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Original Article

Genetic diversity and population structure of farmed and wild Nile tilapia (*Oreochromis niloticus*) in Uganda: The potential for aquaculture selection and breeding programs

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ABSTRACT

Nile tilapia is one of the most important aquaculture species globally, providing high-quality animal protein for human nutrition and a source of income to sustain the livelihoods of many people in low- and middle-income countries. This species is native to Africa and nowadays farmed throughout the world. However, the genetic makeup of its native populations remains poorly characterized. Additionally, there has been important introgression and movement of farmed (as well as wild) strains connected to tilapia aquaculture in Africa, yet the relationship between wild and farmed populations is unknown in most of the continent. Genetic characterization of the species in Africa has the potential to support the conservation of the species as well as supporting selective breeding to improve the indigenous strains for sustainable and profitable aquaculture production. In the current study, a total of 382 fish were used to investigate the genetic structure, diversity, and ancestry within and between Ugandan Nile tilapia populations from three major lakes including Lake Albert (L. Albert), Lake Kyoga (L. Kyoga) and Lake Victoria (*L. Victoria*), and 10 hatchery farms located in the catchment regions of these lakes. Our results showed clear genetic structure of the fish sourced from the lakes, with L. Kyoga and L. Albert populations showing higher genetic similarity. We also observed noticeable genetic structure among farmed populations, with most of them being genetically similar to L. Albert and L. Kyoga fish. Admixture results showed a higher (2.55–52.75%) contribution of L. Albert / L. Kyoga stocks to Uganda's farmed fish than the stock from L. *Victoria* (2.12–28.02%). We observed relatively high genetic diversity across both wild and farmed populations, but some farms had sizable numbers of highly inbred fish, raising concerns about management practices. In addition, we identified a genomic region on chromosome 5, harbouring the key innate immune gene *BPI* and the key growth gene *GHRH,* putatively under selection in the Ugandan Nile tilapia population. This region overlaps with the genomic region previously identified to be associated with growth rate in farmed Nile tilapia.

1. Introduction

Fish is a major source of food for humans, providing to over 4.5 billion people a nutritious source of protein and high-quality nutrients [1,2]. In addition to food and nutritional security, fish also serve as an important source of livelihood by creating business and employment opportunities for up to 59 million people involved in the fish value chain [3]. In Uganda, fish is an important resource contributing ~USD 900 million to the national economy annually [4]. This contribution is projected to increase due to the growing demand for fish within Uganda and the East African region as a consequence of the rapidly growing population [5,6]. Fish in Uganda is predominantly sourced from wild habitats such as L. Albert, L. Kyoga, *L. Victoria*, L. Edward, L. Kyoga, and River Nile [5]. Even under controlled fishing restrictions ongoing on these

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water bodies, the in the country and the region has resulted into the depletion of certain fish species in multiple locations [6,7]. Aquaculture offers a more sustainable and viable solution to alleviate the stress on wild aquatic ecosystems and meet the current and future food demands in the region [6,8]. As a result, the aquaculture industry in Uganda has experienced significant growth in recent years and now accounts for 20% of the national annual fish production [6,8–10].

Globally, Nile tilapia (*Oreochromis niloticus*) is the third most cultured freshwater species accounting for 9% of the total inland aquaculture output [3]. Similarly, Nile tilapia is also an important aquaculture species in Uganda, contributing approximately 49% of national annual aquaculture production output [8–10]. Unfortunately, the productivity of the industry, especially of small and medium-sized farms, remains constrained by the lack of improved Nile tilapia strains with enhanced growth rates and feed assimilation [11,12]. Though there are efforts by numerous private and public hatcheries to produce Nile tilapia fry for commercial farmers, advances in production have been minimal due to strong reliance on unimproved wild Nile tilapia as a source of breeding stock for the aquaculture industry [13,14]. Additionally, like most aquaculture production systems in Africa, there is little application of modern genomics and genetics tools in most of the hatcheries in the country, further contributing to the slow improvement of the indigenous strain for profitable aquaculture [13]. Genomic characterization of farmed and wild populations has the potential to support selective breeding for genetically enhanced tilapia strains for aquaculture through monitoring the levels of inbreeding and genetic diversity of the breeding populations as well as identifying and recruiting suitable diverse founder broodstock [15,16]. With respect to the wild populations, genomic characterization supports the sustainable conservation of such populations through monitoring of their genetic diversity and monitoring genetic admixture with escaping farmed strains that could impact their fitness to survive in natural habitats [15–17]. There have been efforts to pursue genetic characterization of both wild [18–21] and farmed [18] Nile tilapia populations in Uganda. Genetic structure analyses from these studies have revealed genetic relationships between fish from the different major wild habitats in Uganda. For example, close genetic similarities were observed between fish from L. Albert and L. Kyoga, while L. *Victoria* fish was genetically unique from fish from the former two lakes. These observations suggest that the stocks used to introduce Nile tilapia into L. Kyoga were most likely sourced from L.Albert [18,20,21]. With respect to farmed Nile tilapia populations in Uganda, Tibihika et al. 2020 demonstrated genetic overlap of these populations with the wild populations, with L. Albert, L. Kyoga and River Nile, as well as L. Edward, L. George and Kazinga Chanel populations showing predominant genetic contribution to the farmed populations included in that study. However, *L. Victoria* had very little contribution to the genetics of the farmed populations considered in that study. Together, these studies provide knowledge has that has potential application in the species conservation and development programs. However, it is worth highlighting that these studies relied on a few randomly amplified polymorphic DNA [20], and microsatellite [19,21] genomic markers that are likely to provide minimal genome coverage for overall genomic characterization of the studied populations.

