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Genetic Characterization of Nile Tilapia (*Oreochromis niloticus*) Strains from Senegal for Sustainable Local Aquaculture Production

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Abstract

The optimization of aquaculture production requires the selection of efficient strains. Thus, genetic improvement has become one of the important levers to boost the development of aquaculture. The objective of this study was to carry out genetic characterization of Nile tilapia *Oreochromis niloticus*, one of the most cultivated fish species in Senegal, in order to select an efficient strain to optimize local fish production. Thus, fish from five different populations (Richard-Toll, ANA, ITACA, Mbodiene and Sauvage) were analyzed, with 15 individuals per population. Genetic diversity and population structure were assessed using molecular genetic analyses by sequence characterized amplified region (SCAR) and microsatellite (SSR) markers. The analyses of the SCARII marker were conducted on four populations (ANA, ITACA, Sauvage and Mbodiene) while microsatellite analyses were conducted on all five populations. The results show high levels of polymorphism of SSR markers, and a high level of observed heterozygosity (Ho), indicating a high within-population genetic variability. These results are in agreement with the AMOVA results, which indicated a high within-population genetic variability (94%). The genetic structure analysis by DAPC indicates that the five populations analyzed are structured into four groups, which are highly heterogeneous because they share common allele individuals. The analysis of the genetic structure by AMOVA showed a low degree of differentiation between the populations (6%), in agreement with the genetic differentiation index (Fst = 0.059). The heterogeneity of studied populations implies a genetic flow over time, which may have existed between the original populations. The overall negative Ta-

jima D values and low genetic differentiation indicate an excess of rare mutations in the populations studied, resulting from a recent population expansion from a limited number of initial breeders isolated in locale hatcheries. Thus, further studies with a much larger panel of markers are required to better differentiate the strains and identify the most efficient ones for sustainable local aquaculture production.

Keywords

Oreochromis niloticus, Polymorphism, Heterozygosity, Genetic Diversity, Genetic Structure

1. Introduction

Capture fisheries and aquaculture play important roles in the global economy through their contribution to food security, job creation, and increased incomes for people [1] [2] [3] [4] [5]. Indeed, capture fisheries reached a production of nearly 171 million tons in 2016, of which 88% was for human consumption while aquaculture produced about 80 million tons. Capture fisheries production has been relatively stable since the late 1980s, while the demand for fish for human consumption is largely met by aquaculture. Today, half of the fish consumed in the world is supplied by aquaculture [2] [6] [7] [8]. Aquaculture production is mainly dominated in the world by carp, followed by tilapia with a total production of 4.2 million tons in 2016, or 8% of the world production [2] [9] [10]. In Senegal, fish production for human consumption increased from 193.3 tons in 2011 to 468 tons in 2017 and most of this production is Nile tilapia [11].

The success of Nile tilapias in aquaculture is mainly due to some of their biological and ecological characteristics such as plasticity, opportunistic diet, tolerance to environmental factors, sexual dimorphism of growth and genetic characteristics, which are very important assets in fish farming [12]. While food and water quality are important factors for fish production of *O. niloticus*, its plasticity and opportunistic diet allow for appropriate food supply at all levels of rearing intensification [13]. Sexual dimorphism in growth (males grow faster than females) and ease of reproduction are currently the main problems in op energy available to the fish is invested in behavioral and physiological interactions between the sexes and in egg production at the expense of growth [12]. Furthermore, this unwanted reproduction leads to overpopulation, competition for resources, and decreased growth performance [12].

To overcome this problem, studies on monosex reproduction of males have been and continue to be conducted through research projects on sexual inversion especially with the hormone 17-α methyl testosterone. However, studies on genetic aspects (availability of genetically more productive stocks) of Nile tilapia *O. niloticus* native to Senegal have not yet been developed, hence the interest of the present study. The main objective of this study was therefore to carry out a

genetic characterization of different populations of Nile tilapia from Senegal in order to determine their diversity and genetic structure with the aim of improving their zootechnical performance to promote their breeding and farming in the whole country. The genetic characterization of these populations could allow to identify the most efficient strain for local fish farming but also to evaluate the impact of their maintenance in captivity on their genetic diversity. Indeed, genetic characterization of strains and species of tilapia has been widely used for the identification of economically desired traits for aquaculture production [14] [15] [16] [17] [18]. Most of studies on genetic characterization on Nile tilapia used the variations of various genetic markers [16] [19] [20] including microsatellites and SCARs [16] [21] [22] [23] [24] [25] to assess and compare genetic diversity and differentiation of farmed populations. In this study, five SSR and one SCAR (SCARII) markers were used to assess the genetic diversity and structure of five populations of from different localities of Senegal.

2. Materials and Methods

2.1. Sample Collection

This study was conducted on individuals of Nile tilapia *O. niloticus* from five different localities in Senegal. The terms "strain" or "population" are used in this study to designate these localities but there is no evidence that they are genetically different. Among these strains, four (Mbodiene, ANA, Sauvage, ITACA) originate from the experimental hatchery of Institute of Research for Development (IRD) in Hann Bel Air in Dakar, Senegal. The fifth strain (Richard-Toll) was collected in the fish farm of the University Gaston Berger (UGB). The Mbodiene strain is from broodstock of natural population maintained in captivity for several generations in the Mbodiene private fish farm, located in the department of Mbour in the region of Thiès. The ANA strain is also from initial broodstock of a natural parental population maintained for several generations in the ANA fish farm of Richard-Toll (Dagana Department, Saint-Louis Region). The individuals of this population analyzed in this study were maintained in captivity in the IRD experimental hatchery for one and a half years. The individuals of the Richard-Toll strain also come from the ANA fish farm but were collected at the UGB fish farm where they were maintained in captivity for five months prior to the experiment. For the ITACA population, the initial broodstock were collected at Technopole, Dakar. This population was then maintained in captivity for several generations in the IRD fish farm. As for the Sauvage strain, it is from the Senegal River, Saint-Louis. It was then maintained in captivity in the IRD Bel-Air fish farm for experimental studies on sex reversal and the production of male mono sex population.

Fish from these five populations (Richard-Toll, ANA, ITACA, Mbodiene and Sauvage), were sampled. Four populations (ANA, ITACA, Sauvage, and Mbodiene) were analyzed for the SCARII marker whereas all five populations were studied with the SSR markers.

2.2. DNA Extraction and Quality Control

DNA from each sampled individual was extracted using the commercial "Quik DNA Miniprep Kit". Thus, a 20 mg piece of tissue was taken from each pelvic fin sample. It was then cut into small pieces and placed in a mini mortar with quartz and crushed until a homogeneous powder was obtained. The powder from each sample was placed in a 2 ml eppendorf tube containing 95 µl of solid tissue buffer (tissue buffer that causes degradation of cell connections), 10 µl of proteinase K (for protein degradation) and 95 µl of pure water. This mixture was homogenized for 15 seconds with a vortex and incubated in a water bath at 55˚C for 3 hours.

