



# Genetic diversity and differentiation of cultured Nile tilapia populations from Ethiopia revealed by ddRAD-seq: implications for better hatchery management

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**Abstract** – Sub-Saharan Africa, including Ethiopia, is a center of native Nile tilapia populations, which are important for conservation and aquaculture development. Nile tilapia aquaculture in Ethiopia is dominated by small-scale fish farming in ponds, with seeds from poorly managed hatcheries and wild sources. Hence, the development of aquaculture in Ethiopia faces a major hurdle owing to the absence of good-quality seeds, largely because of the lack of genetic management practices within hatchery centers. This study aimed to assess the genetic diversity and differentiation among farmed Nile tilapia populations to inform genetic management strategies and support the development of robust strains for aquaculture advancement. Using ddRAD-seq technology for SNP discovery, we assessed genetic diversity metrics across three farmed populations, Sebeta, Batu, and Aweday, comprising 20, 21, and 15 individuals, respectively. Expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ) and nucleotide diversity ( $\pi$ ) estimates indicated moderate within-population genetic diversity (mean:  $H_e = 0.24$ ,  $H_o = 0.25$ ,  $\pi = 0.25$ ). Pairwise  $F_{ST}$  values revealed the highest genetic distance ( $F_{ST} = 0.067$ ) between Batu and Aweday populations, while the lowest genetic distance ( $F_{ST} = 0.027$ ) was observed between Sebeta and Aweday populations. STRUCTURE analysis identified two genetic clusters, with the first cluster including Batu individuals and some from Sebeta and Aweday. Overall, our results show moderate within-population genetic variation and weak genetic differentiation among the populations. This study underscores the importance of documentation of broodstock backgrounds and formulation of reasonable hatchery practices to assist in aquaculture development and conservation of native genetic resources in Ethiopia.

**Keywords:** *O. niloticus* / aquaculture / genetic management / SNP markers

## 1 Introduction

Nile tilapia (*Oreochromis niloticus*) is an economically important fish species native to Africa, spanning the eastern and western regions and extending northward along the Nile River, with a limited presence in parts of the Middle East (Trewavas, 1983). Since 1960, it has been introduced to around 114 countries, both within and outside Africa, mainly for aquaculture (Geletu and Zhao, 2023; El-Sayed and Fitzsimmons, 2023). Often referred to as ‘aquatic chicken’ owing to its favorable aquaculture traits, affordability, and contribution to the livelihood of low-income communities in Africa, Asia, and

South America (El-Sayed and Fitzsimmons, 2023). Currently, it is one of the most important fish species in aquaculture, with a total production of 4.73 million tonnes in 2021 (FAO, 2024).

Nile tilapia aquaculture activities in sub-Saharan Africa, particularly in countries like Ethiopia, predominantly consist of small-scale fish farming in ponds, utilizing fingerlings sourced from wild catch, produced in unregulated spawning ponds, and government hatcheries (Brummett, 2007; Abwao et al., 2023; Workagegn et al., 2020; Hinrichsen et al., 2022). However, relying on seeds sourced from the wild poses significant challenges, including the risk of genetic contamination (such as loss of adaptation to culture conditions) and unpredictability in growth performance (Brummett, 2007). In addition, the existing hatchery centers do not implement proper

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genetic management protocols, which further contributes to the decline in seed quality (Brummett, 2007; Hinrichsen et al., 2022). Furthermore, apart from the scarcity of improved seeds, aquaculture in sub-Saharan Africa faces additional hurdles, such as the unavailability of affordable fish feed, limited technological capacity, and lack of a clear policy for aquaculture expansion (Brummett et al., 2008; Adeleke et al., 2021).

Nonetheless, in recent years, the production of Nile tilapia has demonstrated promising growth in countries such as Uganda, Ghana, and Zambia, with aquaculture alone accounting for 98,471, 82,900, and 39,363 tonnes in 2021, respectively (FAO, 2023). In addition, the use of locally produced better-performing strains (e.g., the Akosombo strain in Ghana) or imported genetically improved Nile tilapia strains (GIFT and its derivatives in Tanzania) has contributed to an increase in tilapia aquaculture production in the region (Moses et al., 2020; Trinh et al., 2021). Yet, the lack of quality seeds remains one of the main factors hindering the progress of aquaculture in sub-Saharan Africa (Abwao et al., 2023; Hinrichsen et al., 2022). In the absence of proper genetic management practices in hatcheries, and strategies to preserve native genetic resources in the wild, imported genetically improved strains may eventually lose their desirable performance traits and compromise the genetic composition of native wild populations because of intentional or accidental escape from aquaculture settings and interbreeding with indigenous stocks (McKinna et al., 2010; Ansah et al., 2014; Anane-Taabeah et al., 2019).

Ethiopia, situated among the countries of sub-Saharan Africa, possesses a well-recognized genetically diverse native Nile tilapia populations widely distributed across its lakes and rivers (Tesfaye et al., 2021). Nile tilapia capture fisheries contribute to about 40% of the total fish production in the country (FAO, 2024). Capture fisheries have reached their maximum potential in major fishing areas, and can no longer satisfy the country's increasing fish demand (Brummett, 2007; Tesfaye and Wolff, 2014). Although it is still at a nascent stage, aquaculture has the potential to increase fish production to meet the growing demand for fish as an alternative protein source (Wakjira et al., 2013).

In recent years, the Ethiopian government has considered aquaculture production as a viable option for alleviating poverty and improving food security in the country (Yalew et al., 2015). However, owing to the lack of selective breeding programs for the production of better-performing strains, inadequate hatchery centers, and lack of access to artificial feed, this sector has not yet achieved the anticipated goal (Yalew et al., 2015; Natea, 2018). Currently, smallholder farmers and government-run fingerling production facilities distribute Nile tilapia fingerlings to fish farmers in some parts of the country (Yalew et al., 2015). Such fingerlings obtained from hatcheries lacking genetic management practices often exhibit reduced genetic diversity and inferior growth performance, even compared to their wild counterparts, which further deters investment in the aquaculture sector (Brummett et al., 2004; McKinna et al., 2010). Effective genetic management in hatcheries is essential for preserving the genetic gains achieved through selection or domestication in both genetically improved and locally used strains

(Brummett et al., 2004). Evaluation of the impact of hatchery practices on genetic diversity is essential to assess the status of existing stocks and limit the loss of genetic variability (inbreeding) (D'Ambrosio et al., 2019). The availability of genetic information on cultured populations may also assist in making informed decisions for producing strains with superior growth performance and dissemination of fingerlings in a way that does not compromise the genetic composition of native populations (Mireku et al., 2017; Shechonge et al., 2018; Anane-Taabeah et al., 2019; Yáñez et al., 2020).

