoA) demonstrated some level of variation between the two cichlid species. This finding provides more insights into the conservation of the genetic resource and better management of these species to minimize inbreeding in aquaculture. We advise that only wild broodstocks should be used for fish restocking in breeding program for sustainable aquaculture production.

Keywords: Cichlid species, cultured, genetic variation, molecular marker, natural

INTRODUCTION

Cichlid species are imperative in aquaculture production and are being cultured in over 120 countries with a global production volume growing beyond 5 million tonnes (FAO 2018). Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) and unidentified tilapia (Wesafu) are cichlid species', commonly found in the tropical and sub-tropical freshwater of Africa, they grow bigger than some other cichlid species and have been successfully domesticated. Megbowon and Fashina-Bombata (2013) reported that the unidentified tilapia grows up to 1.5 kg and dominates Epe lagoon of Lagos State, Nigeria. These species' are valued for their economical and aquaculture relevance and have been widely used for the production of interspecific hybrids, particularly the *O. niloticus*. Considering their aquaculture importance, characterizing the genetic structure of these currently bred species, particularly Wesafu from the wild in the aquaculture industry can be utilized in the selection of strains for broodstock development and management to conserve their genetic diversity by minimizing inbreeding.

Genetic diversity is considered the key resource in the successful breeding of aquatic animals. The genetic diversity within species (between populations as well as among individuals within populations) results in the degree of variation at different levels (nucleotide, gene, chromosome, and genome). This genetic variation plays an important role in species/population's survival in response to environmental changes (Dudu et al. 2015). The loss of genetic diversity is a serious concern for breeding stocks and population pools which enhances the chances of extinction. Thus, the conservation of genetic diversity at all levels and better breeding plans for aquaculture purposes is encouraged.

Microsatellite markers are a suitable tool for genetic tagging of wild broodstocks and farmed populations due to their higher levels of allelic variation that can help to confront genetic diversity issues (Hosseinnia et al. 2014). They represent the most widely applicable DNA technology. Microsatellites are short stretches (tens to hundreds of base pairs) of DNA composed of di-, tri-, or tetra-nucleotide repeats arrayed in tandem. According to Vallecillos et al. (2022), they are highly polymorphic in teleost fish, with as many as 52 alleles observed at one locus in Atlantic salmon (*Salmo salar* Linnaeus, 1758). This variability makes for suitability in a variety of applications in fisheries and aquaculture, particularly where genetic differentiation between populations may be limited. Potential applications in aquaculture include monitoring changes in genetic variation, as a consequence of different breeding strategies, parentage assignment, and estimation of relatedness between potential breeding pairs. Previously, microsatellite markers have been used to characterize wild populations and breeding stocks of farmed fish (Mojekwu 2020). Farmed and hatchery populations of Atlantic salmon

have been reported as having 20-30% lower heterozygosity in several studies when examined using polymorphic enzyme loci (Verspoor 1988). Usman et al. (2017) also observed high levels of genetic variability for both wild and culture *O. niloticus*. Microsatellites have also revealed the genetic variation among individual fish (Lu et al. 2022), to direct matings during the formation of the population base of breeding programes (Fernández et al. 2014) and also to perform a marker-assisted selection for economic traits (Houston et al. 2010). Similarly, Ukenye et al. (2016) used microsatellite makers to assess the genetic diversity of *Tilapia guineensis* Bleeker, 1863 (Guinean tilapia) from some Nigerian coastal waters. According to Palaiokostas et al. (2020), the microsatellite analysis allows for high-resolution studies of genetic diversity and relatedness at both population and species levels.

Comparing farmed and wild populations is essential for the conservation and management of genetic resources for breeding programs. Genetic diversity information is required to conserve the natural populations and is very necessary to uphold their genetic integrity while constant monitoring of broodstocks in fish farms is also essential to minimize inbreeding and overcome genetic decline (Carlson et al. 2014). Previous studies have been carried out on the genetic diversity of wild and cultured populations and several reports on the reduced genetic variability of farm stocks when compared to wild populations have been documented (Hosseinnia et al. 2014; Gao et al. 2023). Berrebi et al. (2021) also reported reduced genetic variability in farmed brown trout (*Salmo trutta* Linnaeus, 1758) and the common carp respectively. This study therefore aimed to: i) check whether aquaculture affects the genetic diversity of tilapia species; ii) check if genetic differences exist between farmed strains as a result of breeding practices and between wild strains; iii) characterize and compare genetic variability of farmed *O. niloticus* and wild Wesafu for conservation of genetic resources and proper management of these cichlid species especially Wesafu in aquaculture through microsatellite analysis.