After incubation, the tubes were centrifuged at 12,000 g for 1 minute to remove insoluble debris. The supernatant was then transferred to a new clean tube and measured. The volume of each tube was then doubled by adding genomic binding buffer and homogenized for 15 seconds using a vortex. After thorough mixing, the content of each tube was poured into a zymo spin column placed on a collection tube and centrifuged at 12,000 g for one minute. The collection tubes were discarded with their content. Each spin column was taken up and placed on a new collection tube. A total volume of 400 µl of DNA prewash buffer was added to each column and centrifuged at 12,000 g for 1 minute. A 700 Ml volume of G-DNA wash buffer was then added to each spin column, and the tubes were centrifuged at 12,000 g for 1 minute and the collection tube and its contents discarded.

A volume of 200 µL of G-DNA wash buffer was added to the spin column, which was replaced on the same collection tube and centrifuged at 12,000 g for one minute. After centrifugation, the column was transferred to a 1.5 ml eppendorf tube and dried to remove any traces of wash buffer. After drying, 32 µl of DNA elution buffer was added directly to the matrix and incubated for 5 minutes at room temperature. The tubes were centrifuged at 12,000 g for one minute to elute the DNA. The resulting DNA solution was then stored in the refrigerator at +4˚C. Quality control and quantification of the extracted DNA were performed by electrophoretic migration through a 1% agarose gel to ensure its integrity (quality) and to estimate its concentration. The estimation was done by comparing the band of each sample with the bands of known concentrations of the control marker or the Smart Ladder.

2.3. Selection of Molecular Markers

A dozen genetic markers were initially selected for the genetic characterization analyses. These markers were selected based on data from the literature of studies that have been conducted on tilapia and other fish species [12] [15] [26] [27] [28] [29]. Preliminary analyses were then performed to ensure their quality and suitability for use in Nile Tilapia *O. niloticus*. As a result of these tests, six markers including one SCARII (sequence characterized amplified regions) marker and five microsatellite markers were selected for this study. The microsatellite markers were analyzed for five populations (Mbodiene, ANA, Sauvage, ITACA

and Richard-Toll) while for the SCARII marker analyses were conducted only on four of the five populations (Mbodiene, ANA, Sauvage and ITACA). The Richard-Toll population was not analyzed for this marker.

The SCARII marker is one of the most stable markers, usually derived from RFLP, RAPD, AFLP markers [15] [20] [30]. The basic principle of this genetic marker is to design specific primers of about 20 bp in length from the acquired sequence information and then reveal a polymorphism by ordinary PCR. Because of its speed, simplicity of application, and low cost, the SCARII marker is particularly suitable for analysis of large numbers of samples Li *et al.*, [22]. However, it requires minimal information about the composition of the sequence to be amplified and enormous effort and additional expense in defining locus-specific primers.

2.4. Amplification of SCARII and SSR Markers

For PCR amplification of the SCARII marker, the conventional amplification protocol that requires a pair of primers (one forward and one reverse primer) was used (**Table 1**). PCR amplification was performed in one well of a 96-well PCR plate with a total reaction volume of 25 µl containing 1 µl of template DNA, 1 µl for each primer at 10 µM (forward and reverse primer), 12.5 µl of 2× Master Mix (MGCL2 buffer mix, Taq, DNTPs), 9.5 µl of ultrapure molecular free water nuclease.

Primer testing and selection of SSR markers (**Table 1**) were performed by acrylamide gel electrophoretic migration after PCR. This PCR amplification assay selects primers that correctly amplify the correct DNA fragment and eliminates those that did not work. Thus, out of seven pairs of SSR primers tested, only one (G12288) was eliminated as monomorphic.

Table 1. Primers of SSR and SCARII markers analyzed. For the primer name, the codes between parentheses correspond to the name in certain scientific articles.

Primer name	Forward (F) and (R) sequences	AT ˚C	%GC	Length (bp)	Accession number
SCARII	F: TGGATGGATGGATTGATGGA R: AGCCAGCGAACCAAGATCTAT	60	45	20	XR269836.4
PRLI-MS04	F: GTTAGCCCCCTCCTCACTCT R: ACCTTGCTCGTCACACCTG	60	60	20	X92380
UNH104 (G12257)	F: GCAGTTATTGTGGTCACTA R:GGTATATGTCTAACTGAAATCC	55	40	20	G12257
G12373 (UNH222)	F: CTCTAGCACACGTGCAT R: TAACAGGTGGGAACTCA	53	52.94	17	G12373
G12330 (UNH136)	F: GTCACACCTCCATCATC R: AGTTGTTTGGTCGTGTAAG	53	52.94	17	G12330
G12362 (UNH211)	F: GGGAGGTGCTAGTCATA R: CAAGGAAAACAATGGTGATA	53	52.94	17	G12362

AT: annealing temperature; bp; base pair; GC: guanine-cytosine.

Amplification of microsatellite markers or SSRs was performed using a conventional PCR protocol but all direct primers were labeled with a fluorescent dye (M13-Primer) for detection of the amplified fragment. The PCR amplification reaction consisted of a 5 ng/l DNA template and a master mix composed of TP 10 × (85 µl), DNTP 2000 (85 µl), $MgCl_2$ 50 mm (34 µl), AMF-M13 10 µm (7 µl), AMR 10 µm (7 µl), labeled M13 (Dye 700 or Dye 800): 10 µm (7 µl), Taq 2 U/µL (8 µl), and H_2O (191 µl). Amplification was performed in a thermal cycler according to the following program: initial denaturation at 94˚C for 5 minutes, denaturation cycles at 94˚C for 45 s, hybridization at 54˚C - 58˚C for 1 min, elongation at 72˚C for 1 min, final extension at 72˚C for 5 min, hold at 4˚C.

2.5. Sequencing of SCARII Marker and Genotyping of SSR Markers

Sequencing of the DNA fragment of each individual amplified by the SCARII primers was performed by the South African genomics company "Inqababiotec". The first step of the SSR genotyping consists in depositing the DNA on an acrylamide gel to produce an electrophoresis on the Li-Cor system (sequencer). Preparation of the acrylamide gel consisted of pouring 20 ml of cold acrylamide (LR) into a small beaker and adding 175 µl of 10% APS (ammonium persulfate) and 25 µl of temed (tetra-methyl-ethylene-diamine). After gel polymerization (approximately 1.5 hours), 2 µl of PCR product from each sample was plated onto a 96-well plate into which 8 µL of urea blue is added. PCR-derived microsatellite amplicons migrated through the gel mesh using an ultrahigh-voltage electric field (1500 V). The fragments marked during amplification emit fluorescence when excited by laser diodes at 2 different wavelengths (680, 780 nm). An IR camera detects these signals and the system retranscribes a migration profile on the computer interface connected to Li-Cor. At the end of the migration, the images are recorded and processed with the XNView software.