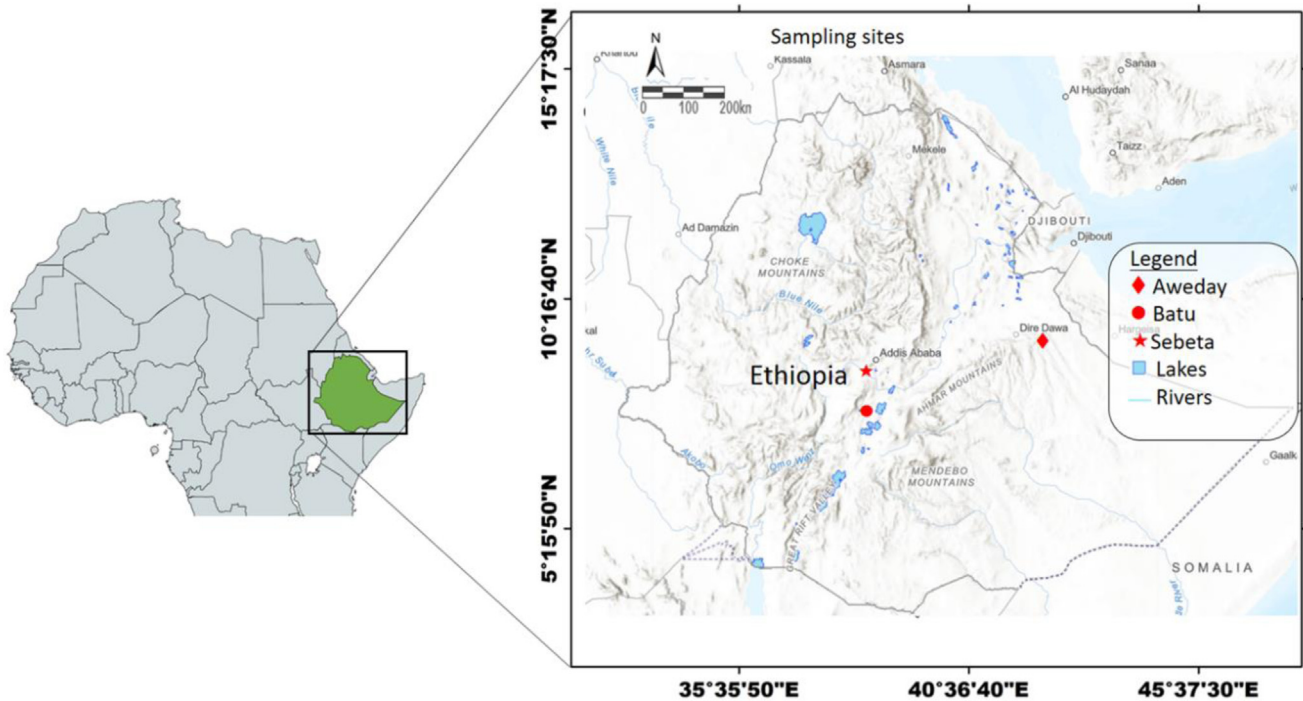
Understanding the genetic background of existing genetic resources is an indispensable first step in revealing the genetic problems associated with aquaculture strains, conserving valuable stocks/strains, and designing selective breeding programs to produce genetically robust strains for sustainable aquaculture (Taniguchi, 2003; Diyiye et al., 2021; Li, 2022). In aquaculture, genetic variation is a vital component of the genetic improvement (breeding) process, as selection acts directly on it (Taniguchi, 2003; Longo et al., 2024).

Genetic diversity studies of farmed populations of *O. niloticus* using SNP markers have been reported from countries such as Egypt (Nayfa et al., 2020), Tanzania (Kajungiro et al., 2019; Moses et al., 2020), Benin (Fagbémi et al., 2021), Bangladesh and Philippines (Hamilton et al., 2020), USA (Delomas et al., 2019), Brazil and Costa Rica (Yoshida et al., 2019), and Uganda (Robledo et al., 2024), and their implications for conservation and aquaculture development have been discussed. This is mainly due to the continuous advancement and affordability of next-generation sequencing techniques, such as ddRAD-seq, for the discovery of genome-wide SNP markers, which provide higher resolving power in population genetic/genomic analysis than traditional molecular markers such as microsatellites (Sunde et al., 2020). ddRAD-seq has been shown to accurately estimate genetic diversity even in populations represented by as few as eight individuals, particularly when thousands of SNPs are used (Nazareno et al., 2017). The objective of this study was to assess the genetic diversity and differentiation of existing Nile tilapia aquaculture populations from Ethiopia using ddRAD-seq for genome-wide SNP discovery, to provide insights that support the development of fast-growing strains and highlight the need for better genetic management practices in hatcheries.

## 2 Materials and methods

### 2.1 Ethical considerations

Sample collection was conducted after consultation with the Ethiopian Biodiversity Institute (EBI) and permission was obtained from responsible personnel from the respective sample collection sites. Permission to export the samples to Shanghai Ocean University, China, for genomic DNA analysis was obtained from the Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia (permission number: EBI-71/2023/2015). Caudal fin-clip samples (approximately 1 cm × 1 cm) were collected from each fish following sedation in MS-222 (50 mg/L). After recovery in aerated tanks, the fish were returned to culture ponds.



**Fig. 1.** Geographic locations of sampling sites in Ethiopia.

## 2.2 Background of the sampled populations

Three locations were selected for fin-clip sample collection for genomic DNA analysis (Fig. 1). Two of the sampling locations: (1) the National Fisheries and Other Aquatic Life Research Center in Sebete and (2) Batu Fisheries and Other Aquatic Life Research Center, are central and regional government-run fisheries and other aquatic life research and hatchery centers, respectively. Apart from other fisheries-related activities, these research centers are also actively engaged in Nile tilapia fingerling production and distribution to fish farmers, as well as stocking into artificial and natural water bodies (lakes, rivers, dams, and irrigation reservoirs). Hence, the majority of Nile tilapia farmed in Ethiopia originate from the aforementioned research centers. The founding individuals of these farmed strains were sourced from lakes in the Rift Valley region of Ethiopia (Adugna and Goshu, 2010; Workagegn and Gj oen, 2012). The third sampling site is located on the outskirts of Aweday town in eastern Ethiopia. Nile tilapia fingerlings were translocated from Sebete and Batu research and hatchery centers to farmers in Aweday by the Agriculture Office of the district for the purpose of aquaculture. No records were found on the dates and number of translocated fish.

Caudal fin-clip samples (approximately 1 cm × 2 cm) were collected from adult fish weighing >70 g. In total, 56 fin-clip samples were collected, and each sample was immediately stored in a 1.5 ml Eppendorf tube containing 99% (v/v) ethanol (Tab. 1). The samples were kept at room temperature during transportation and later stored in a −20 °C refrigerator at the laboratory until DNA extraction.

## 2.3 Genomic DNA extraction

Genomic DNA was extracted from parts of collected fin-clips (approximately 50 – 100 mg) using DNeasy Blood and Tissue Kit (Qiagen, Germany) according to manufacturer's protocol. The extracted DNA samples were quantified using Qubit fluorometer (ThermoFisher Scientific, USA). Samples were standardized in TE buffer to 20 ng/ L followed by gel electrophoresis (1% agarose gel) to assess DNA quality.

