

# Genetic diversity of four broodstocks of tilapia (*Oreochromis* sp.) from Antioquia, Colombia

*Diversidad genética de cuatro grupos de reproductores de tilapia (*Oreochromis* sp.) de Antioquia, Colombia*

*Diversidade genética de quatro estoques de reprodutores de tilápia (*Oreochromis* sp.) de Antioquia, Colombia*

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## Abstract

**Background:** Tilapia is the most farmed fish in Colombia. However, the genetic diversity and structure of broodstocks in the hatcheries of Antioquia province remains unknown.

**Objective:** To analyze the genetic diversity and structure of one Nile and three red tilapia broodstocks in Antioquia, Colombia.

**Methods:** Fish were genotyped using 24 microsatellite markers of 13 linkage groups in five multiple reactions. Genetic diversity metrics were estimated and null alleles were detected. Analysis of Molecular Variance and analysis of number of clusters were used to describe the relationship between broodstocks.

**Results:** Two microsatellites could not be amplified, and 22 were polymorphic. Average number of alleles per locus ranged 5.77 to 7.91. Locus UNH211 had the most alleles (17), whereas OMO032 had the fewest (4). Except for GM234 and OMO032, the analyzed loci had at least one private allele per population. Average effective number of alleles (3.37–4.03) was always less than the number of observed alleles. Significant deviations from Hardy-Weinberg equilibrium with heterozygote deficiencies were registered. Nine markers showed evidence of null alleles. The expected heterozygosity (0.65 to 0.67 per broodstock) was significantly higher than the observed heterozygosity (0.601 to 0.649) in the four populations. The fixation index for all broodstocks (excluding null alleles) was 0.0766 (95% confidence interval, 0.05092 to 0.10289). According to the molecular variance analysis, the greatest variation was between individuals rather than between groups of broodstocks or individuals within broodstocks. The genetic distance between the Nile and red broodstocks ranged from 0.43 to 0.54.

**Conclusions:** Overall, these findings provide baseline information about the genetic diversity and structure of tilapia broodstocks in Antioquia, Colombia, useful for the management of hatcheries.

**Keywords:** *aquaculture; aquiculture; assisted selection; fish; fisheries; genetic diversity; molecular markers; stock management; tilapia.*

## Resumen

**Antecedentes:** La tilapia es el pez más cultivado en Colombia; sin embargo, hay gran desconocimiento sobre la estructura genética actual de los reproductores.

**Objetivo:** Analizar la diversidad y estructura genética de los reproductores de tres granjas de tilapia roja y una de tilapia Nilótica en Antioquia, Colombia.

**Métodos:** Se utilizaron 24 microsátélites de 13 grupos de ligamiento amplificados en cinco reacciones múltiples. Se calcularon diferentes medidas de diversidad y se detectaron alelos nulos. Se utilizó un análisis de varianza molecular y uno de número de grupos para describir las relaciones entre las granjas de reproductores.

**Resultados:** Dos marcadores no fueron amplificados y los 22 restantes fueron polimórficos. El promedio de alelos por locus varió entre 5,77 y 7,91. El mayor número de alelos (17) se encontró en el locus UNH 211, mientras que el menor se observó en OMO032 (cuatro). Veinte loci presentaron por lo menos un alelo privado. El número de alelos efectivos promedio fue menor al número de alelos observado y estuvo entre 3,37 y 4,03. Se registraron desviaciones significativas en el equilibrio Hardy-Weinberg, en su mayoría con deficiencias de heterocigotos. Se encontraron evidencias de alelos nulos en nueve marcadores. La heterocigosidad observada estuvo entre 0,601 y 0,649. El índice de fijación fue de 0.0766 (intervalo de confianza de 95%, entre 0,05092 y 0,10289). Según el análisis de varianza molecular, la mayor fuente de variación se encontró entre individuos. El valor de la distancia de Nei entre los reproductores Nilóticos y rojos estuvo entre 0,43 y 0,54.

**Conclusión:** Los resultados de la presente investigación proveen una línea base acerca de la diversidad y estructura genética de los reproductores de tilapia en Antioquia, Colombia, y son útiles para el manejo de granjas dedicadas a la reproducción de tilapia.

**Palabras clave:** *acuicultura; acuicultura; diversidad genética; manejo de reproductores; marcadores moleculares; pesca; pez; selección asistida; tilapia.*

## Resumo

**Antecedentes:** A tilápia é o peixe mais cultivado na Colômbia. É importante examinar a diversidade genética de peixes reprodutores.

**Objetivo:** Avaliar a diversidade e estrutura genética de três estoques de reprodutores de tilápias vermelhas e um de tilápia Nilótica em Antioquia, Colômbia.

**Métodos:** Utilizaram-se 24 microssatélites de 13 grupos de ligação em cinco reações múltiplas. Métricas de diversidade genética foram estimadas e alelos nulos foram detectados. Análise da Variância Molecular e análise do número de clusters foram utilizados para descrever a relação entre os estoques.