Therefore, the objective of the current study was to utilize genome-wide SNP markers to genetically characterize farmed and wild Nile tilapia from hatcheries across the country, and three major lakes (Albert, Kyoga, and Victoria) in Uganda. We investigated the genomic inbreeding, diversity, and admixture across 13 Ugandan populations, as well as the genetic structure within and between these populations. We also investigated the potential introgression of GIFT-derived strain into Uganda's Nile tilapia populations. In addition, we performed genome-wide runs of homozygosity analysis to investigate potential genomic regions that could be foci for important traits via natural or artificial selection in the Ugandan Nile tilapia populations.

2. Materials and methods

2.1. Ethical approval

All procedures and protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Makerere University (SVAR_IACUC/133/2022) and registered by the Uganda National Council for Science and Technology (A269ES).

2.2. Fish sampling and genotyping

A total of 382 Nile tilapia fish were sampled in March of 2023 from open waters of the three major lakes in Uganda including L. *Victoria* ($n = 37$), L. Kyoga ($n = 24$), and L. Albert ($n = 37$) as well as Nile tilapia hatchery farms ($n = 286$) in the catchment areas of these three lakes (Fig. 1 and Table 1). Wild fish were sampled using a fishing net of 0.5 in. mesh size, 2 m width and 40 m in length, while farmed fish were sampled using a seine net of 0.5 in. mesh, 2 m width and 25 m length. The fish were humanely anesthetized by immersing them in a Tricaine Methane Sulphate (MS-222) solution of 100 mg/l for one minute and subsequently, fish were then weighed, and measured for heart girth, standard length, and total length as summarized in Table 1. Then an anal fin clip of 0.5cm^2 was collected from each fish using sharp scissors. The collected tissue was immediately preserved in 1.5 ml of 97% ethanol and thereafter stored in a fridge at 4 °C. The collected fin clip tissue was divided into two halves, of which approximately 0.25cm^2 was transferred into cryo-vials containing fresh 97% ethanol and shipped to Neogen Ltd.'s genotyping laboratory in Nebraska, USA. Briefly, genomic DNA was extracted from each individual fin clip tissue using magnetic bead DNA extraction method [22], and DNA concentration of 15-20 ng/ul was considered sufficient for the genotyping process. DNA was also evaluated for quality using the Thermo Scientific NanoDrop Spectrophotometer (Thermo Fisher Scientific, Cleveland, OH, USA), and DNA samples were considered acceptable for genotyping if they had A260/A280 and A260/A280 absorbance ratios of 2.0–2.2 and 1.8–2.2 respectively. Subsequently, each fish was genotyped for 60,785 genome-wide SNP markers on a custom-made Thermofisher Axiom Nile tilapia array (Axiom_OreNil50), designed by Benchmark Genetics (Bergen, Norway) and shared in-kind for this study. To promote the conservation of Uganda's indigenous Nile tilapia strains and stocks, the importation of GIFT-derived strains is prohibited in the country, however, there are speculations of the importation of GIFT strains into the country by some hatcheries that could potentially jeopardize the conservation efforts. In this regard, Benchmark Genetics (Bergen, Norway) shared the genotype data of 30 Nile tilapia fish from their GIFT-like strain breeding program, which were used as an outgroup population for independent comparisons, as well as estimation of potential GIFT introgressions into Ugandan Nile Tilapia populations in the current study. These samples are referred to as the BMK population in this study. These fish were genotyped on the same array (Axiom_OreNil50) as the Ugandan populations.