## 2.4 ddRAD library preparation and sequencing

The ddRAD libraries were prepared according to the methodology originally described by Peterson et al. (2012), with some modifications described in Syaifudin et al. (2019) and Di Santo et al. (2022). In brief, each DNA sample (20 ng/ L) was digested with two high-fidelity restriction enzymes *EcoRI* and *MseI* both sourced from New England Biolabs (NEB, Inc.). The digestion took place for about 1 h at 37 °C. Then the digested products were heat-inactivated at 65 °C for about 20 min. Then, sample specific combinations of P1 and P2 adapter, each with unique 5 or 7 base pairs barcode were ligated to the digested DNA samples by adding 1  l *EcoRI* compatible P1 adapter (25 nM), 0.7  l *MseI* compatible P2 adapter (100 nM), 0.06  l 100 mmol/L rATP (Promega, UK), 0.95  l 1 × Reaction Buffer 2 (NEB, inc.), 0.05  l T4 ligase (NEB, 2 × 10<sup>6</sup> U/mL) with reaction volumes made up to 12  l with nuclease-free water for each sample and incubated at 22 °C for 2 h. This was followed by another round of heat inactivation at 65 °C for about 20 min, and the ligation reaction was slowly cooled to room temperature and combined in a

**Table 1.** Location of sampling sites and information of cultured populations in Ethiopia.

Cultured populations	Source	GPS coordinates	Number of samples collected	Number of samples sequenced	Year of collection
Sebeta	Hatchery center	8° 92' N, 38° 64' E	20	20	2022
Batu	Hatchery center	7° 92' N, 38° 72' E	21	21	2022
Aweday	Farmers' ponds	9° 36' N, 42° 01' E	15	15	2022

single pool. Purification of the pooled library was performed with a PCR Purification Kit and eluted in 80  $\mu$ L EB buffer (Qiagen, UK). Size selection of fragments ranging from approximately 250 bp to 450 bp was performed by agarose gel electrophoresis. Gel purification was done by MinElute Gel Extraction Kit (Qiagen, UK) according to the manufacturer's protocol, and the obtained size selected template DNA was amplified by PCR. The amplified product was column purified (MinElute PCR Purification Kit). Then the eluate was subjected to further size-selection cleanup using an equal volume of AMPure magnetic beads (Perkin-Elmer, UK) to remove remnant small DNA fragments (<200 bp) and eluted into EB buffer (Qiagen, UK). The ddRAD libraries were sequenced on Illumina HiSeq-4000 instrument using a paired-end read length of  $2 \times 150$  bp at the BGI genomics center (Shenzhen, China).

## 2.5 Sequence data analysis and SNP genotyping

PCR duplicates were removed and sequence reads were demultiplexed by barcode using Stacks v2.65 (*process\_radtags*) program (Catchen et al., 2013). Reads with quality score less than 30 ( $Q < 30$ ) were removed and reads with a length less than 150 bp were discarded. The retained reads were aligned to the *O. niloticus* reference genome assembly [GenBank accession number GCA\_001858045.2 (Conte et al., 2017)] using bowtie2 (Langmead and Salzberg, 2012). Stacks software v2.65 (Catchen et al., 2013) was used according to Rochette and Catchen (2017) to identify and extract SNPs using gstacks (var-alpha 0.001; gt-alpha 0.001; min-mapq 75) from uniquely aligned reads. Using Stacks v2.65 population module, SNPs with a minor allele frequency (MAF) below 0.05 and with more than 10% missing data were discarded, and only SNPs detected in at least 85% of the samples in each population were retained for further analysis (Catchen et al., 2013).

## 2.6 Analysis of genetic differentiation within and among populations

Mean observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), and individual inbreeding coefficient ( $F_{IS}$ ) were estimated from filtered SNP dataset using population module in Stacks software v2.65 (Rochette et al., 2019). Pairwise  $F_{ST}$  values among all populations were estimated using the R software package v4.3.3, StAMPP v1.6.3 (Pembleton et al., 2013). To visualize the genetic relationship between the populations and individuals, a neighbor-joining phylogenetic

tree was constructed with Treebest v1.9.2 (Vilella et al., 2009). STRUCTURE v2.3.4 software (Pritchard et al., 2000) was used for clustering analysis, with a burn-in of 100,000, followed by 500,000 iterations with three replications for each  $k$  ( $k$  1 to 10). The membership coefficients and determination of the most probable number of clusters (average  $\Delta k$  for each  $k$ ) were determined using STRUCTURE HARVESTER (Earl and vonHoldt, 2012) software package based on Evanno method (Evanno et al., 2005). Moreover, principal component analysis (PCA) was conducted using the R software package *adeigenet* v2.1.3 (Jombart, 2008) to check the presence of genetic clustering.

## 3 Results

### 3.1 Sequence quality control and SNP discovery

The ddRAD sequencing of 56 fish from three locations has resulted in about 101 million paired-end reads (150 bp paired-end reads). After demultiplexing into individual samples and quality control (filtering) steps, 93.4% of the raw reads were retained for alignment to the reference genome. Alignment of the filtered reads to the Nile tilapia reference genome (Assembly Id: GCA\_GCA\_001858045.2) resulted in an average mapping rate of 98.3%. After further quality control steps, 85,267 high quality SNPs in 56 individuals were retained for downstream analysis.

### 3.2 Analysis of genetic diversity and phylogenetic relationship

The expected heterozygosity ( $H_e$ ) and nucleotide diversity ( $\pi$ ) estimates considering variant positions demonstrated very slight differences (0.005–0.01) for all three populations suggesting the consistency of the genetic diversity estimates when assessed at both allelic and sequence levels (Tab. 2). The highest and lowest genetic diversity measurements ( $H_e$ ,  $H_o$ ,  $\pi$ , and  $F_{IS}$ ) values were observed in the samples from Sebeta and Batu fisheries research centers, respectively (Tab. 2). Population from Aweday fishponds showed intermediate values in all genetic diversity parameters (Tab. 2). Overall, the inbreeding level across all populations was low. However, the Sebeta population showed a notably higher inbreeding coefficient ( $F_{IS}$ ) compared to the Batu and Aweday population (Tab. 2).

The measure of genetic distance (population pairwise  $F_{ST}$ ) between the populations revealed moderate to low levels of genetic differentiation (Tab. 3). The highest  $F_{ST}$  value (0.068) was recorded between the populations of Aweday and Batu (Tab. 3). A slightly lower level of genetic differentiation was

**Table 2.** Genetic diversity parameters for the three farmed Nile tilapia populations.

Population	$N$	$H_e$ (SE)	$H_o$ (SE)	$\pi$ (SE)	$F_{IS}$ (SE)
Sebeta	20	0.28 (0.001)	0.28 (0.04)	0.29 (0.001)	0.032 (0.01)
Batu	21	0.19 (0.001)	0.20 (0.06)	0.20 (0.001)	0.006 (0.01)
Aweday	15	0.26 (0.001)	0.27 (0.05)	0.27 (0.001)	0.007 (0.01)

Note: (SE)=standard error;  $N$ =mean number of individuals genotyped per population;  $H_e$ =expected heterozygosity;  $H_o$ =observed heterozygosity;  $\pi$ =nucleotide diversity; and  $F_{IS}$ =inbreeding coefficient.