**Resultados:** Dois marcadores não foram amplificados e vinte e dois microssatélites analisados mostraram-se polimórficos. O número médio de alelos por locus variou entre 5,77 e 7,91. O Locus UNH211 apresentou o maior número de alelos (17), enquanto o OMO032 apresentou o menor número (4). Exceto GM234 e OMO032, os loci analisados mostrou um pelo menos um alelo privado por população. O número efetivo médio de alelos (3,37-4,03) foi sempre menor do que o número de alelos observados. Foram observados desvios significativos do equilíbrio Hardy-Weinberg e deficiência de heterozigotos. Nove loci mostraram evidências de alelos nulos. A heterozigosidade esperada (0,6504-0,6748 por população) foi significativamente maior do que a heterozigosidade observada (0,601-0,649). O índice de fixação foi de 0,0766 (intervalo de confiança de 95%, 0,05092-0,10289). De acordo com a análise da variância molecular, a maior variação foi entre indivíduos. Adistância genética entre o Nilo e os reprodutores vermelhos variou de 0,43 a 0,54. **Conclusão:** No geral, esses resultados fornecem informação básica sobre diversidade e estrutura genética de reprodutores de tilápia em Antioquia, Colômbia, e são significativos para o manejo de plantéis de reprodutores.

**Palavras-chave:** *aquicultura; diversidade genética; gestão de plantéis de peixes; marcadores moleculares; pescaria; peixe; seleção assistida; tilapia.*

## Introduction

Tilapia is the second most important fish in aquaculture after carp. Global production of *Oreochromis niloticus* reached 3,670,260 tons in 2014 (FAO, 2016). Furthermore, *Oreochromis* spp. are the most economically important aquaculture species in Colombia (Ministry of Agriculture and Rural Development of Colombia, unpublished data), and this country was the second largest exporter of fresh tilapia fillet to the United States in 2015, with 5,329 tons valued in USD \$44,119,211 (NMFS, 2016).

Colombia has a long history of successive introductions of tilapia. Importation of *O. niloticus* began around 1979 to promote aquaculture, followed by red varieties of *Oreochromis mossambicus* and *Oreochromis urolepis*, Nile strains, and red hybrids (Gutiérrez *et al.*, 2012). As a result, several tilapia species are cultured in this country. However, maintaining genetic variation is important because of its critical role in a population's adaptative potential to cope with variable environmental conditions, and its usefulness when genetic improvement through selective breeding is anticipated (Lind *et al.*, 2012).

Aquaculture stocks derived from small founder populations, show after few generations, loss of genetic diversity as well as depression related to inbreeding (Doyle, 2016). Therefore, the gene pool needs to be refreshed periodically (McKinna *et al.*, 2010). For tilapia, total female fertility and male reproductive success are affected by the level of endogamy (Fessehaye *et al.*, 2009). Thus, it is important to monitor broodstock genetic diversity in aquaculture farms responsible for fry production (Petersen *et al.*, 2012). Microsatellites are among the best genetic markers for analyzing pedigree, population structure, genomic variation, and evolutionary processes, as well as for verifying the quality of selective breeding programs in aquaculture (Abdul-Muneer, 2014).

Genetic diversity of *Oreochromis* spp. has been extensively studied using microsatellites worldwide (Petersen *et al.*, 2012; Gu *et al.*, 2014). Diversity metrics such as heterozygosity, allelic richness and inbreeding coefficient are highly variable between studies. Previous work by Brinez *et al.*, (2011) evaluated the genetic diversity of six populations of red hybrid tilapia from several regions of Colombia. However, the genetic diversity and structure of broodstocks from the Antioquia region remains unstudied.

Accordingly, the purpose of this study was to assess the genetic variability and population structure of broodstocks in the most important tilapia hatcheries in Antioquia, Colombia. In addition, we discuss current trends and directions for future research in the study of genetic diversity of tilapia using microsatellites.

## **Materials and Methods**

### ***Ethical considerations***

Handling procedures followed the section seven of the Aquatic Animal Health Code about welfare of farmed fish (OIE, 2016).

### ***Tissue sampling***

The farms were selected according to the following criteria: a. they had their own breeders and hatcheries, and b. their high contribution to tilapia production in Antioquia (Colombia). Terminal tailfin fragments were collected randomly from tilapia broodstock in one Nile and three red tilapia farms. Samples were stored in 98% ethanol until processing.

### ***DNA isolation and amplification***

Genomic DNA was extracted from samples using DNEasy® blood and tissue kits in the QIAcube® automated system (QIAGEN), in accordance with manufacturers' protocols. Total DNA was quantified, and its purity (260-nm/230-nm ratio) was determined using a NanoDrop® spectrophotometer (Thermo Fisher Scientific). Next, DNA integrity was visualized with 1.2% agarose gel using SYBR® Safe gel stain (Thermo Fisher Scientific).