2.3. Data analyses

2.3.1. Genotype data processing and cleaning

Plink software version 1.9 [23] was used to remove fish samples that had low genotyping rates (< 90%; --mind 0.1). In addition, SNP markers that had high missing genotype rates (> 5%; --geno 0.05), very low minor allele frequencies (< 0.005; --maf 0.005) or deviated from Hardy-Weinberg equilibrium ($P < 1E\text{-}4$; --hwe 1E-4) were also removed from the final dataset.

2.3.2. Population genetic structure and admixture analysis

To investigate genetic structure within and between the Nile tilapia populations in this study, we performed discriminant analysis of principal components (DAPC) [24] in R v4.2.1 [25] using the adegenet v2.1.1 package [26]. Firstly, a vcf file of the SNP genotypes was

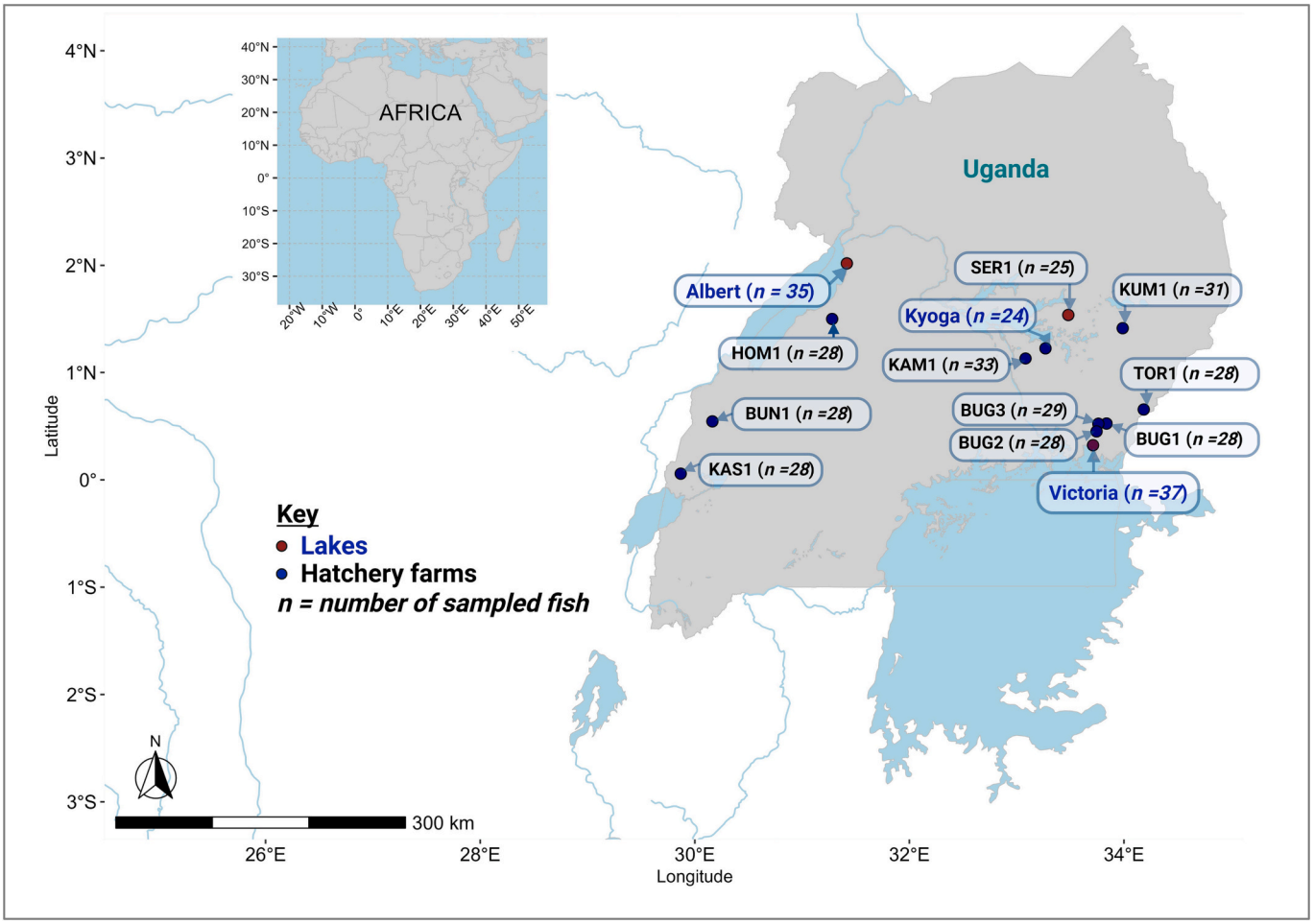

Fig. 1. Map showing sample collection sites including 10 hatchery farms and three lakes (Albert, Kyoga, and Victoria).