**Table 3.** Population pairwise  $F_{ST}$  values (confidence interval 95%) of the farmed Nile tilapia populations.

	Sebeta	Batu	Aweday
Sebeta	0		
Batu	0.054	0	
Aweday	0.028	0.068	0

observed between Sebeta and Batu populations ( $F_{ST}=0.054$ ) and the lowest genetic distance ( $F_{ST}=0.028$ ) was observed between Sebeta and Aweday populations (Tab. 3). In the phylogenetic tree analysis, two main clusters (Cluster 1 and Cluster 2) were identified, with Cluster 2 further subdivided into sub-clusters 2a and 2b after excluding a few outgroup samples (Fig. 2). Cluster 1 consists exclusively of samples from Aweday and Sebeta. Cluster 2 includes samples from all the three populations, with sub-cluster 2a containing only samples from Aweday and Sebeta, and sub-cluster 2b comprising all Batu samples along with a considerable number of samples from Sebeta (Fig. 2).

### 3.3 Analysis of population genetic structure

The STRUCTURE analysis suggested that the presence of two genetic clusters ( $K=2$ ) was the most probable number of genetic clusters (Supplementary information-1, SI-1). However, we also present the results for  $K=3$  and  $K=4$  to explore potential sub-structuring within the populations, as these values may still provide insights into finer genetic differentiation (Fig. 3). Here, all samples from Batu were grouped in one cluster (blue bars), while some samples from Sebeta and Aweday formed the second category showing marked levels of admixture (blue and orange bars). The remaining samples from Sebeta and Aweday (10 and 3, respectively) were grouped in the same category as samples from Batu. When the number of clusters was increased ( $K=3$  or  $K=4$ ), the 3rd cluster (full green bars) comprised samples from Batu, indicating the relative genetic distinctness of this population, in a similar manner to the results from genetic distance ( $F_{ST}$ ) and phylogenetic tree analysis.

For further verification of population genetic structure, principal component analysis (PCA) was performed to visualize genetic relationships between individuals by plotting the first two principal components (PC1 and PC2), while three-dimensional PCA (3DPCA) was used to visualize relationships using PC1, PC2, and PC3 (Fig. 4, SI-II). The first and second

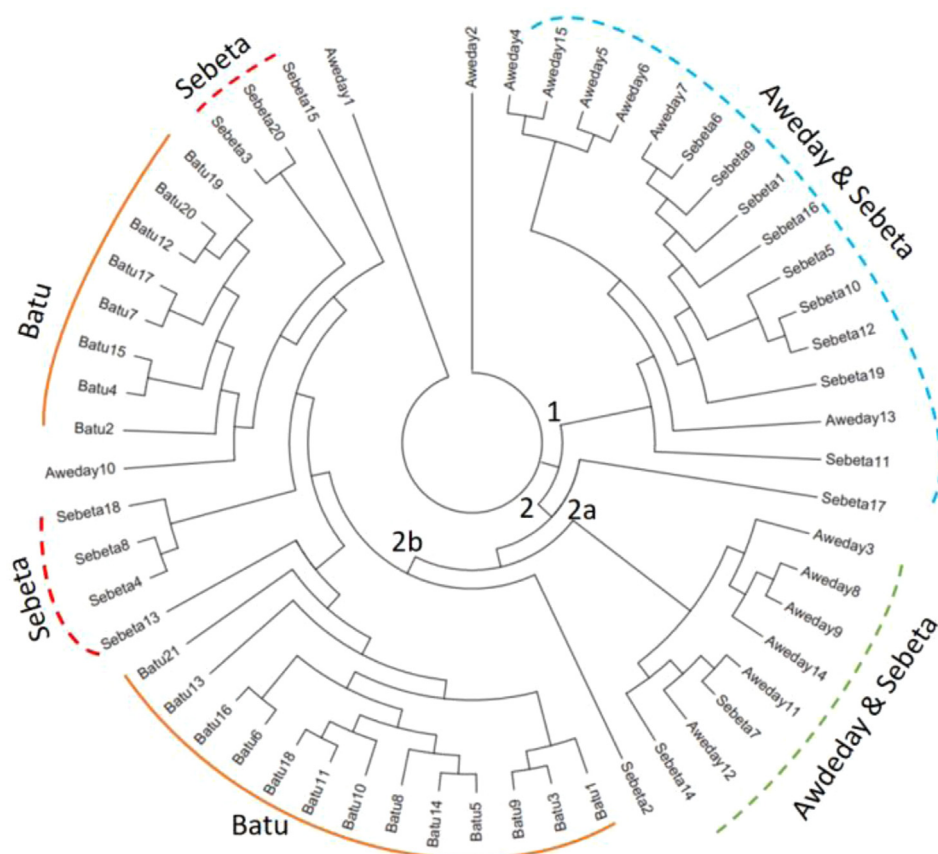
principal components (PC1 & PC2) accounted for 18.4% and 8.5% of total variations, respectively (Fig. 4). Here, according to PC1, the first cluster (cluster 1) comprises all of the samples from Batu together with some samples from Sebeta and Aweday, while the second cluster (cluster 2) comprises only individuals from Sebeta and Aweday (Fig. 4).

## 4 Discussion

### 4.1 Genetic diversity and differentiation

The results of the genetic diversity metrics (mean  $H_e=0.24$  and mean  $H_o=0.25$ ) indicate a moderate level of genetic diversity and a slight excess in heterozygosity, suggesting potential factors such as gene flow or hybridization in the studied cultured populations of Nile tilapia in Ethiopia. This estimate was higher than that of cultured Nile tilapia populations from Tanzania genotyped using ddRAD-seq method (Kajungiro et al., 2019). In another study on genetically improved Nile tilapia strains from Tanzania, Moses et al. (2020) reported observed and expected heterozygosity estimates comparable to our results (mean  $H_e=0.24$  and mean  $H_o=0.23$ ). However, Barria et al. (2023) reported a higher level of genetic diversity ( $H_e=0.35-0.41$  and  $H_o=0.3-0.413$ ) in selectively bred Nile tilapia populations from Asia and Africa. Usually, a high level of genetic diversity is expected in genetically improved strains produced by well-designed breeding programs that incorporate individuals from diverse genetic backgrounds into their founding population (Moses et al., 2020; Villanueva et al., 2022). In agreement with this, a study by Diyie et al. (2021) on cultured Nile tilapia populations from Ghana revealed higher observed and expected heterozygosity values in genetically improved Akosombo strain (produced by selective breeding program) compared to unimproved locally used farmed strains. The possible reasons for either a reduction in genetic diversity in improved strains from Tanzania or the maintenance of a moderate level of genetic diversity in cultured populations from Ethiopia may include poor broodstock management practices in Tanzania or the occasional addition of new wild broodfish to cultured stocks in Ethiopia, which may have helped to maintain a moderate level of within-population genetic diversity (Brummett, 2007).