MATERIALS AND METHODS

Sample collection

A total number of forty fish samples (twenty Wesafu and twenty *O. niloticus* species') were collected from two stations; Wesafu from Epe lagoon (latitude N04° 27.200[1] and longitude E07° 19.618[1]) obtained from the fishermen at the landing site and *O. niloticus* selected randomly from four concrete tanks in Aquaculture hatchery of Nigerian Institute for Oceanography and Marine Research Lagos, Nigeria. The samples were transported in ice chest to the Biotechnology laboratory of Nigerian Institute for Oceanography and Marine Research Lagos, Nigeria at -2°C, where extraction started immediately to avoid DNA denaturation.

DNA extraction and Polymerase Chain Reaction (PCR) amplification

Genomic DNA extraction was carried out from the caudal fin (1 g) using phenol-chloroform protocol according to Sambrook et al. (2001). The purity check was done to ascertain the quality of extracted DNA using a Nano-drop spectrophotometer (Shimadzu Corporation Japan, MODEL UV-1800, 2000 series) at the absorbance of 260/280nm. Polymerase chain reaction amplification was done using ten microsatellite primers originally developed for tilapia by Lee et al. (2005). Out of the ten, eight amplified while two did not amplify. A total volume of 20 µL sample reaction consisting of 4 µL Solis Biodyne 5x fire pol (master mix with 12.5 mM MgCl), 13.1 µL dd H_2O, 0.5 µL dNTP (0.2 mM; nucleotides), 0.2 µL forward primer, 0.2 µL reverse primer, and 2 µL of template DNA (10 ng) was run on a Thermocycler (Biorad, module 170 -8731). The program for PCR amplification was: 2 min initial 96°C denaturation, 30 cycles of 94°C for 30sec, 30sec at the annealing temperature shown in Table 1, and 30sec at 72°C, followed by a 6min final extension step at 72°C. The amplicons were separated on Polyacrylamide Gel Electrophoresis (PAGE) at 6% concentration and 80 V for 2 h in a 1x TBE buffer. This was followed by the scoring of the gel bands by two researchers, independently to reduce or rule out error due to improper scoring.

Table 1. SSR Primer code, sequences, annealing temperature and band size

Primer code	Sequence	Annealing temp. (°C)	Molecular size (bp)
GM531	Forward 5' AAAGCCAACGGTCTGAATTG 3' Reverse 5' AGCAGAGGACACCCCTCAT 3'	55	150-200
GM538	Forward 5' CAGCATGTTGTCTGGATCTTG 3' Reverse 5' TTTGTTGCTGTGGTCTGTTCTT 3'	55	100-200
UNH104	Forward 5' GCAGTTATTTGTGGTCACTA 3' Reverse 5' GGTATATGTCTAACTGAAATCC 3'	50	100-200
UNH207	Forward 5' ACACAACAAGCAGATGGAGAC3' Reverse 5' CAGGTGTGCAAGCAGAAGC 3'	55	100-200
UNH185	Forward 5' CAGACACACTAGACACATTCTA 3' Reverse 5' GTGTTTCCATGTGTCTGTAC 3'	55	100-150
UNH146	Forward 5' CCACTCTGCCTGCCCTCTAT 3' Reverse 5' AGCTGCGTCAAACTCTCAAAAG 3'	55	100-150
UNH123	Forward 5' CATCATCACAGACAGATTAGA 3' Reverse 5' GATTGAGATTTCATTCAAG 3'	50	100-150
UNH995	Forward 5' CCAGCCCTCTGCATAAAGAC 3' Reverse 5' GCAGCACAACCACAGTGCTA 3'	55	150-250

Data analysis

The number of alleles per SSR locus, an effective number of alleles, Shannon information index, observed and expected heterozygosity were calculated from the generated genetic data and analyzed with POPGENE version 1.32 software while the Polymorphic Information Content (PIC), genetic diversity and Inbreeding coefficient were determined using powerMarker v.3.6. Molecular data were subjected to the Principal Coordinate Analysis (PCoA) that was used to determine variation among cichlid species.