2.6. Genetic Analyzes

Data obtained from SCARII sequencing and SSR genotyping were cleaned to correct any errors. They were then used to assess genetic variability (within- and between-population genetic diversity) and the genetic structure of populations.

Genetic diversity was assessed using GENALEX 6.501 [30] by analyzing the following parameters: polymorphism rate, allele frequency at each locus, number of alleles per locus (NA), average number of alleles or allelic richness, number of effective alleles (NE), observed heterozygosity (Ho) and expected heterozygosity (He). The Fis index (differentiation of individuals within populations) or inbreeding coefficient was used to assess happlotypic diversity and to assess the degrees of inbreeding within each population using DNASP 6. The typical Haplex Network was generated by the Haplotype Network.

Total genetic diversity (HT), within-population genetic diversity, between-population genetic diversity (Dst), and genetic differentiation coefficient (Gst) were calculated from the allelic frequencies of microsatellite loci for the five pop-

ulations. Genetic differentiation among populations was assessed using AMOVA, Fst, (the correlation between alleles within a population versus all subpopulations), differentiation index (differentiation of individuals from the total), and discriminant analysis of principal component (DAPC).

The DAPC was used to illustrate the genetic structuring of populations. In the DAPC approach, data are first analyzed using principal component analysis (PCA) and then clusters are identified using discriminant analysis (DA). The performance of this method was evaluated using simulated data, which was also analyzed using the structure as a reference.

Nei's genetic distance was used on the SCARII marker to assess intra- and inter-population genetic structure [31]. Tajima's neutral test was used to illustrate the allelic frequency distribution of nucleotide sequence data [32]. Tajima's D was used to test the null hypothesis of mutation-drift equilibrium and constant population size by considering the difference of nucleotide sequence pair (π) and the number (s) of segregating sites.

3. Results

3.1. Polymorphism and Diversity of SCARII and SSR Markers

After extraction, all individuals showed good profiles (**Figure 1(A)**, **Figure 1(B)**), indicating that the quality and quantity of DNA extracted from these individuals was sufficient for all PCR amplifications that were to be performed in this study. The PCR amplification of the SCARII marker (**Figure 1(C)**) and SSR (**Figure 1(D)**) also showed that all individuals were amplified and that the amount of amplified DNA as well as its quality was good enough to perform all future analyses.

3.2. Polymorphism and Diversity of SCARII and SSR Markers

A total of 59 haplotypes were determined, with 15 phaplotypes per population (**Table 2**). All haplotypes are different from each other with happlotypic diversity

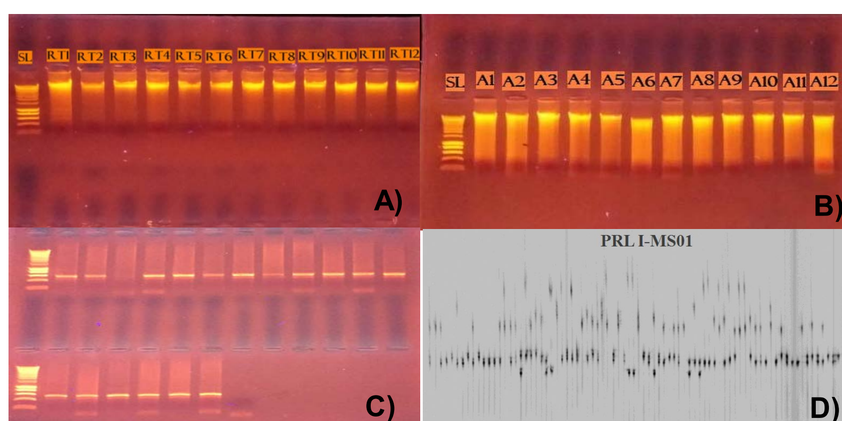

Figure 1. DNA concentration estimation ((A) Richard-Toll; (B) ANA) using control marker concentration values (smart ladder) and PCR amplified DNA profiles using the SCARII marker (C) and the PRLI-MS01 SSR marker (D).

Table 2. Nucleotide and haplotype diversities of four populations analyzed with the SCARII marker.

POPULTIONS	H	Hd	S	Pi	K
ANA	15	1	188	0.10224	42.8381
ITACA	15	1	99	0.07158	29.99048
Mbodiene	15	1	178	0.10544	44.18095
Sauvage	14	1	111	0.08075	33.83516
TOTAL ESTIMATE	**59**	**1**	**233**	**0.08907**	**37.32145**

H: number of haplotypes; Hd: haplotypic diversity; S: polymorphic sites; Pi: nucleotide diversity; K: nucleotide differences.

(HD) equal to 1 for each population. The highest number of polymorphic sites (S) was observed for ANA, followed by Mbodiene (188 and 178 polymorphic sites, respectively) and the lowest were noted for the ITACA and Sauvage populations (99 and 111 polymorphic sites, respectively) (Table 2). As for nucleotide diversity (Pi), it ranged from 0.07158 for the ITACA population to 0.10544 for the Mbodiene population. Thus, it was higher for ANA and Mbodiene populations compared to ITACA and Sauvage. The nucleotide difference (K) was higher ANA and Mbodiene populations (42.8381 and 44.18095, respectively) and lower for of ITACA (29.99048) and Sauvage (33.83516) (Table 2).

For the five microsatellites or SSR markers used, a total of 52 different alleles were identified in the five populations. All markers showed a high rate of polymorphism (100%). The average number of alleles per locus (Na) varied from 5.000 to 7.600 with an average value of 6.920 (Table 3, Figure 2). The most polymorphic marker was G12362 with 14 alleles and the least polymorphic was G12330 with six alleles. The most diverse populations were ANA and Mbodiene (Na = 7.600), followed by ITACA and Sauvage (Na = 7.200) and the least genetically diverse population was Richard-Toll (Na = 5.000). The number of effective alleles (Ne) varied from 3.405 to 5.404 (Table 3, Figure 2). The number of effective alleles (Ne) varied from 3.405 (Richard-Toll) to 5.404 (IACA) (Table 3, Figure 2). The Ne was higher for ITACA (5.404), followed by ANA (4.393) and Mbodiene (4.222) and lower for Richard-Toll (3.405). The number of unique allele to a single population (*No. private allele*) was higher for the ANA (4 alleles) compared to Richard-Toll, ITACA and Sauvage (Figure 2). The *No* was null for the Mbodiene population. The number of different alleles with a frequency ≥ 5% (*Na Freq.* ≥ 5%) was not significantly different between populations (Figure 2). The *No*. LComm alleles (≤50%), number of locally common allele (Freq. ≥ 5%) found in 50% or fewer populations was higher Mbodiene, Sauvage and ITACA and lower for ANA and Richard-Toll (Figure 2). As for the number of alleles with a frequency ≥ 5% (Na Fq), it was higher for ITACA, ANA and Mbodiene and lower for Sauvage and Richard-Toll (Figure 2). The Shannon-Weaver index (I) varied between 1.341 and 1.743 (Figure 2). It was slightly higher for ANA, ITACA and Mbodiene and lower for Richard-Toll and Sauvage.