The inbreeding coefficient ( $F_{IS}$ ) is important for inferring the presence of inbreeding, which occurs due to mating with closely related individuals and severely affects offspring fitness (Kardos et al., 2015). Our results show a slight loss of heterozygosity in the Sebeta population ( $F_{IS}=0.032$ ), while the remaining populations show close to neutral values



**Fig. 2.** Phylogenetic tree depicting genetic relationships among samples from the three populations.

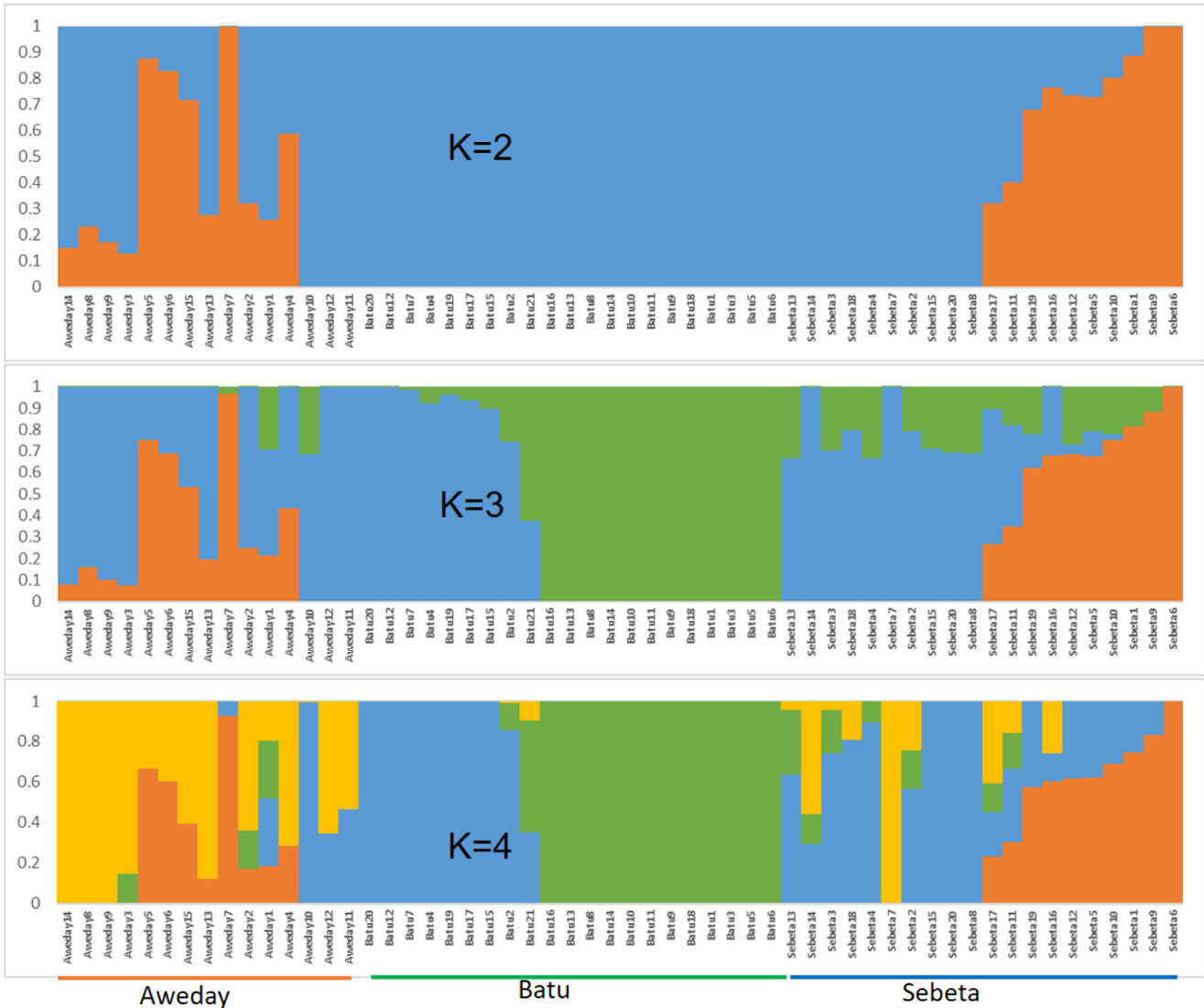
( $F_{IS} = 0.006/0.007$ ). This could be due to the relatively longer domestication history of Nile tilapia in Sebeta, since it is the earliest fish culture center in Ethiopia. The observed  $F_{IS}$  values were consistent with previous results obtained from locally produced cultured strains of Nile tilapia (not produced by selective breeding) (Kajungiro et al., 2019). A previous study conducted on aquaculture strains produced by selective breeding programs revealed negative inbreeding coefficient values characterized by excess of heterozygosity (Barria et al., 2023), which is expected because the founding individuals are usually sourced from diverse genetic backgrounds.

The loss of genetic diversity in aquaculture strains usually emanates from poor hatchery practices, founder effects (narrow genetic background of the founding populations), and the number of broodfish required to maintain healthy genetic variability (effective population size ( $N_e$ ) less than 100–150) (Brummett, 2007; Frost et al., 2006; Romana-Eguia et al., 2005). Genetic differentiation between the populations in the present study was relatively low (mean  $F_{ST} = 0.05$ ), suggesting relatedness in the gene pool of the source individuals. The founding individuals from which the cultured populations were sourced were usually located in the Ethiopian Rift Valley region, particularly in lakes such as Hora, Ziway, Hawassa, and Abaya-Chamo (pers. communication). A molecular genetic diversity study on these wild populations (from the Ethiopian Rift Valley region) by Tesfaye et al. (2021) using nuclear microsatellite markers, revealed a low level of

genetic differentiation based on population pairwise  $F_{ST}$  values.

#### 4.2 Population genetic structure and clustering

Population genetic structure and clustering analysis help to identify genetically distinct populations within a species and are crucial for understanding patterns of genetic differentiation and gene flow both within and between populations (Zhu et al., 2022). Population genetic structure analysis results from STRUCTURE, PCA, and phylogenetic tree suggest weak genetic differentiation. Results from all three analyses revealed genetic similarity between the Sebeta and Aweday populations, consistent with the history of translocation of strains from Sebeta to Aweday. Based on STRUCTURE analysis ( $K = 2$ ), except for three samples from Aweday and ten samples from Sebeta, the remaining samples show either complete similarity or some level of admixture with the Batu population (blue bars). However, about 57% of the Batu population (12 individuals) formed a unique genetic cluster at  $K = 3$  and  $K = 4$ , indicating further differentiation of the Batu population. The reason for the presence of genetically differentiated individuals among the Batu population could be due to the recent supplementation of new broodfish from Lake Chamo and its possible crossbreeding with existing stock due to stock mixing practices (pers. communication). Since broodfish supplementation involves the addition of new wild individuals to existing