RESULTS AND DISCUSSIONS

Genetic characterization of microsatellite loci

In the present study, eight microsatellite markers were utilized to investigate the genetic diversity of farmed *O. niloticus* and Wesafu from the wild. The eight microsatellite loci used revealed polymorphism across the species with the number of alleles which ranging from 2.00 to 3.00 with an average of 2.38. The Polymorphic Information Content (PIC) values ranged from 0.25 at locus GM531 to 0.56 at locus GM538 and UNH104, respectively with an average value of 0.39. All the loci recorded a total number of 19 alleles and showed negative values for the inbreeding coefficient, except locus GM531 (Table 2). The level of diversity revealed by the studied loci ranged from 0.29 to 0.63 with an average of 0.47. The highest heterozygosity was obtained by locus UNH104 (He = 0.97) while locus GM531 (0.29) had the lowest.

Genetic differences between farmed and wild cichlid species

The microsatellite loci revealed 4 maximum allele numbers from both species with both of them having the same number of alleles (Na = 2). The effective number of alleles varied from 1.64 farmed *O. niloticus* to 1.75 for Wesafu from the wild, which was lower than the observed number of alleles in both species. The observed Heterozygosity (He) mean for *O. niloticus* was 0.60 and 0.68 for Wesafu while the expected (GD) heterozygosity mean for *O. niloticus* was 0.35 and 0.40 for Wesafu respectively. Shannon index was observed higher in ecotype, Wesafu (I=0.59), from the wild than in farmed *O. niloticus*, (I=0.55). *Oreochromis niloticus* showed more polymorphism (87%) than Wesafu (75%) (Table 3). These genetic variability results revealed that Wesafu from the wild had higher genetic diversity than farmed *O. niloticus* as evidenced by the effective number of alleles, Shannon information index, observed and expected heterozygosity.

Figure 1 shows a gel image of an amplified fragment produced by primer UNH995. GM538, UNH104 and UNH123 detected the highest number of alleles (3 alleles) respectively suggesting that these markers were sufficiently robust to differentiate specifically different species of the same family. The Principal Coordinate Analysis (PCoA) recovered 35.18% of the total variation in the first Principal Component analysis (PC1), and 14.88% in the second (PC2). The two cichlid species were separated by the principle coordinate analysis. However, *O. niloticus* formed a separate cluster and differentiated from Wesafu as presented in Figure 2.

Table 2. Genetic performance of microsatellite loci analyzed

Marker	Na	GD	He	PIC	Inb
GM531	2.00	0.29	0.29	0.25	0.04
GM538	3.00	0.63	0.90	0.56	-0.41
UNH104	3.00	0.63	0.97	0.56	-0.52
UNH207	2.00	0.45	0.69	0.35	-0.52
UNH185	2.00	0.33	0.42	0.28	-0.24
UNH146	2.00	0.49	0.57	0.37	-0.15
UNH123	3.00	0.57	0.87	0.48	-0.51
UNH995	2.00	0.33	0.42	0.28	-0.25
Mean	2.38	0.47	0.64	0.39	-0.36

Note: Na: number of alleles, GD: Genetic Diversity, He: Heterozygosity, PIC: Polymorphic Information Content, Inb: Inbreeding coefficient