Table 3. Average number of alleles (allelic richness) per locus and per population (NA), and the number of effective alleles (Ne).

locus		Richard-Toll	ANA	ITACA	Mbodiene	Sauvage
PRLI-MS01	NA	4	9	7	9	5
	Ne	3.103	6.429	5.422	5.556	3.041
UNH104	NA	4	7	6	9	9
	Ne	2.217	3.383	3.982	4.215	2.601
G12373	NA	6	7	8	6	7
	Ne	3.409	3.689	5.844	3.814	5.294
G12330	NA	4	4	4	4	5
	Ne	3.000	2.036	3.061	1.899	3.041
G12362	NA	7	11	11	10	10
	Ne	5.294	6.429	8.711	5.625	6.426
Average NA		**5.000**	**7.600**	**7.200**	**7.600**	**7.200**
Average Ne		**3.405**	**4.393**	**5.404**	**4.222**	**4.081**

Na: average number of alleles; Ne: effective number of alleles of PRLI-MS01, UNH104, G12373, G12330, G12362 markers.

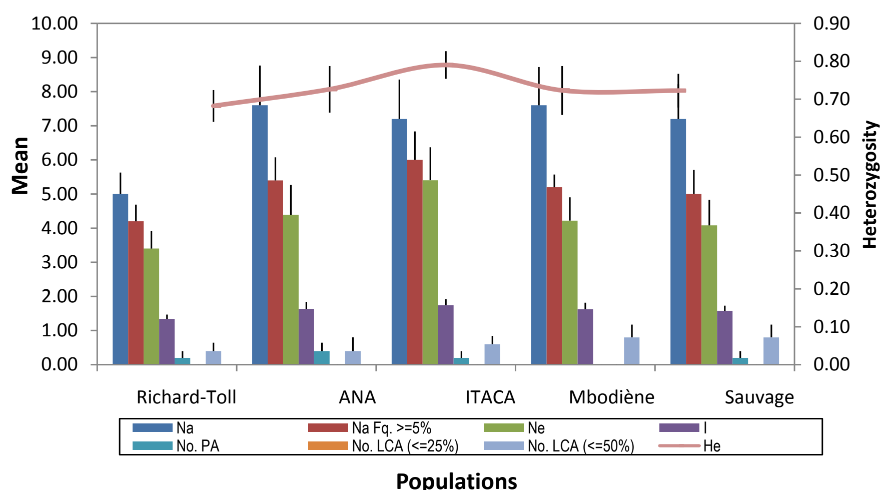

Figure 2. Genetic diversity parameters and heterozygosity of captive stocks and natural population of Nile tilapia *Oreochromis niloticus* from Senegal.

The values of expected heterozygosity (He) and observed heterozygosity (Ho) are presented in Table 4. The Ho values ranged from 0.267 for UNH104 (ITACA) to 1.000 for PRLI-MS01 (ANA) and G12373 (all populations). As for He values, they ranged from 0.473 for G12330 (Mbodiene) to 0.885 for G12362 (ITACA). The mean Ho values range from 0.68 to 0.81 for Richard-Toll and ANA, respectively. The mean values of He range from 0.71 for Richard-Toll population to 0.82 for ITACA. The mean He was significantly higher for ITACA compared to the other populations (Table 4, Figure 2).

Table 4. Observed heterozygosity rate (Ho) and expected heterozygosity (He) by locus and population.

	Richard-Toll	ANA	ITACA	Mbodiene	Sauvage
PRLI-MS01					
N	15	15	15	15	15
Ho	0.333	1.000	0.867	0.933	0.533
He	0.678	0.844	0.816	0.820	0.671
UNH104					
N	15	15	15	14	15
Ho	0.667	0.800	0.267	0.786	0.533
He	0.549	0.704	0.749	0.763	0.616
G12373					
N	15	15	15	15	15
Ho	1.000	1.000	1.000	1.000	1.000
He	0.707	0.729	0.829	0.738	0.811
G12330					
N	15	15	15	15	15
Ho	0.600	0.400	0.733	0.467	0.667
He	0.667	0.509	0.673	0.473	0.671
G12362					
N	15	15	14	15	14
Ho	0.800	0.867	0.714	0.667	0.857
He	0.811	0.844	0.885	0.822	0.844
Average					
N	15	15	15	15	15
Ho	0.68	0.81	0.71	0.77	0.72
He	0.71	0.75	0.82	0.75	0.75

N: number; Ho: observed heterozygosity; He: expected heterozygosity.

3.3. Allelic Frequencies

The allelic composition (10 alleles in total) and the distribution of their frequencies for the PRLI-MS01 locus showed that the ANA and Mbodiene populations have the highest number of alleles (9 alleles in total) (**Figure 3**). They are followed by ITACA, Sauvage and Richard-Toll (7, 5 and 4, respectively). Alleles 2, 3 and 4 have the highest frequencies (20% to 40%) in these populations. Allele 8 is rarer and was only found in the ANA population, followed by alleles 10 and 11 which are present in two populations (ANA/Mbodiene and ITACA/Mbodiene, respectively), but with low frequencies (<10%) (**Figure 3**).

For the UNH104 marker, 12 alleles were identified in total, two of which (alleles 3 and 4) are present in all populations (**Figure 4**). The most polymorphic

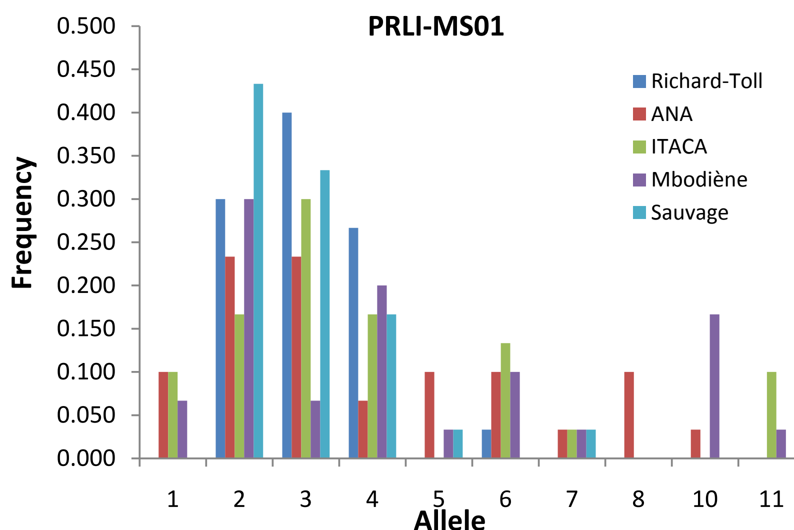

Figure 3. Comparison of allele frequencies of PRLI-MS01 locus between *O. niloticus* populations from Senegal.