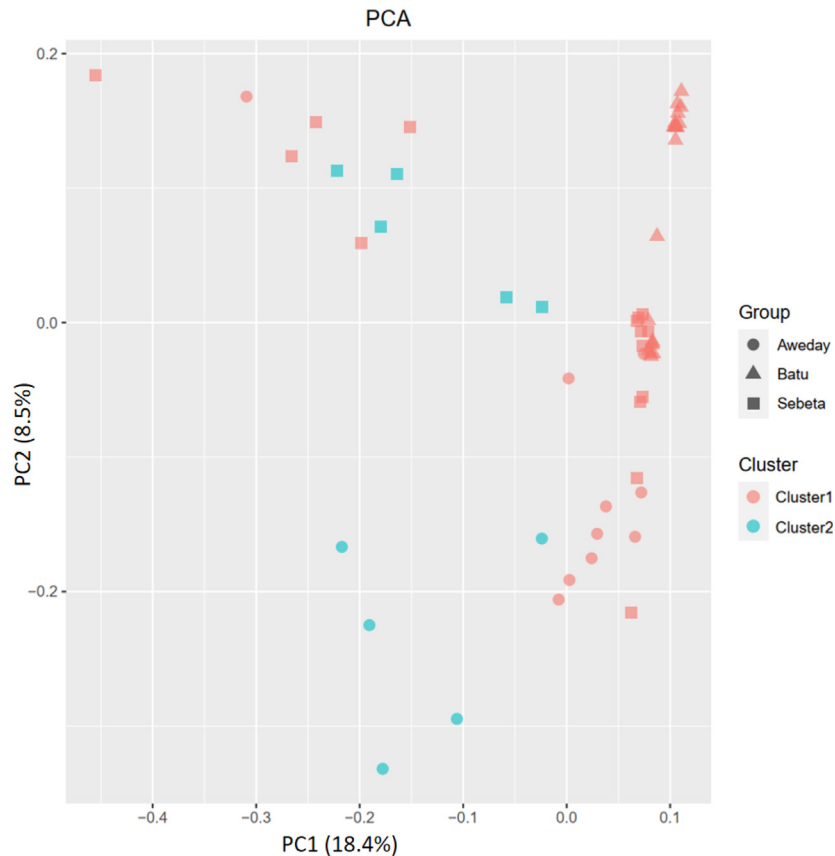
**Fig. 3.** STRUCTURE analysis showing  $K = 2, 3,$  and  $4$  for the three farmed populations. Each bottom label with vertical bars represents a single individual/sample and the proportion of colors in each bar represents the likelihood of a membership of an individual to a respective cluster.

stocks, this practice also common in many African countries (Brummett, 2007). Newly domesticated individuals from Lake Chamo have demonstrated better growth performance than existing stocks obtained from Lake Ziway and Sebeta; therefore, they are used for fingerling production and distribution to fish farmers (pers. communication; Workagegn et al., 2020). However, this result (better growth performance of newly domesticated seeds) should be considered with caution, as wild sourced seeds have been observed to exhibit superior growth performance compared with domesticated seeds raised in hatcheries lacking proper genetic management (Brummett et al., 2004; Brummett, 2007).

### 4.3 Suggestions for better hatchery management and development of synthetic strain

Hatchery managers are responsible for ensuring that high-quality seeds reach fish farmers and contribute to the growth of

the aquaculture sector. The decisions on fry production should include basic genetic principles, such as pedigree information and performance of individuals or stocks (Migaud et al., 2013; Hosoya et al., 2018; Longo et al., 2024). It is also worth realizing that in hatcheries without proper broodstock management, genetic problems (e.g., inbreeding and loss of genetic diversity) occur after a few generations, resulting in poor performance, and continuous supplementation with wild stocks only alleviates the problem temporarily (Brummett et al., 2004; Brummett, 2007; Longo et al., 2024). In addition, fish farmers need to continuously replace fish stocks with newly produced fingerlings from recognized hatcheries rather than relying on small broodfish of their own or unauthorized suppliers for subsequent productions (Shikuku et al., 2021). Given the current practices, genetic diversity measurements (revealed by this study) of the present cultured strains will change shortly, with unpredictable consequences. To address this unpredictability and meet market demand, the government should prioritize capacity building and consider outsourcing



**Fig. 4.** Principal component analysis (PCA) of the cultured populations. Each plot represents an individual sample from a respective site and similar colors indicate clustering into the same category.

seed production to licensed hatcheries (Brummett, 2007; Shikuku et al., 2021). Moreover, the development of new synthetic strains should be pursued, incorporating existing stocks and outcrossing with selected wild populations to establish good-quality broodstock (Brummett, 2007; Migaud et al., 2013; Guo et al., 2022; Longo et al., 2024). Incorporating wild individuals, potentially sourced from regions outside Rift Valley, into the base population would enhance the genetic diversity of the synthetic strain. This step is necessary as existing cultured populations exhibit high relatedness (Fig. 3) and also demonstrate lower within-population genetic diversity estimates compared to the improved strains studied by Barría et al. (2023) (mean  $H_e$ , 0.24 vs 0.37). The selection process may benefit from existing genetic information obtained from genetic diversity studies of both farmed (this study) and wild populations (Tesfaye et al., 2021), along with performance evaluation experiments conducted on wild populations (Workagegn and Gjøen, 2012; Workagegn et al., 2020). In addition, genomic information from cultured populations can help prevent the loss of native genetic diversity. For instance, by providing insights into the genetic background of these populations, it can guide decisions to avoid translocations to areas where native populations have unrelated genetic backgrounds (Kajungiro et al., 2019).

## 5 Conclusion

This study provides valuable insights into the genetic background of cultured Nile tilapia populations in Ethiopia. Overall, the cultured populations demonstrated moderate levels of genetic diversity and weak genetic differentiation. Genetic problems such as high inbreeding levels and very low levels of genetic diversity were avoided, likely due to broodstock supplementation practices from wild populations. However, these practices may not substitute for proper broodstock management aimed at maintaining long-term genetic diversity. The absence of distinct genetic lines (strains) with better growth performance and pedigree records in Ethiopian hatchery centers emphasizes the challenge of accessing good-quality seeds. Hence, this study may serve as an impetus for the establishment of a good broodstock management strategy and development of Nile tilapia strains with a broader genetic background in Ethiopia.

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## Conflicts of interest

The authors declare no conflict of interest.

## Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

## Supplementary material

**Fig. SI-1.** Details on STRUCTURE analysis and determination of most probable of genetic clusters.

The Supplementary Material is available at <https://www.alr-journal.org/10.1051/alr/2024018/olm>.