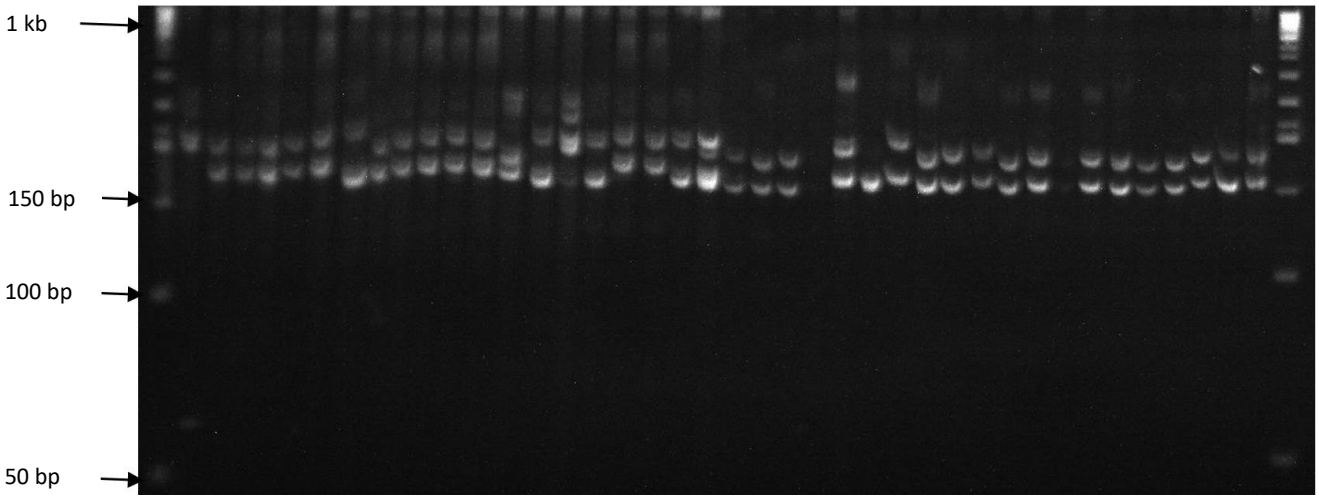

Figure 1. Results of polyacrylamide gel electrophoresis of the amplified microsatellite loci using UNH995 primer. 1-40 specimen

Table 3. Estimates of genetic diversity in farmed *O. niloticus* and wild wesafu populations

Population		Na	Ne	I	He	GD	F	Pp (%)
O. niloticus	Mean	2.000	1.642	0.553	0.603	0.359	-0.549	87.50
	SE	0.327	0.311	0.129	0.158	0.085	0.138	
Wesafu	Mean	2.000	1.756	0.596	0.682	0.400	-0.720	75.00
	SE	0.378	0.304	0.137	0.152	0.089	0.098	
Total	Mean	2.000	1.699	0.575	0.642	0.380	-0.628	81.25
	SE	0.242	0.211	0.091	0.106	0.060	0.085	6.25

Note: Na: Number of alleles, Ne: Number of effective alleles, I: Shannon's information Index, He: Observed heterozygosity, GD: Gene Diversity or expected heterozygosity, F: Fixation index, SE: Standard Error, Pp: Percentage polymorphism

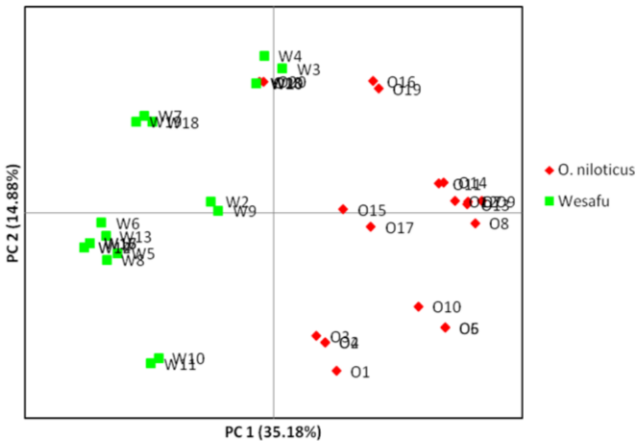

Figure 2. Principal Coordinates Analysis (PCoA) between *O. niloticus* and ecotype, Wesafu