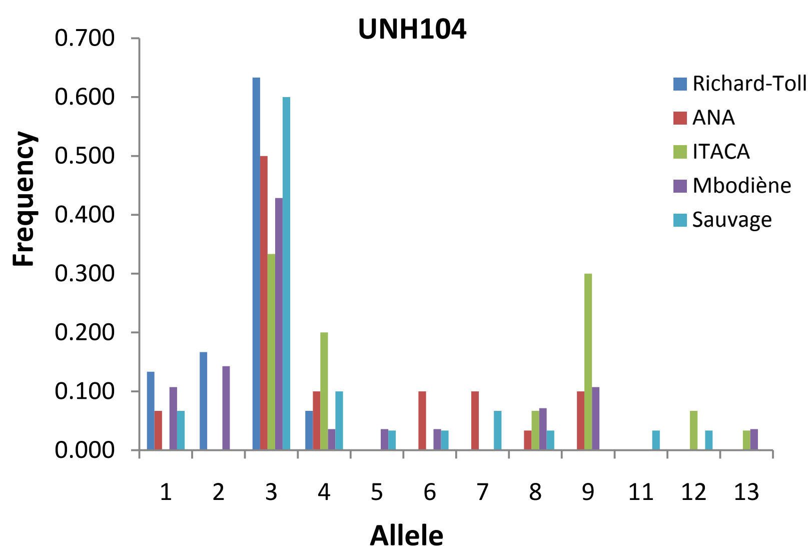

Figure 4. Comparison of allele frequencies of UNH104 locus between *O. niloticus* populations from Senegal.

populations are Sauvage and Mbodiène (9 alleles each), followed by ANA (7 alleles) and ITACA (6 alleles). The least diverse population is the Richard-Toll population with three alleles in total. Allele 11 is present only in the Sauvage population while alleles 2, 5, 7, 12 and 13 are all found in two of the five populations analyzed (Figure 4). Alleles 1 and 8 are present in four populations while alleles 6 and 9 are found in three of the five populations (Figure 4). In terms of frequency, allele 3 has the highest frequency in all populations (40% - 60%), in contrast to the other alleles that are present with a frequency < 20%.

A total of 10 alleles were identified for locus G12373 across the five populations studied (Figure 5). The most polymorphic population for this locus is ITACA (8 alleles), followed by ANA and Sauvage (7 alleles each) and finally

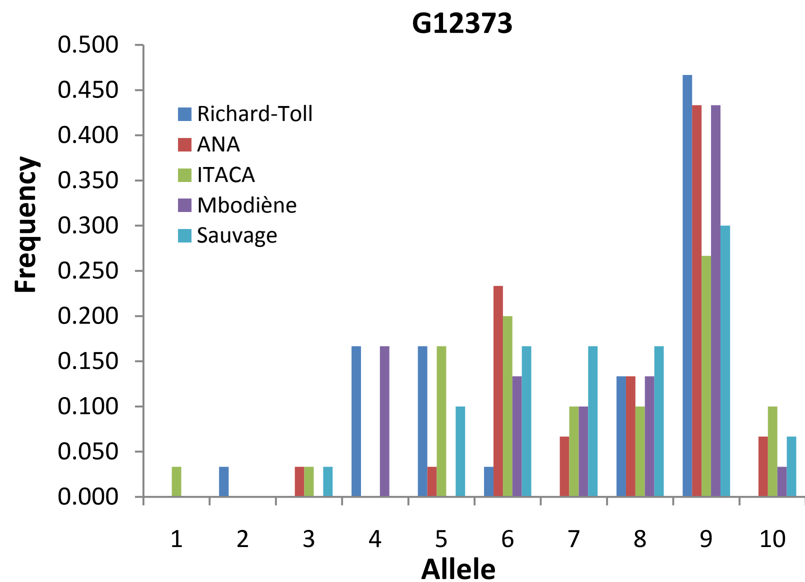

Figure 5. Comparison of allele frequencies of G12373 between five populations of *O. niloticus* from Senegal.

Mbodiene and Richard-Toll (6 and 5 alleles, respectively). Alleles 6, 8 and 9 are present in all populations but allele 9 has the highest frequency. In contrast, alleles 1 and 2 are rarer because they are present in only one population (ITACA and Richard-Toll, respectively). Allele 4 is found in only two populations (Richard-Toll and Mbodiene) while allele 3 is present in three of the five populations (ANA, ITACA and Sauvage). The remaining alleles (5, 7 and 10) are found in four of the five populations, but with low frequency (**Figure 5**).

Locus G12330 has six alleles, two of which (alleles 1 and 2) are found in all populations, followed by allele 5 which is present in four of the five populations (**Figure 6**). Alleles 4 and 6 are rarer as they are found in only two of the five populations. Allele 3 is present in three populations (Richard-Toll, Mbodiène and Sauvage). The frequency of allele 1 was high in all populations (40% - 60%) while that of the other alleles 2, 3, 4, 5 and 6 was low (**Figure 6**). The most diverse population for this locus is Sauvage with a total of five alleles, followed by the Richard-Toll, ANA, ITACA and Mbodiene populations, which have four alleles each (**Figure 6**).

Locus G12362 has 14 alleles of which 2 (10 and 12) are present in all populations with relatively high frequencies (**Figure 7**). For this locus, the ANA and ITACA populations are more diverse (11 alleles), followed by Mbodiene and Sauvage (10 alleles). The least diverse population is Richard-Toll with 7 alleles (**Figure 7**). Allele 14 is rarer because it is present only in the ANA population. It is followed by alleles 2 and 5 found in the ITACA and Sauvage populations with the lowest frequencies. Alleles 3, 4, 9, 10 and 11 are present with high frequencies (>20) in the ANA, Richard-Toll, Sauvage and Mbodiene populations, respectively, while the other alleles are present with relatively low frequency (<20) in all populations.