Twenty-four microsatellite loci located in 13 different linkage groups were analyzed with forward primers tagged with fluorochrome markers FAM, VIC, NED, or PET (Table 1). Five sets of primer tags in multiple reactions (Table 2) were amplified, such that all fragments could be uniquely marked and separated within a single capillary without overlap. PCR was carried out in reaction volumes of 10.0 to 12.3 µl with a thermal profile of initial denaturing at 94 °C for 15 min, followed by 30 cycles of 30 s at 94 °C, 90 s at 56 °C, 90 s at 72 °C, and 10 min at 72 °C. Fragment lengths were determined with a GeneScan® 500 ROX® Size Standard (Applied Biosystems) in an Applied Biosystems 3130 automated capillary genetic sequencer. DNA sizing and quality allele calls were analyzed with GeneMapper software version 4.0 (Applied Biosystems).

### ***Data analysis***

Data were transformed and alleles were grouped using TANDEM software version 2.0 (Matschiner and Salzburger, 2009). SPAGeDi software version 1.5 (Hardy and Vekemans, 2002) was used to calculate the number of alleles, effective number of alleles, observed heterozygosity, expected unbiased heterozygosity (Nei, 1978), allelic richness, and individual endogamy coefficient. Genepop software version 4.1 (Rousset, 2008) was used to determine deviations from Hardy–Weinberg equilibrium (HWE) across populations within loci and across loci within populations via the Markov chain method, with heterozygote deficit being specified as the alternative hypothesis. Markov chain length was 1,000 iterations plus 100 subsequent groups of 2,000 iterations. Genepop version 4.1 (Rousset, 2008) was used to analyze allele fixation and linkage disequilibrium via the G-test with 10,000 dememorizations, 100 groups, and 5,000 iterations per group. Genetix software version 4.02 (Belkhir *et al.*, 1996) was used to calculate the number of private alleles per locus, Wright's fixation index ( $F_{ST}$ ) (Weir and Cockerham, 1984), and Nei's distance (Nei, 1972). To detect artifacts ("stuttering"), loss of alleles, and null alleles, Micro-Checker software version 2.2.3 was used (Van Oosterhout *et al.*, 2004). Hierarchical subdivision of genetic diversity was determined via Analysis of Molecular Variance (AMOVA) using Arlequin software version 3.5.2.2 (Excoffier and Lischer, 2010). To determine the number of groups and to assign individuals to specific groups, Structure software version 2.3.4 was used (Pritchard *et al.*, 2000). The analysis was performed in accordance with recommendations of Gilbert *et al.* (2012). For Markov chain Monte Carlo, 100,000 burn-in iterations and repetitions for each run, were used. To confirm parameter suitability, value convergence in the statistical

summary was determined, as recommended in the Structure User Manual (Pritchard *et al.*, 2010). Twenty independent replicas with one to seven groups were run in the Structure software program. To select the number of clusters (K), the method of Evanno *et al.*, (2005) was applied by using the online software application Structure Harvester version 0.6.94 (Earl and vonHoldt, 2012).