## References

- Abwao J, Jung'a J, Barasa JE, Kyule D, Opiyo M, Awuor JF, Ogello E, Munguti JM, Keya GA. 2023. Selective breeding of Nile tilapia, *Oreochromis niloticus*: a strategy for increased genetic diversity and sustainable development of aquaculture in Kenya. *J Appl Aquacult* 35: 237–256.
- Adeleke B, Robertson-Andersson D, Moodley G, Taylor S. 2021. Aquaculture in Africa: A comparative review of Egypt, Nigeria, and Uganda Vis-À-Vis South Africa. *Rev Fish Sci Aquacult* 29: 167–197.
- Adugna BT, Goshu G. 2010. Integrating aquaculture with traditional farming system: Socioeconomic assessment in the Amhara Region, Ethiopia. *Ecohydrol Hydrobiol* 10: 223–230.
- Anane-Taabeah G, Frimpong E, Hallerman E. 2019. Aquaculture-mediated invasion of the genetically improved farmed tilapia (Gift) into the lower Volta Basin of Ghana. *Diversity* 11: 188.
- Ansah YB, Frimpong EA, Hallerman EM. 2014. Genetically-improved tilapia strains in Africa: potential benefits and negative impacts. *Sustainability* 6: 3697–3721.
- Barria A, Peñaloza C, Papadopoulou A, Mahmuddin M, Doeschl-Wilson A, Benzie JAH, Houston RD, Wiener P. 2023. Genetic differentiation following recent domestication events: a study of farmed Nile tilapia (*Oreochromis niloticus*) populations. *Evol Appl* 16: 1220–1235.
- Brummett RE. 2007. Freshwater fish seed supply: Africa regional synthesis. In: M.G. Bondad-Reantaso (ed.). Assessment of freshwater fish seed resources for sustainable aquaculture (pp. 41–58). FAO Fisheries Technical Paper. No. 501. Rome, FAO. 2007. 628p.
- Brummett RE, Angoni DE, Pouomogne V. 2004. On-farm and on-station comparison of wild and domesticated Cameroonian populations of *Oreochromis niloticus*. *Aquaculture* 242: 157–164.
- Brummett RE, Lazard J, Moehl J. 2008. African aquaculture: realizing the potential. *Food Policy* 33: 371–385.
- Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. 2013. Stacks: an analysis tool set for population genomics. *Mol Ecol* 22: 3124–3140.
- Conte MA, Gammerding WJ, Bartie KL, Penman DJ, Kocher TD. 2017. A high quality assembly of the Nile tilapia (*Oreochromis niloticus*) genome reveals the structure of two sex determination regions. *BMC Genomics* 18: 341.
- D'Ambrosio J, Phocas F, Haffray P, Bestin A, Brard-Fudulea S, Poncet C, Quillet E, Dechamp N, Frasin C, Charles M, Dupont-Nivet M. 2019. Genome-wide estimates of genetic diversity, inbreeding and effective size of experimental and commercial rainbow trout lines undergoing selective breeding. *Genet Sel Evol* 51: 26.
- Delomas TA, Gomelsky B, Vu N, Campbell MR, Novelo ND. 2019. Single-nucleotide polymorphism discovery and genetic variation in YY-male and mixed-sex strains of Nile tilapia available in the United States. *North Am J Aquacult* 81: 183–188.
- Di Santo LN, Hoban S, Parchman TL, Wright JW, Hamilton JA. 2022. Reduced representation sequencing to understand the evolutionary history of Torrey pine (*Pinus torreyana parry*) with implications for rare species conservation. *Mol Ecol* 31: 4622–4639.
- Diye RL, Agyarkwa SK, Armah E, Amonoo NA, Owusu-Frimpong I, Osei-Atweneboana MY. 2021. Genetic variations among different generations and cultured populations of Nile Tilapia (*Oreochromis niloticus*) in Ghana: application of microsatellite markers. *Aquaculture* 544: 737070.
- Earl DA, vonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* 4: 359–361.
- El-Sayed A-FM, Fitzsimmons K. 2023. From Africa to the world—The journey of Nile tilapia. *Rev Aquacult* 15: 6–21.
- Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol Ecol* 14: 2611–2620.
- Fagbémi MNA, Pigneur L, André A, Smitz N, Gennotte V, Michaux JR, Mélard C, Lalèye PA, Rougeot C. 2021. Genetic structure of wild and farmed Nile tilapia (*Oreochromis niloticus*) populations in Benin based on genome wide SNP technology. *Aquaculture* 535: 736432.
- FAO. 2024. Fishery and Aquaculture Statistics – Yearbook 2021. *FAO Yearbook of Fishery and Aquaculture Statistics*. Rome. <https://doi.org/10.4060/cc9523en>
- FAO. 2023. Fishery and Aquaculture Statistics. Global aquaculture production 1950–2021 (FishStatJ). In: *FAO Fisheries and Aquaculture Division [online]*. Rome. Updated 2023. [www.fao.org/fishery/en/statistics/software/fishstatj](http://www.fao.org/fishery/en/statistics/software/fishstatj)
- Frost LA, Evans BS, Jerry DR. 2006. Loss of genetic diversity due to hatchery culture practices in barramundi (*Lates calcarifer*). *Aquaculture* 261: 1056–1064.
- Geletu TT, Zhao J. 2023. Genetic resources of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) in its native range and aquaculture. *Hydrobiologia* 850: 2425–2445.
- Guo X-Z, Chen H-M, Wang A-B, Qian X-Q. 2022. Population genetic structure of the yellow catfish (*Pelteobagrus fulvidraco*) in China inferred from microsatellite analyses: implications for fisheries management and breeding. *J World Aquacult Soc* 53: 174–191.