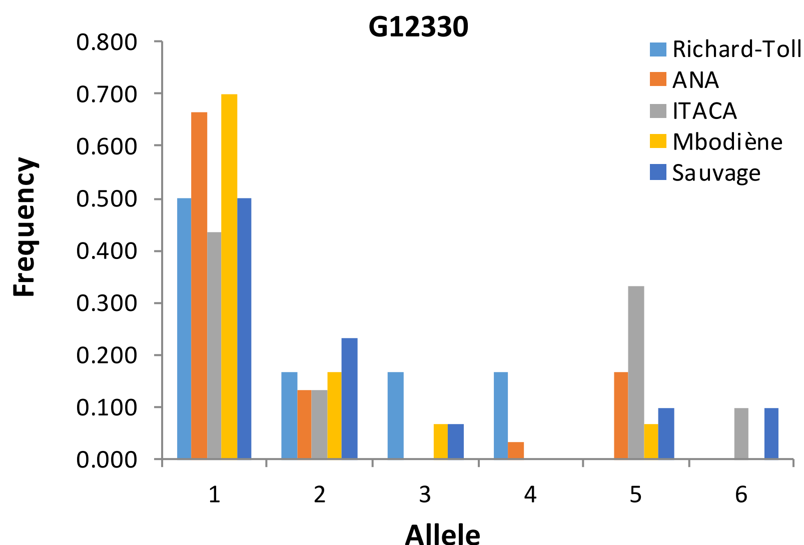

Figure 6. Comparison of allele frequencies of G12330 locus between five populations of *O. niloticus* from Senegal.

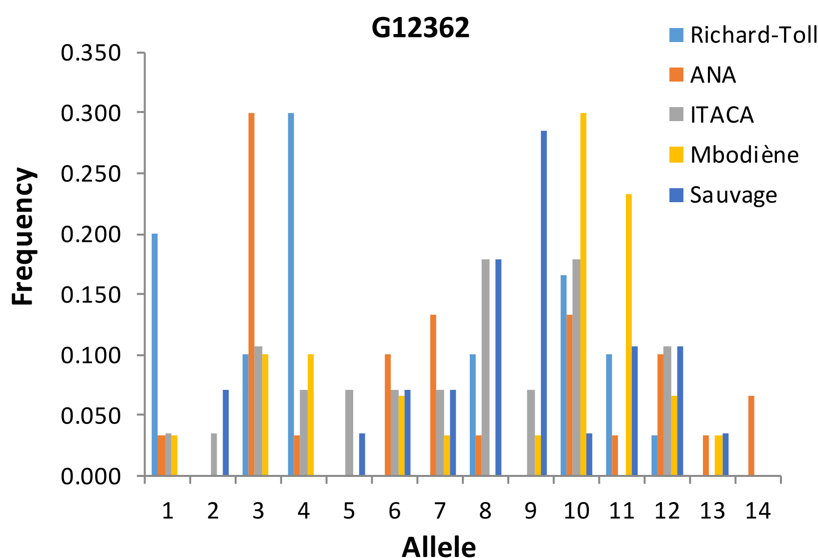

Figure 7. Comparison of allele frequencies of G12362 locus between five populations of *O. niloticus* from Senegal.

3.4. Within and among Population Diversities

Total genetic diversity (HT), within-population genetic diversity (HS), between-population genetic diversity (Dst), and coefficient of genetic differentiation (Gst) are shown in Table 5. The HT varied from 0.638 to 0.911 with an average of 0.779 whereas the HS ranged from 0.621 to 0.875 with an average of 0.759. The Dst and Gst are low as evidenced by their values that ranged from 0.012 to 0.036 (average: 0.020) and from 0.019 to 0.049 (average: 0.032), respectively (Table 5). The contribution of within-population genetic diversity (average HS = 0.759) to total genetic diversity (average HT = 0.779) is greater than the contribution of genetic diversity between populations (Dst = 0.020).

Table 5. Nei diversity indices estimated in five populations of *O. niloticus* for the five microsatellites.

SSR Loci	HT	HS	Dst	Gst
PRLI-MS01	0.836	0.809	0.017	0.026
UNH104	0.737	0.711	0.017	0.029
G12373	0.794	0.781	0.012	0.019
G12330	0.638	0.621	0.017	0.034
G12362	0.911	0.875	0.036	0.049
Average	**0.779**	**0.759**	**0.020**	**0.032**

HT: total genetic diversity; HS: intra population genetic diversity; Dst: inter population genetic diversity; Gst: gene differentiation coefficient.

The AMOVA based on the five populations showed that the vast majority of genetic diversity (94%) occurs within populations (**Table 6**), while diversity between populations accounted for only 6% of total genetic variation. Multiple comparisons of Wright's F statistics from all microsatellite data showed that the index of genetic differentiation Fst was 0.059 (P value = 0.0001) whereas the Fis and Fit were 0.026 and 0.051, respectively.

Although genetic differentiation between populations was significant, genetic variation within populations was maintained. The SSR results confirmed that the observed genotypic frequencies in *O. niloticus* are consistent with the expected frequencies, indicating that there was no significant deviation from the Hardy-Weinberg hypothesis. They also showed that panmictic interbreeding within populations is relatively high. The estimated migration rate between populations was 4.74, suggesting low gene flow between populations.

3.5. Genetic Structure

Genetic distance was higher for the ITACA/Richard-Toll pairwise populations (Nei genetic distance = 0.312) which are more genetically distant compared to the other populations (**Table 7**). The lowest genetic distance was noted for the Mbodiene/ANA pairwise populations (Nei genetic distance = 0.126). Comparison of genetic distances also indicated that the ITACA/ANA, Sauvage/ANA pairwise populations have equivalent genetic distances, which are slightly lower than that of Sauvage/ITACA, Mbodiene/Sauvage and Mbodiene/ITACA.

The haplotype network (**Figure 8**) showed that all haplotypes appear with equivalent frequency. The network did not show a haplotype representing a center of differentiation. Otherwise, there is no haplotype variant shared by two or more individuals belonging to different populations or localities. All haplotypes are unique to different populations. The Tajima neutrality test was obtained from the total number of polymorphic sites (278) and the total number of nucleotide differences K (41.44). The value of D obtained is negative equal to −1.72.

Table 6. AMOVA results for *O. niloticus* individuals based on 5 microsatellites analyzed in five populations (ANA, ITACA, Mbodiene, Richard-Toll and Sauvage).

Partitioning	df	Sum of Squares	Variance component	Percentage of variance	F-statistic
Inter population	4	25.261	0.205	**6**	**Fst = 0.059***
Intra population	70	226.799	3.239	**94**	**P-value = 0.0001**
Total	74	252.061	3.445		

Table 7. Nei genetic distance (above the diagonal) and identity of Nei per pair (below the diagonal).