**Table 1.** Microsatellites used in the present study.

Locus	Repetition motif	Linkage group	Direction	Primer	GenBank access no.	Linkage group reference
GM234	GT	22	Forward	5'ATCGCTGTGTATTTCTATCAC 3'	BV005371.1	
			Reverse	5'ATGCCACCCTATTCTGT 3'		
GM407	CA	12	Forward	5'GGCTCGTCCATCTCGT 3'	BV005443.1	
			Reverse	5'CTTGGCTCTTTGTTGGTTATT 3'		
OMO032	GAAAA	13	Forward	5'GGTAAGCGGTGCAAAGTTCA 3'	GR695143.1	
			Reverse	5'GTTGGCTAACCTCTGTCTCACTCC 3'		
OMO039	ATT	11	Forward	5'CTGGCTGCCTCAGGACTCATCA 3'	JX204854	Liu <i>et al.</i> (2013)
			Reverse	5'GCACGGCCGCAGGTAATG 3'		
OMO175	GT	4	Forward	5'CAACCTGCCTGAAAATCACCACAC 3'	JX204934	
			Reverse	5'AAACCCAAAACCCAGCAACACAA 3'		
OMO194	GT	2	Forward	5'TCAGGAAAGGGAACCCGAGATG 3'	JX204949	
			Reverse	5'GGACCTGGTTGGACGTGCTTCT 3'		
OMO228	CA	22	Forward	5'CCACGATTCCTTCGACAGCCAT 3'	JX204972	
			Reverse	5'AACGCGCACATATTGCCTTTTCA 3'		
UNH104	CA	1	Forward	5'GCAGTTATTTGTGGTCACTA 3'	G12257.1	
			Reverse	5'GGTATATGTCTAACTGAAATCC 3'		
UNH106	CA	3	Forward	5'CCTTCAGCATCCGTATAT 3'	G12259.1	Lee <i>et al.</i> (2005)
			Reverse	5'GTCTCTTTCTCTCTGCACAAG 3'		
UNH108	CA	2	Forward	5'GGGATCAGCTGTTAAGTTT 3'	G12261.1	
			Reverse	5'TGAGTTGATTATTAATTTCTGA 3'		
UNH118	GT	-	Forward	5'CAGAAAGCCTGATCTAATATT 3'	G12271.1	N/A
			Reverse	5'TTTCAGATACATTTTATAGAGGG 3'		
UNH123	CA	12	Forward	5'CATCATCACAGACAGATTAGA 3'	G12276.1	Lee <i>et al.</i> (2005)
			Reverse	5'GATTGAGATTTCAATCAAG 3'		
UNH124	GT	4	Forward	5'AATTTGGCAGCTTCTTTT 3'	G12277.1	Moen <i>et al.</i> (2004)
			Reverse	5'CCCACAAGCATAGTAACT 3'		
UNH129	CA	8	Forward	5'AGAAGTCGTGCATCTCTC 3'	G12282.1	
			Reverse	5'TGTACATCATCTGTGGG 3'		
UNH159	CA	2	Forward	5'TTGTTTTAGGAGCTTCTTTTGTG 3'	G12311.1	
			Reverse	5'ATATTCATCTGGATTGGCTCTAA 3'		
UNH160	CA	6	Forward	5'CCATTGGCTTTACATC 3'	G12312.1	
			Reverse	5'GATAGCATTTCTGTAGTTATGG 3'		
UNH166	CA	9	Forward	5'CCCTCACACACTCTT 3'	G12318.1	Lee <i>et al.</i> , (2005)
			Reverse	5'GATAACGACACGACAGTAC 3'		
UNH172	CA	4	Forward	5'AATGCCTTTAAATGCCTTCA 3'	G12324.1	
			Reverse	5'CTTTTATAGTCGCCCTTTGTTA 3'		
UNH207	CA	6	Forward	5'ACACAACAAGCAGATGGAGAC 3'	G12358.1	
			Reverse	5'CAGGTGTGCAAGCAGAAGC 3'		
UNH208	CA	19	Forward	5'CTTCTTGGCTACAATTT 3'	G12359.1	Cnaani <i>et al.</i> (2004)
			Reverse	5'CAGATGGGTGATAGCAA 3'		

UNH211	CA	19	Forward	5' GGGAGGTGCTAGTCATA 3'	G12362.1	Lee <i>et al.</i> (2005)
			Reverse	5' CAAGGAAAACAATGGTGATA 3'		
UNH216	CA	23	Forward	5' GGGAACTAAAGCTGAAATA 3'	G12367.1	
			Reverse	5' TGCAAGGAATATCAGCA 3'		
UNH222	CA	2	Forward	5' CTCTAGCACACGTGCAT 3'	G12373.1	
			Reverse	5' TAACAGGTGGGAACTCA 3'		
UNH231	CA	6	Forward	5' GCCTATTAGTCAAAGCGT 3'	G12382.1	
			Reverse	5' ATTTCTGCAAAGTTTCC 3'		

**Table 2.** Volumes or concentrations of components for multiple reactions.

Multiplex reaction	Locus	Primer (pmol $\mu\text{L}^{-1}$ )	Water <sup>1</sup> ( $\mu\text{L}$ )	Buffer ( $\mu\text{L}$ )	Taq pol <sup>2</sup> ( $\mu\text{L}$ )	Mix ( $\mu\text{L}$ )	DNA ( $\mu\text{L}$ )	Total ( $\mu\text{L}$ )
A	UNH208	6	0.8	4.0	0.05	8.2	1.8	10
	UNH207	2.5						
	GM407	6						
	OMO032	6						
	UNH123	13						
B	UNH104	6	0	4.0	0.06	10.26	2	12.26
	UNH159	10						
	UNH108	6						
	UNH160	6						
	UNH222	18						
	OMO194	6						
C	UNH166	6	2.65	4.0	0.05	8.5	1.5	10
	GM234	6						
	UNH118	6						
	UNH216	6						
D	UNH172	6	1.1	4.0	0.05	8.5	1.5	10
	UNH124	12						
	UNH106	3.5						
	UNH129	6						
	OMO039	6						
E	UNH231	6	1.85	4.0	0.05	8.5	1.5	10
	OMO175	8						
	UNH211	6						
	OMO228	6						

<sup>1</sup> Nuclease-free water from Applied Biosciences was used. Other manufacturers are as described in the Methods. <sup>2</sup> Taq pol stock solution was 5 U  $\mu\text{L}^{-1}$ .