Discussion

Information on genetic diversity is of great significance for formulating conservation strategies for the management of genetic resources in aquaculture particularly in fish breeding and artificial propagation (Hosseinnia et al 2014). Adequate genetic information from wild populations is therefore considered necessary before any aquaculture activity. In this study, eight microsatellite markers were utilized to assess the genetic diversity of two cichlid species (farmed *O. niloticus* and Wesafu from the wild). Our results revealed that the genetic diversity of cultured populations was lower than that of wild populations suggesting that aquaculture could generally reduce the genetic diversity of many cultured populations. Overall, Wesafu from the wild demonstrated higher genetic diversity than farmed *O. niloticus* as evidenced by higher values of effective allele, Shannon information index, observed and expected heterozygosity. Low heterozygosity detected in the farmed populations might be due to selection of parents' stock for breeding exercise, poor breeding management, reduction in population size and inbreeding as a result of consistent farming (Sekino et al. 2004). This indicates that artificial breeding inhibited the genetic characteristics to affect the genetic diversity and population structure of the farmed species. Similarly, Nyinondi et al. (2020) observed that the farmed populations of tilapia species ranked among the lowest in terms of heterozygosity values in their study. Hence, proper management and conservation are required to minimize inbreeding which might lead to inbreeding depressions and consequently a reduction in growth rate. In both wild and farmed populations, the observed heterozygosity was higher than the expected heterozygosity. As a consequence, the inbreeding coefficient was negative. High polymorphism was revealed among both species. The genetic polymorphism detected between *O. niloticus* and Wesafu corroborates the findings of El-Kader et al. (2013) who reported high polymorphism among three Tilapia species (*Tilapia zillii* Gervais, 1848, *Sarotherodon galilaeus* Linnaeus, 1758 and *O. niloticus)* including *O. niloticus* that recorded low percentage of polymorphism than *T. zilli* and *S. galilaeus.* However, *O. niloticus* was observed to be more polymorphic than Wesafu in the present study which is contrary to the report of El-Kader et al. (2013). The higher gene diversity detected in Wesafu indicated that this species has a higher proportion of heterozygous genotypes than *O. niloticus* populations which is important for the long-term survival of a species. The level of genetic diversity within the wild populations is also considered as a cause for an increase in adaptability to environmental variation. This finding is consistent with the results of Mahboob et al. (2019) where the genetic diversity within the Madhepura populations was higher than the Pama populations. Considering the differentiation within the wild and between the farmed populations, it is suggested that only wild broodstock should be used for fish restocking in breeding program for sustainable aquaculture production. It is worth mentioning that the genetic diversity information gathered from either farmed or wild populations can assist in suitable management and also increase the efficiency of conservation strategies.

The inbreeding coefficient that was observed negatively expressed excess in heterozygotes which confirms that microsatellite markers are naturally co-dominant markers since they could identify heterozygotes in both species. This result did not corroborate Hassanien and Al-Rashada (2021) who obtained a positive value of the inbreeding coefficient at almost all the loci across grouper species. The fixation index is commonly used to express the degree of genetic differentiation between populations. Allendorf et al. (2013) stated clear rules for defining obvious genetic differentiation among populations (FST <0.05, low; 0.05<FST<0.15, medium; FST>0.15, high). The low fixation index observed in the present study indicated that genetic differentiation was low between the two cichlid species studied. The Fixation index (F) values were found to be negative at all loci except one locus in both species' populations reflecting the considerable presence of heterozygotes in both species. This suggests insignificant chances of inbreeding particularly in Wesafu from the wild. Our finding is consistent with the results of Tewari et al. (2013) who also reported a negative fixation index in the genetic diversity analysis of *Labeo gonius* Hamilton, 1822.

The level of polymorphism detected by the PIC value (mean = 0.39) is moderate which is contrary to the result of Wang et al. (2021) who found a high polymorphic value (PIC>0.5) in *Lateolabrax maculatus* McClelland, 1844 populations with microsatellite loci. Thus, the PIC value in

this study indicated that all these primers were moderately informative and had good merit to distinguish between genotypes. The Principal Coordinate Analysis (PCoA) results that separated *O. niloticus* and Wesafu into different clusters showed differentiation between wild and farmed populations and revealed lower similarity between them which might reflect some level of genetic variation that might be attributed to the difference in species. This is following the findings of Sokenu et al. (2020) who reported a lower similarity between *O. niloticus* and *Sarotherodon melanotheron* Rüppell, 1852 from the wild.

In conclusion, the current study revealed that Wesafu from the wild demonstrated higher genetic diversity than farmed *O. niloticus* with low genetic differentiation between them indicating that aquaculture practices affect the genetic diversity. This provides more insights into the conservation of the genetic resource and better management of these species to minimize inbreeding in aquaculture considering their high nutritional and economic values. Furthermore, an increase in the number of parents and population size should be encouraged to strengthen genetic diversity in the cultural system.

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