- Hamilton MG, Lind CE, Barman BK, Velasco RR, Danting MJ, Benzie JA. 2020. Distinguishing between Nile tilapia strains using a low-density single-nucleotide polymorphism panel. *Front Genet* 11: 594722.
- Hinrichsen E, Walakira JK, Langi S, Ibrahim NA, Tarus V, Badmus O, Baumüller H. 2022. *Prospects for aquaculture development in Africa: A review of past performance to assess future potential*, ZEF Working Paper Series, No. 211, University of Bonn, Center for Development Research (ZEF), Bonn. <https://nbn-resolving.de/urn:nbn:de:101:1-2022021001555935838790>
- Hosoya S, Kikuchi K, Nagashima, H, Onodera J, Sugimoto K, Satoh K, Matsuzaki K, Yasugi M, Nagano AJ, Kumagayi A, Ueda K, Kurokawa T. 2018. Assessment of genetic diversity in Coho salmon (*Oncorhynchus kisutch*) populations with no family records using ddRAD-seq. *BMC Res Notes* 11: 548.
- Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24: 1403–1405.
- Kajungiro RA, Palaiokostas C, Pinto FAL, Mmochi AJ, Mtolera M, Houston RD, De Koning DJ. 2019. Population structure and genetic diversity of Nile tilapia (*Oreochromis niloticus*) strains cultured in Tanzania. *Front Genet* 10: 1269.
- Kardos M, Luikart G, Allendorf F. 2015. Measuring individual inbreeding in the age of genomics: marker-based measures are better than pedigrees. *Heredity* 115: 63–72.
- Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9: 357–359.
- Li C. 2022. Conservation of genetic resources for sustainable aquaculture. *J World Aquacult Soc* 53: 4–7.
- Longo A, Kurta K, Vanhala T, Jeuthe H, de Koning D-J, Palaiokostas C. 2024. Genetic diversity patterns in farmed rainbow trout (*Oncorhynchus mykiss*) populations using genome-wide SNP and haplotype data. *Anim Genet* 55: 87–98.
- McKinna EM, Nandlal S, Mather PB, Hurwood DA. 2010. An investigation of the possible causes for the loss of productivity in genetically improved farmed tilapia strain in Fiji: inbreeding versus wild stock introgression. *Aquacult Res* 41: e730–e742.
- Migaud H, Bell G, Cabrita E, McAndrew B, Davie A, Bobe J, Herráez MP, Carrillo M. 2013. Gamete quality and broodstock management in temperate fish. *Rev Aquacult* 5: S194–S223.
- Mireku KK, Kassam D, Changadeya W, Attipoe FYK, Adinortey CA. 2017. Assessment of genetic variations of Nile Tilapia (*Oreochromis niloticus* L.) in the Volta Lake of Ghana using microsatellite markers. *Afr J Biotechnol* 16: 312–321.
- Moses M, Mtolera MSP, Chauka LJ, Lopes FA, De Koning DJ, Houston RD, Palaiokostas C. 2020. Characterizing the genetic structure of introduced Nile tilapia (*Oreochromis niloticus*) strains in Tanzania using double digest RAD sequencing. *Aquacult Int* 28: 477–492.
- Natea G. 2018. Aquaculture potential, status, constraints and future prospects in Ethiopia: a review. *Int J Adv Res* 7: 336–343.
- Nayfa MG, Jones DB, Benzie J, Jerry DR, Zenger KR. 2020. Comparing genomic signatures of selection between the Abbassa strain and eight wild populations of Nile tilapia (*Oreochromis niloticus*) in Egypt. *Front Genet* 11: 567969.
- Nazareno AG, Bemmels JB, Dick CW, Lohmann L. 2017. Minimum sample sizes for population genomics: an empirical study from an Amazonian plant species. *Mol Ecol Resour* 17: 1136–1147.
- Pembleton LW, Cogan NOI, Forster JW. 2013. StAMPP: An R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Mol Ecol Resour* 13: 946–952.
- Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One* 7: e37135.
- Robledo D, Ogwang J, Byakora E, Nascimento-Schulze JC, Benda KK, Frasin C, Salisbury S, Solimo M, Mayega JF, Peter B, Masembe C, Houston R, Mukibi R. 2024. Genetic diversity and population structure of farmed and wild Nile tilapia (*Oreochromis niloticus*) in Uganda: the potential for aquaculture selection and breeding programs. *Genomics* 116: 110781.
- Rochette N, Catchen J. 2017. Deriving genotypes from RAD-seq short-read data using Stacks. *Nat Protoc* 12: 2640–2659.
- Rochette NC, Rivera-Colón AG, Catchen J. 2019. Stacks 2: Analytical methods for paired-end sequencing improve RAD-seq-based population genomics. *Mol Ecol* 28: 4737–4754.
- Romana-Eguia MRR, Ikeda M, Basiao ZU, Taniguchi N. 2005. Genetic changes during mass selection for growth in Nile tilapia, *Oreochromis niloticus* (L.), assessed by microsatellites. *Aquacult Res* 36: 69–78.
- Shechonge A, Ngatunga BP, Tamatamah R, Bradbeer SJ, Harrington J, Ford AGP, Turner GF, Genner MJ. 2018. Losing cichlid fish biodiversity: genetic and morphological homogenization of tilapia following colonization by introduced species. *Conserv Genet* 19: 1199–1209.
- Shikuku M, Tran N, Joffre OM, Islam AHMS, Barman BK, Ali S, Rossignoli CM. 2021. Lock-ins to the dissemination of genetically improved fish seeds. *Agric Syst* 188: 103042.
- Sunde J, Yıldırım Y, Tibblin P, Forsman A. 2020. Comparing the performance of microsatellites and RADseq in population genetic studies: Analysis of data for pike (*Esox lucius*) and a synthesis of previous studies. *Front Genet* 11: 218.
- Syaifuludin M, Bekaert M, Taggart JB, Bartie KL, Wehner S, Palaiokostas C, Khan MGQ, Selly S-LC, Hulata G, D'Cotta H, Baroiller J-F, McAndrew BJ, Penman DJ. 2019. Species-specific marker discovery in tilapia. *Sci Rep* 9: 13001.
- Taniguchi N. 2003. Genetic factors in broodstock management for seed production. *Rev Fish Biol Fish* 13: 177–185.
- Tesfaye G, Curto M, Meulenbroek P, Englmaier GK, Tibihika PD, Alemayehu E, Getahun A, Meimberg H. 2021. Genetic diversity of Nile tilapia (*Oreochromis niloticus*) populations in Ethiopia: insights from nuclear DNA microsatellites and implications for conservation. *BMC Ecol Evol* 21: 113.
- Tesfaye G, Wolff M. 2014. The state of inland fisheries in Ethiopia: a synopsis with updated estimates of potential yield. *Ecohydrol Hydrobiol* 14: 200–219.
- Trewavas E. 1983. Tilapiine fishes of the genera *Sarotherodon*, *Oreochromis*, and *Danakilia*. Publication No. 898. British Museum of Natural History, London, UK.
- Trinh TQ, Agyakwah SK, Khaw HL, Benzie JAH, Attipoe FKY. 2021. Performance evaluation of Nile tilapia (*Oreochromis niloticus*) improved strains in Ghana. *Aquaculture* 530: 735938.
- Vilella AJ, Severin J, Ureta-Vidal A, Heng L, Durbin R, Birney E. 2009. EnsemblCompara GeneTrees: complete, duplication-aware phylogenetic trees in vertebrates. *Genome Res* 19: 327–335.
- Villanueva B, Fernández A, Peiró-Pastor R, Peñaloza C, Houston RD, Sonesson AK, Tsigonopoulos CS, Bargelloni L, Gamsız K, Karahan B, Gökçek EÖ, Fernández J, Saura M. 2022. Population structure and genetic variability in wild and farmed Mediterranean populations of gilthead seabream and European seabass inferred from a 60K combined species SNP array. *Aquacult Rep* 24: 101145.

- Wakjira M, Tolemariam T, Kim TJ, Kim KR. 2013. Aquaculture development in Ethiopia: review on potential and strategy. *J Agric Life Environ Sci* 25: 20–25.
- Workagegn KB, GjØen HM. 2012. Comparison studies on growth performance of four juvenile *O. niloticus* strains in pond culture, Ethiopia. *Int J Aquacult* 2: 40–47.
- Workagegn KB, Natarajan P, Gedebo A. 2020. Genetic parameters and genotype by environment interaction of the Nile tilapia (*Oreochromis niloticus*) reared in two test environments. *Aquacult Int* 28: 2263–2273.
- Yalew A, Dejen E, Spliethoff P. 2015. *Investment opportunities in the Ethiopian aquaculture sub-sector*. <https://www.rvo.nl/sites/default/files/2015/11/Rapport%20Aquaculture%20Ethiopia.pdf>
- Yáñez JM, Joshi R, Yoshida GM. 2020. Genomics to accelerate genetic improvement in tilapia. *Anim Genet* 51: 658–674.
- Yoshida GM, Barria A, Correa K, Cáceres G, Jedlicki A, Cadiz MI, Lhorente JP, Yáñez JM. 2019. Genome-wide patterns of population structure and linkage disequilibrium in farmed Nile tilapia (*Oreochromis niloticus*). *Front Genet* 10: 745.
- Zhu W, Fu J, Luo M, Wang L, Wang P, Liu Q, Dong Z. 2022. Genetic diversity and population structure of bighead carp (*Hypophthalmichthys nobilis*) from the middle and lower reaches of the Yangtze River revealed using microsatellite markers. *Aquacult Rep* 27: 101377.

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