## Results

Between 32 and 47 fish per farm were genotyped. Two microsatellites (UNH208 and UNH222) could not be amplified. The remaining 22 microsatellites were polymorphic. Across the four broodstocks, the number of alleles per locus varied from four (locus OMO032 in all four broodstocks) to 12 (UNH118 in red 1 broodstock). Average number of alleles per locus was between 5.77 (Nile) and 7.91 (red 3). The highest total number of alleles was 17 in locus UNH211, and the lowest was 4 in locus OMO032. Allelic range was 101 to 143 (locus UNH207), and 303 to 359 (locus OMO175). Allelic range amplitude varied from six (locus GM234) to 88 (locus GM407). Average effective number of alleles was always lower than observed number of alleles and fell between 3.37 (red 3) and 4.03 (Nile). Half of the loci (44/88) showed significant deviations from HWE, and 42 of these loci showed heterozygote deficiency. Loci OMO032, OMO194, and UNH159 were in HWE in all four broodstocks. Loci OMO228, UNH106, UNH160, and UNH216 were in HWE in three of the four broodstocks.

At least one private allele per broodstock was found in the 20 loci (except in GM234 and OMO032), and locus OMO228 had seven private alleles. The Nile population had the highest number of private alleles (22). Micro-Checker analysis showed evidence of null alleles in loci GM407, OMO175, UNH104, UNH108, UNH118, UNH123, UNH124, UNH129, and UNH166.

Eliminating loci with null alleles, linkage disequilibrium analysis for all populations showed six loci pairs with  $p < 0.05$ . Loci pairs UNH159-OMO194 and UNH207-UNH231 had infinite  $\chi^2$  values and highly significant  $p$  values. Thus, loci UNH159, OMO194, and UNH207 were excluded from further analysis. Table 3 shows the genetic diversity descriptors per broodstock for the 10 microsatellites, excluding loci with null alleles and linkage disequilibrium (GM234, OMO032, OMO039, OMO228, UNH106, UNH160, UNH172, UNH211, UNH216, and UNH231). The calculated value of unbiased expected heterozygosity varied depending on the microsatellite marker group (Table 4). Differences between calculations were generally smaller when 10 and 13 microsatellite markers were used than when 22 markers were used. Expected heterozygosity based on Hardy–Weinberg proportions ranged between 0.6504 (Nile) and 0.686 (red 2). Observed heterozygosity was different from the expected heterozygosity in all four broodstocks ( $p=0.000$ , standard deviation =0.000).

**Table 3.** Genetic diversity per broodstock from 10 microsatellites, excluding null alleles and loci with linkage disequilibrium.

Broodstock	Na	Nae	AR	Fis
Red 1	6.5	3.29	5.4	0.039
Red 2	7.1	3.45	5.75	0.097
Red 3	6.7	3.63	5.68	0.094
Nile	5.5	3.14	4.96	0.078

Na - number of alleles; Nae - effective number of alleles; AR - allelic richness; Fis - individual endogamy coefficient.

The  $F_{ST}$  value for all broodstocks together (excluding null alleles and linkage disequilibrium) was 0.0777 (95% confidence interval, 0.05763 to 0.09718), reflecting the low average substructuring. AMOVA results showed that the greatest variation was between individuals. Variation

**Table 4.** Expected heterozygosity (He) and observed heterozygosity (Ho) per population calculated from different microsatellite groups.

Broodstock	22 Microsatellites		13 Microsatellites, no null allele loci		10 Microsatellites, no null allele and linkage disequilibrium loci	
	He	Ho	He	Ho	He	Ho
Red 1	0.7159	0.6166	0.6784	0.6419	0.6748	0.649
Red 2	0.7243	0.6174	0.6956	0.6308	0.686	0.62
Red 3	0.7169	0.5787	0.6809	0.6299	0.6705	0.609
Nile	0.6827	0.6004	0.6716	0.6051	0.6504	0.601

percentage from the source “between groups” was very low (0.80%). Sources “between broodstocks within groups” and “between individuals within broodstocks” showed negative variation percentages, because their values could be close to zero (Table 5).

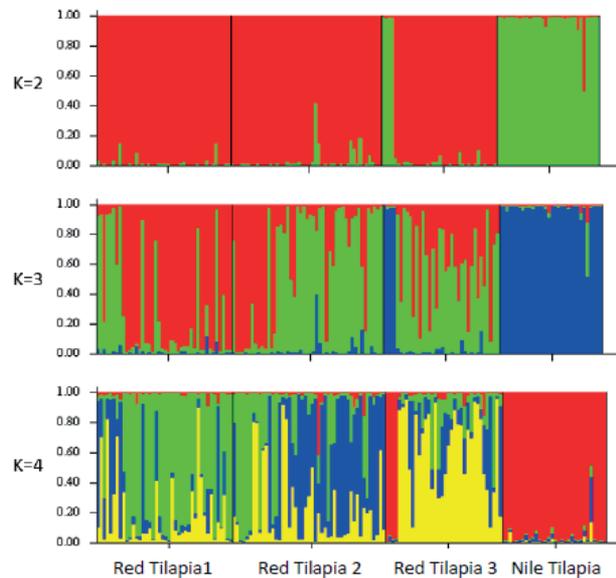
The trend of the logarithm curve for the probability of number of clusters K began to plateau at K = 3, with a value of -4049.155. However, the most appropriate number of clusters based on estimates of  $\Delta K$  was two ( $\Delta K = 798.053$ ). Figure 1 shows a graphical representation of the assignment of each individual to K = 2, 3 or 4. Nile broodstock was the only that retained an assignment proportion greater than 90% in just one cluster among all values of K evaluated (results for K = 5, 6, and 7 not shown). In red broodstocks, the maximum assignment proportion decreased with an increase in K value. Finally, red broodstocks were closer to each other (Nei’s distance: 0.03 to 0.08), than they were to the Nile broodstock (Nei’s distance: 0.433 to 0.54).