Richard-Toll	**ANA**	**ITACA**	**Mbodiene**	**Sauvage**	
	0.215	0.312	0.172	0.191	**Richard-Toll**
0.807		0.167	0.126	0.176	**ANA**
0.732	0.847		0.267	0.214	**ITACA**
0.842	0.882	0.765		0.218	**Mbodiene**
0.826	0.838	0.807	0.804		**Sauvage**

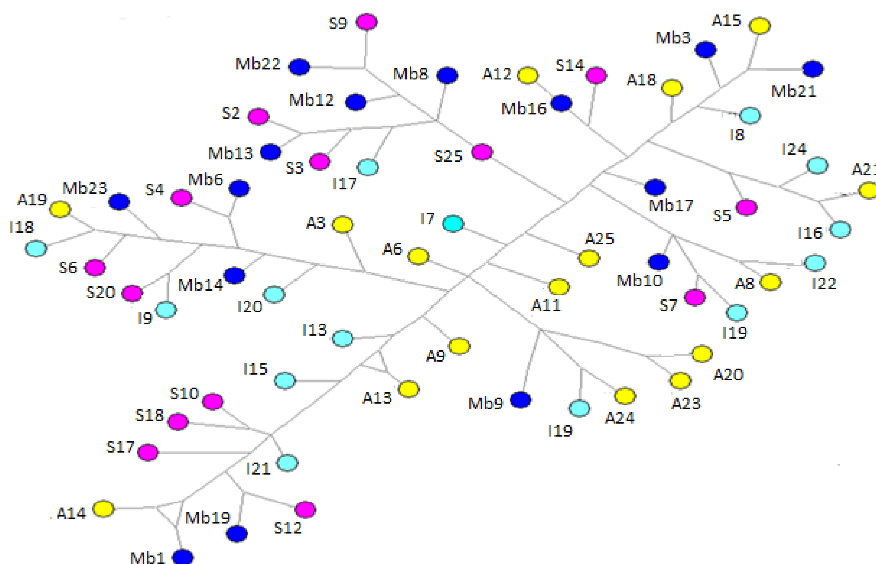

Figure 8. Haplotype network generated by the haplotype network software. ANA (A) population in yellow, ITACA (I) in green, Mbodiene (Mb) in blue and Sauvage (S) in pink. The Richard-Toll population was not analyzed for the SCARII marker.

The principal component discriminant analysis (**Figure 9**) performed on the basis of allelic positions indicates that axes 1 (43.9%) and 2 (32.36%) contribute approximately 92.04% of the total variance. The analysis reveals four population groups (**Figure 9**). Group 1 is made up of individuals from the ANA, ITACA, Mbodiene and Sauvage populations, while group 2 includes individuals from the Richard-Toll, ITACA, Mbodiene and Sauvage populations. As for group 3, it is formed by individuals from Richard-Toll, ANA, ITACA, Mbodiene and Sauvage

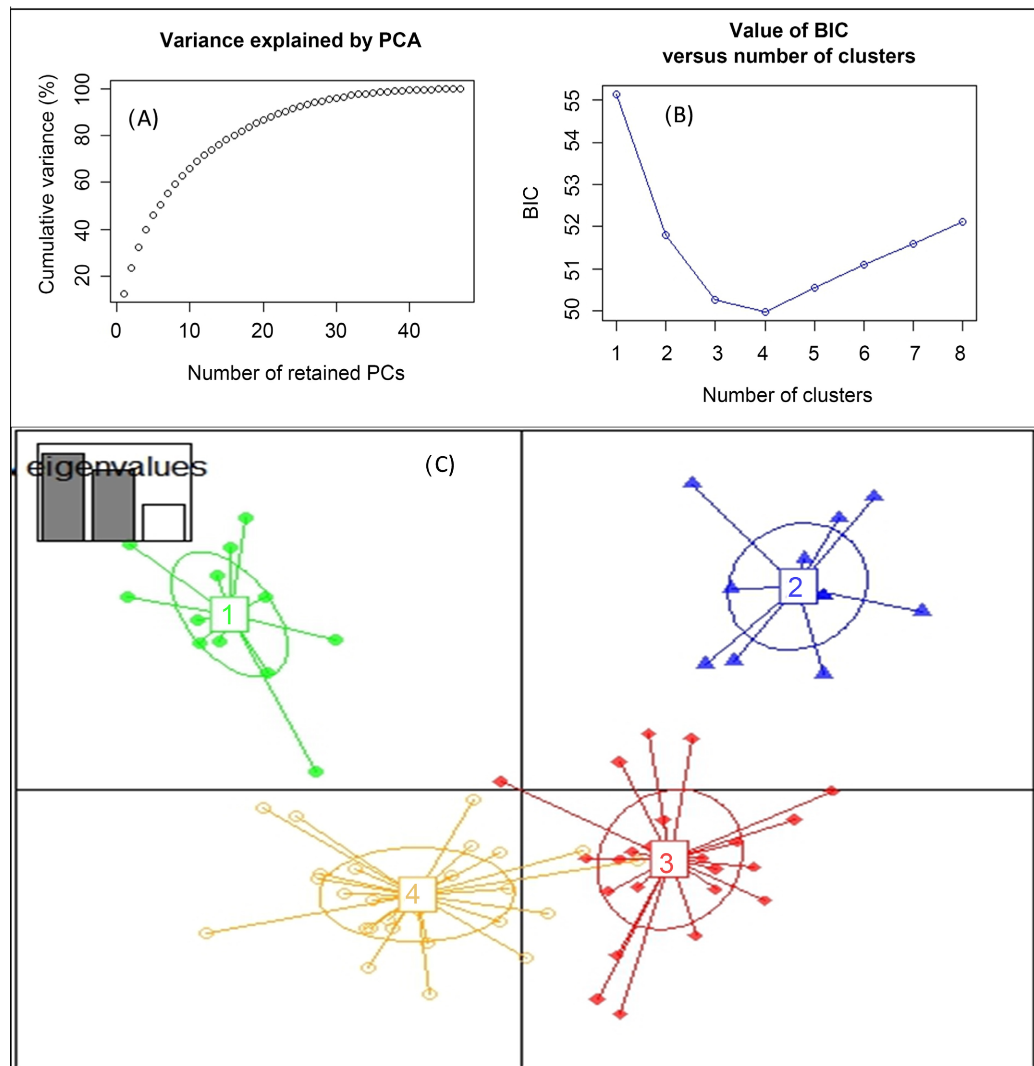

Figure 9. Discriminant analysis of principal component (DAPC) of the five study populations for the five microsatellite markers. (A) Variance explained by the PCA; (B) value of BIC versus number of clusters; (C) scatter plot. Dots represent individuals and the colors denote the genetic cluster (group).

and group 4 is made up of individuals from the Richard-Toll, ANA, ITACA, Mbodiene and Sauvage. These results show that there is heterogeneity in the populations studied.

4. Discussion

The growth performance of fish species in aquaculture may be due to its genetic potential or to the farming conditions. It is therefore necessary to perform genetic characterization of the strains when comparing aquaculture performance individuals in order to know whether the measured performance is determined by the genetic component or by the rearing conditions. Although *O. niloticus* is the most cultivated fish species in Senegal, comparison of aquaculture performance and characterization of strains locally exploited has not been carried out. The selection of a strain with better growth performance linked by the genetic

component could allow to increase the production in local farms. This study was conducted to characterize and measure the level of genetic variability of different strains of *O. niloticus* exploited in aquaculture in Senegal. The results show that the genetic variability of the cultured populations can be different due to a genetic difference of parental populations or a reduction of genetic variability in hatcheries.