**Table 5.** Results of molecular variance analysis (AMOVA).

Comparison between:	DoF	SS	VC	VP	F index	p
Groups (Reds vs. Nile)	1	0.455	0.00248	0.80	$F_{CT} 0.00795$	$0.24735 \pm 0.00797$
Broodstocks within Groups (Reds or Nile)	2	0.371	-0.00115	-0.37	$F_{SC} -0.00371$	$0.55919 \pm 0.00956$
Individuals within broodstocks	153	42.894	-0.03020	-9.68	$F_{IS} -0.09726$	$0.97156 \pm 0.00349$
All Individuals	157	53.500	0.34076	109.26	$F_{IT} -0.09257$	$0.96825 \pm 0.00270$

DoF - degrees of freedom; SS - sum of squares; VC - variance components; VP - variance percentage; F index - fixation index.

## Discussion



**Figure 1.** Assignment of each individual from the four broodstocks to  $K = 2, 3,$  or  $4$ . Each color represents one cluster. Length of each bar was the estimated assignment proportion of a particular cluster. Graphs were obtained from the Structure software package.

On a global level, different panels of microsatellites have been used for diversity analysis of wild and farmed populations of *Oreochromis* sp. (particularly of *O. niloticus*, see Table 6). However, these results are not comparable across studies. First, the estimation of levels of population genetic diversity (allelic richness, expected heterozygosity, coefficient of inbreeding), structure ( $F_{ST}$ ), and genetic distances (Nei) from microsatellite data is depending on number of loci and the strategy of marker selection (Orozco-terWengel *et al.*, 2011; Blanco *et al.*, 2012; Queirós *et al.*, 2015). Second, there are differences in detection and scoring of microsatellites between techniques such as: agarose gel, slab gel and capillary electrophoresis (Vemireddy *et al.*, 2007; Stewart *et al.*, 2011). Third, most studies did not test the neutrality of markers, the occurrence of null alleles and linkage disequilibrium. Fourth, the comparisons among populations with appreciable size differences between alleles may be biased because longer microsatellite alleles mutate at a higher rate (Putman and Carbone, 2014). Finally, although some of the microsatellite loci used in the present study were also used in previous works, this is the first report using this panel. Our contrasts therefore need to be interpreted with caution.

A median of nine microsatellites have been used in genetic diversity studies for Nile and red tilapia populations (range: 4–20 markers, see table 6), which represents a slightly smaller number than the one of microsatellites used in the present study (10). According to Koskinen *et al.* (2004), a possible disadvantage of using a small number of loci is an increased standard deviation of genetic distances across populations.

The studies listed in Table 6 found that the mean number of alleles per locus per population ranged from three (Karuppannan *et al.*, 2013) to 50 (Bhassu *et al.*, 2004) with a median of 5.87, which is in accordance with the present results. However, we obtained decreased values of the mean number of alleles compared to previous studies in Colombia and Brazil (6.45 in the present study vs. 8.37 in Brinez *et al.*, 2011 and 10,46 in Petersen *et al.*, 2012, respectively). Nineteen different panels of microsatellite loci in several tilapia populations worldwide showed expected heterozygosity values between 0.170 Bezaul *et al.* (2011) and 0.968 Bhassu *et al.* (2004), and observed heterozygosity values between 0.150 (Dias *et al.*, 2016) and 0.90 (Hassanien and Gilbey, 2005) with a median of 0.686 and 0.649, respectively (Table 6). Our results of  $H_e$  (0.6504 to 0.686) and  $H_o$  (0.601 to 0.649) are consistent with these reports.

The farms sampled in the present study did not keep records of broodstocks origin and pedigree, and

the genetic background of broodstocks was unknown. Moreover, the farmers carry out replacement of broodstocks based on mass selection for coloration and growth (red or Nile tilapia, respectively) of some groups that are not sex-reversed derived mostly from their own farms. These practices could decrease the genetic diversity of broodstock after only a few generations. For example, Romana-Eguia *et al.* (2005) reported a reduction in the mean number of alleles (from 10 to 7.4) and mean  $H_e$  (from 0.752 to 0.691) in selected and control stocks of mass-selected Nile tilapia after four generations.