4.1. Genetic Variability of SCARII and Microsatellite Markers

Molecular analysis by the SCARII marker showed a high nucleotide diversity (polymorphism) ranging from 0.0715 for the ITACA population to 0.105 for the Mbodiene population. These results are in agreement with those of Ndiwa *et al.* [29] who found nucleotide diversities ranging from 0 to 0.051 for the Senegal River strain inhabiting the Chelaba source. However, they revealed higher polymorphism at nucleotide sites compared to the results of Ndiwa *et al.* [29]. The SSR markers also showed a high genetic polymorphism with a total of 52 alleles for the five populations studied and a number of alleles per locus ranging from 6 (locus G12330) to 14 (locus G12362). This high number of alleles is sufficient to reduce the standard error committed for an estimation of genetic distances according to Takezaki and Nei [33], who suggested at least 4 alleles per locus. The levels of allele variability observed in this study are similar to those of Moreira *et al.* [28] who found a high level of polymorphism with a total of 37 alleles ranging from 8 alleles for loci UNH104 and UNH118 to 7 alleles for loci UNH108, UNH222 (G12373) and UNH231. Melo *et al.* [27] found similar results in the same species with a total of 39 alleles ranging from 6 alleles for loci UNH108 and UNH169 to 11 alleles for locus UNH160. Similarly, when studying 4 strains of Nile tilapia, Rutten *et al.* [15] obtained a lower number of alleles per locus (5 to 7.5 alleles), which can be explained by the fact that they worked only on farmed populations. On the contrast, Romana-Eguia *et al.* [16] observed a higher number of alleles (10 to 14.8 alleles per locus on average). The difference in the average number of alleles per locus between these two species with similar reproductive systems could be explained by the fact that the individuals sampled by Romana-Eguia *et al.* [16] belonged to domestic strains including genetically improved breeding strains and Nile hybrid tilapia strains. The number of alleles in this study is also lower than that observed in wild *O. mossambicus* strains (ranging from 8.937 to 15.751. These differences may be explained by the plurality of rivers sampled for Mozambique (Limpopo, Umbeluzi, incomati and sabié) that promote high genetic diversity [34].

4.2. Genetic Diversity between Populations

The estimating of Ho and He provides information on the degree of genetic diversity within populations. In this study, the average He ranged from 0.71 to 0.83 while that of Ho ranged from 0.68 to 0.81. Consistent with these results, Rutten *et al.* [15] in a genetic characterization study conducted on four strains of Nile

tilapia, found a relatively higher expected heterozygosity (He = 0.624 to 0.711) than observed heterozygosity (Ho = 0.612 to 0.696). These results are of the same order of magnitude but significantly lower than those found in the current study, which can be explained by a reduction in genetic variability under hatchery conditions due to a limited number of broodstock. Indeed, Rutten *et al.* [15] sampled captive populations whereas the individuals analyzed in this study were from both captive and wild populations. This is consistent with the high He and Ho (0.819 to 0.911 and 0.900 to 1.000, respectively) reported in wild populations of *O. mozambicus* from the Limpopo, Umbeluzi, incomati, and Sabie rivers [34].

The mean He obtained in the present study is higher than the Ho, suggesting a heterozygosity deficiency in the populations analyzed. This reduction in Ho relative to He may be explained by gene flow, allele reading error, or inbreeding. In addition, the observed genotypic frequencies are consistent with expected frequencies, suggesting a lack of significant deviation from Hardy-Weinberg imbalance (Fit = 0.051). The low Fis (0.026) found in this study also suggests the absence of Hardy-Weinberg deviation in the five populations analyzed and indicates that the tilapia populations studied are not affected by inbreeding. In addition, the migration rate between populations was 4.74, suggesting low gene flow between populations. Thus, the high heterozygosity and allelic frequency found in this study may be due to a common origin of some populations (ANA, Richard-Toll, Mbodiene, and Sauvage), which may promote random mating within a Hardy-Weinberg parental population.

4.3. Genetic Structure

Tajima's D parameter obtained with the neutral test compares the average number of differences per nucleotide pair with the number of segregation sites [32]. The neutral test gave negative Tajima values ranging from −1.508 to −0.540 for the ANA, ITACA, Mbodiene and Sauvage populations. The lowest Tajima D value was noted for the ANA and Mbodiene pairwise populations and the highest for the ITACA and Sauvage. SSR results showed moderate genetic differentiation among populations (Fst = 0.059; p = 0.0001), consistent with the strong contribution of intra-population genetic diversity (HS) to total genetic diversity (HT). In agreement with our results, Simbine *et al.* [34] revealed moderate differentiation (STF = 0.006, p < 0.001) between *O. mossambicus* strains from different rivers. Discriminant analysis of principal component indicates two axes that contribute to approximately 92.04% of the total variance. It reveals four groups gathering 80% to 100% of the individuals of the studied populations, which are discriminated by alleles 1, 2, 11, 14 and 8. However, alleles 1, 2 and 11 have a stronger discriminating power, followed by allele 14 and then allele 8. These four groups are very heterogeneous, which can be explained by a common origin and a recent geographical separation, probably by the establishment of aquaculture farms and the isolation of the breeders of these farms. These results

are consistent with those obtained with the genetic diversity, which indicated high within-population genetic diversity, but low among-population genetic diversity. The negative Tajima values indicate an excess of rare mutations in the studied populations, a result of recent population expansion [35]. Thus, the low genetic differentiation observed in this study could due to a limited number of initial breeders and their isolation in the hatcheries. They have then expanded in the hatcheries during their years of captivity.

5. Conclusion

Analysis of the five microsatellite loci showed a total of 52 alleles with high polymorphism and revealed a high level of heterozygosity (Ho) within the study of Nile tilapia *O. niloticus* populations from Senegal. Allele frequency analysis showed that 80% - 100% of populations carried at least high frequencies of alleles 1, 2, 3 and 9. These analyses showed high genetic diversity among individuals within *O. niloticus* populations. The AMOVA results revealed strong differentiation within populations and weak but significant genetic differentiation between populations in agreement with the DAPC results that showed differentiation of the five populations studied into four genetic groups. The overall results suggest that the captive stocks of *O. niloticus* exploited in aquaculture systems in Senegal originated from a single population. This parental population was further divided into four distinct genetic groups following the isolation of a limited number of broodstock in local hatcheries. However, further studies using much larger samples from the major breeding regions with a much broader panel of genetic markers are needed to better quantify the levels of genetic differentiation within and between strains of *O. niloticus*. It would also be interesting to compare at the same time the zootechnical performance of these strains and ultimately select the best strains for sustainable breeding of the species.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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