Comparisons of the deviations from HWE obtained in the present work with those of previous studies (Table 6) confirms that some wild populations and unselected stocks of tilapia conformed to the HWE (Romana-Eguia *et al.*, 2004; Bezault *et al.*, 2011; Sukmanomon *et al.*, 2012), while departure from HWE equilibrium was detected in most of the farmed populations due to a significant deficit of heterozygotes. Deviation of the HWE in hatchery populations could be attributed to the aquaculture practices because a limited number of adults are spawned and an unequal sex ratio is applied (Napora-Rutkowski *et al.*, 2017). The  $F_{IS}$  values obtained in this study (0.031–0.121) indicate a deficit of observed heterozygotes. These results reflect those obtained previously by Rutten *et al.* (2004), Nyingi *et al.* (2009), some populations in Bezault *et al.* (2011) and Gu *et al.* (2014). In contrast, Melo *et al.* (2006), Moreira *et al.* (2007) and Dias *et al.* (2016) found negative values of  $F_{IS}$  (see Table 6). Furthermore, our results are similar to the positive values of  $F_{IS}$  in tilapia populations from Colombia ( $F_{IS} = -0.345$  in Brinez *et al.*, 2011) and Brazil ( $F_{IS} = 0.160$ – $0.330$  in Petersen *et al.*, 2012;  $F_{IS} = 0.192$ – $0.401$  in Rodriguez-Rodriguez *et al.*, 2013).

The  $F_{ST}$  value of 0.078 (95% confidence interval, 0.05763 to 0.09718) obtained in this study suggests a low level of genetic differentiation. This result is comparable with the  $F_{ST}$  values of tilapia populations from Philippines ( $F_{ST} = 0.069$ , Romana-Eguia *et al.*, 2004), Egypt ( $F_{ST} = 0.035$ , Hassanien and Gilbey, 2005), Africa ( $F_{ST} = 0.09$ , Bezault *et al.*, 2011), Thailand ( $F_{ST} = 0.087$ , Sukmanomon *et al.*, 2012) and India ( $F_{ST} = 0.089$ , Shyamala *et al.*, 2014). On the contrary, six commercial stocks of tilapia from Brazil exhibited large genetic difference ( $F_{ST} = 0.326$ , Melo *et al.*, 2006).

The AMOVA results in the present study indicated that almost the entire variation occurred between individuals. These findings differ from those obtained by Brinez *et al.* (2011) for four Colombia provinces; excluding Antioquia, because they reported variation percentages of 17.32% between populations, 28.55% within populations, and 54.12% between individuals. The AMOVA results of the present study were similar to those of Romana-Eguia *et al.* (2004), who reported 99.33% variation between individuals and only 0.00331% between groups of red and Nile tilapia. In contrast, Bezault *et al.* (2011) found in natural populations of *O. niloticus* that 49.8% of the genetic variability was partitioned among hydrographic basins and 3.5% was detected among populations within basins.

We found evidence of null alleles at nine of 22 loci. This situation represents the absence of a PCR product due to a mutation in the region flanking the microsatellite. Also, it creates a false homozygosity reading in individuals that could be heterozygotes and, therefore, introduces biases in estimates of genetic diversity and differentiation across populations (Chapuis and Estoup, 2007). The presence of null alleles in microsatellite data of tilapia have been previously reported by Nyingi *et al.* (2009), McKinna *et al.* (2010), and Sukmanomon *et al.* (2012). Our result showed that after eliminating loci with null alleles the  $H_e$  values decreased. However, Bezault *et al.* (2011) identified in natural populations of tilapia that the exclusion of loci with potential null alleles had minor effects on  $F_{IS}$ .

Linkage disequilibrium is the non-random association of alleles at distinct loci. It creates pseudo-replication for analyses in which loci are assumed to be independent samples of the genome. In order to avoid increased Type I error, one locus in the pair should be discarded if significant disequilibrium is found consistently between loci (Selkoe and Toonen, 2006). Therefore, we discarded the loci UNH159, OMO194, and UNH207. Nevertheless, after eliminating these loci the  $H_e$  values slightly decreased. Sukmanomon *et al.* (2012) and Rodriguez-Rodriguez *et al.* (2013) observed linkage disequilibrium in different pairs of microsatellite loci in tilapia. However, these authors did not apply any correction to

the data. Conversely, McKinna *et al.* (2010) found no evidence to suggest that four microsatellite loci used in their tilapia study deviated from linkage equilibrium.

In the present study, the most appropriate number  $\Delta K$  value was obtained for  $K=2$  (i.e., one red and one Nile cluster), and the values of Nei's distances were higher between the three red and the Nile broodstocks. This outcome is contrary to that of Shyamala *et al.* (2014), who found a Nile tilapia population clustered with red tilapias due to breeding management schemes. Nonetheless, our results are similar to those of Romana-Eguia *et al.* (2004), in which Nile tilapias formed a distinct cluster from red tilapias.

In conclusion, ten microsatellite loci allowed the characterization of the genetic diversity and structure of three red and one Nile tilapia broodstocks from Antioquia, Colombia. This molecular analysis suggests that the four broodstocks are not affected by major loss of genetic diversity and it shows a clear separation between three red and the Nile broodstocks. Our results provide basic information that could be helpful for the management of tilapia hatcheries and the design of future breeding schemes. We would encourage researchers to standardize and calibrate a panel of microsatellite loci (including adaptive and neutral markers) in multiplex PCRs for tilapia that helps the comparison of information.

**Table 6.** Comparison of results obtained by previous microsatellite studies in wild and cultivated tilapia populations. No. of popul – number of population; Ho - observed heterozygosity, He - unbiased expected heterozygosity;  $F_{IS}$  inbreeding coefficient;  $F_{ST}$  - Wright's fixation index, gen – generations, CI – confidence interval.

Loci	No. of popul.	Ho	He	$F_{IS}$	$F_{ST}$	Region	Reference
UNH 104, 106, 123, 132, 146, 168, 190, 212, 214	1	0.500	0.612	–	–	Fiji	Appleyard <i>et al.</i> (2001)
UNH 104, 106, 132, 146, 149, 160, 178, 190, 208, 211, 212, 214, 222, 231	4	0.612– 0.696	0.624– 0.711	0.007– 0.093	0.178 (95% CI: 0.146–0.218)	Thailand-Germany	Rutten <i>et al.</i> (2004)
UNH 123, 147, 172, 216, 222	11	0.636– 0.734	0.666– 0.813	–	0.069	Philippines	Romana-Eguia <i>et al.</i> (2004)
UNH 106, 145, 146, 155, 160, 166, 168, 172, 188, 190, 207	5	0.651	0.968	–	–	Malaysia	Bhassu <i>et al.</i> (2004)
UNH 104, 106, 136, 178, 211, 222	5 (native wild)	0.493– 0.90	0.843– 0.886	–	0.035	Egypt	Hassanien and Gilbey (2005)
UNH 104, 108, 136, 160, 169	6	0.385– 0.815	0.416– 0.693	(-0.345)– 0.254	0.326	Brazil	Melo <i>et al.</i> (2006)
UNH 104, 108, 118, 222, 231	2	0.712– 0.763	0.794– 0.953	(-0.324)– (-0.07)	0.131	Brazil	Moreira <i>et al.</i> (2007)
UNH 860, 874, 887, 1003, Pr11AC, Pr11GT	6 (native wild)	0.346– 0.763	0.515– 0.813	0.071– 0.638	–	Kenya and Uganda	Nyingi <i>et al.</i> (2009)
UNH 115, 129, 142, 146, 154, 162, 189, 211, 216	10 (native wild)	0.17– 0.69	0.17– 0.74	(-0.03)– 1.15	0.09	Africa	Bezault <i>et al.</i> (2011)
UNH 106, 123, 172, 216, 222	6	–	0.586– 0.837	0.345	–	Colombia	Brinez <i>et al.</i> (2011)
UNH 104, 108, 136, 160	3	0.530– 0.660	0.590– 0.620	0.160– 0.330	0.130	Brazil	Petersen <i>et al.</i> (2012)
UNH 132, 138, 153, 160, 172, 173, 192, 211, 212, 213, 216, 222, 982, 1004	3 (feral)	0.610– 0.740	0.620– 0.770	–	0.087 (99% CI, 0.066–0.109)	Thailand	Sukmanomon <i>et al.</i> (2012)

UNH 104, 111, 120, 145, 146, 149, 162, 166, 172, 173, 189, 190, 194, 207, 211, 213, 214, 216, 231, 233	4	0.704– 0.783	0.561– 0.585	–	–	Malaysia	Karuppannan <i>et al.</i> (2013)
UNH 140, 159, 160, 162, 163	1 (4 gen.)	0.391– 0.531	0.636– 0.716	0.192– 0.401	–	Brazil	Rodriguez-Rodriguez <i>et al.</i> (2013)
GM 211, 531, 538; UNH 104, 106, 123, 146, 185, 207, 995	1	0.839	0.755	–	–	Egypt	Saad <i>et al.</i> (2013)
UNH 104, 106, 123, 147, 172, 211, 213, 216, 222	9	0.663– 0.779	0.777– 0.873	–	0.089	India	Shyamala <i>et al.</i> (2014)
GM 024, 677, 119; UNH 178, 738, 860, 932, 954, 971, 995	6 (feral)	0.31– 0.583	0.397– 0.698	0.096– 0.282	–	China	Gu <i>et al.</i> (2014)
UNH 005, 009, 103, 104, 123, 203, 828, 829, 866; GM672	4	0.15– 0.58	0.46– 0.53	(-0.11)– 0.71	–	Brazil	Dias <i>et al.</i> (2016)
GM234; OMO032, 039, 228; UNH106, 160, 172, 211, 216, 231	4	0.601– 0.649	0.6504– 0.686	0.031– 0.121	0.078 (95% CI, 0.05763– 0.09718)	Colombia	Present Study

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## Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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