Table 1
Morphometric details of the fish samples from the 10 hatchery farms and 3 lakes; Weight = average total body weight at sampling, TL = average total length, SL = average standard length, HG = average heart girth, SE = standard error.

Population	District	Source (Farmed/Wild)	Sample size (*n*)	Weight (g) ± SE	SL (cm) ± SE	TL (cm) ± SE	HG (cm) ± SE	Age (months)
L. Albert	Buliisa	Wild	35	132.53 ± 6.25	15.42 ± 0.28	19.38 ± 0.33	14.92 ± 0.4	4
BUG1	Bugiri	Farmed	28	93.71 ± 2.35	13.55 ± 0.14	17.02 ± 0.21	12.66 ± 0.16	–
BUG2	Bugiri	Farmed	28	100.71 ± 5.46	13.7 ± 0.27	17.23 ± 0.34	13.16 ± 0.26	14
BUG3	Bugiri	Farmed	29	99.62 ± 5.35	13.54 ± 0.27	17.04 ± 0.34	13.15 ± 0.26	4
BUN1	Bunyangabu	Farmed	28	112.68 ± 6.10	14.96 ± 0.26	18.73 ± 0.29	14.11 ± 0.27	4
HOM1	Hoima	Farmed	28	126.42 ± 31.5	14.09 ± 0.42	17.66 ± 0.48	12.96 ± 0.38	7
KAM1	Kamuli	Farmed	33	198.74 ± 11.56	18.41 ± 0.35	28.84 ± 5.83	24.49 ± 8.14	7
KAS1	Kasese	Farmed	28	64.46 ± 2.83	12.66 ± 0.23	15.8 ± 0.27	11.54 ± 0.2	5
KUM1	Kumi	Farmed	31	97.33 ± 3.67	14.81 ± 0.19	17.89 ± 0.27	12.9 ± 0.18	–
L. Kyoga	Buyende	Wild	24	116.32 ± 37.2	12.04 ± 1.12	15.24 ± 1.43	12.29 ± 1.17	4
SER1	Serere	Farmed	25	87.12 ± 3.87	14.02 ± 0.2	23.65 ± 6.02	12.45 ± 0.18	8
TOR1	Tororo	Farmed	28	318.45 ± 10.62	21.55 ± 0.23	26.66 ± 0.27	18.49 ± 0.22	7
L. Victoria	Bugiri	Wild	37	27.01 ± 1.48	9.82 ± 0.16	11.79 ± 0.18	9.71 ± 0.17	–

generated using Plink software version 1.9 (Purcell et al. 2007), the VCF file then imported into R for analysis using vcfR v1.14.0 package [27]. To choose the optimal number of principal components (P-axes) to retain for DAPC models, the 'adegenet' cross-validation function 'xval-Dapc' was applied across 500 replicates. The maximum number of P-axes was set to one less than the number of sampling sites, mitigating potential overplotting and subsequent overestimation of spatial structuring in the tested populations [28]. Visualization of DAPC model results was performed using the R package ggplot2 v3.4.2 [29]. The first and second principal components from the discriminatory analysis were used to visualize genetic structure and variation of within and between the investigated populations using the R package ggplot2 version 3.4.2 [29]. To further assess genetic differentiation between pairs of the studied populations, we computed the pairwise average fixation indexes (F_{ST}) using Weir and Cockerham's method implemented in the R package dartR version 2.7.2 [30] using the gl.fst.pop() function with the number of bootstraps ("*nboots*") set at 100 and a confidence interval ("*percent*") of 95%. Corrected *P*-value for Fst estimate was calculated as the proportion of bootstrapped FST values ≤ 0 from the bootstrapping FST estimations. Subsequently visualized as a heatmap using the R package ggplot2 version 3.4.2 [29]. Reynold's genetic distances between the populations were estimated from the genotype data using

dartR version 2.7.2 [30], these distances were then used to construct an unrooted phylogenetic tree for the studied populations using Splitstree version 4.19.0 [31]. To assess genetic ancestries of each of the studied fish, we performed unsupervised admixture/ancestry in ADMIXTURE software version 1.3.0 [32]. To identify the most likely number of background ancestries in the sampled fish, we performed five-fold cross-validation admixture analyses from two (K = 2) to 14 (K = 14) ancestral populations, and the K value with the lowest cross-validation error was selected as the optimal number of ancestral populations within our studied fish.

2.3.3. Genetic diversity and inbreeding analysis

To investigate within-population genetic diversity in these Nile tilapia populations, we estimated genomic inbreeding coefficient (Fis) of each fish based on expected and observed genomic homozygosity as implemented in Plink version 1.9 [23] using the *–het* flag. In addition, we estimated individual fish's expected and observed heterozygosity using the *gl.report.heterozygosity()* function in the dartR version 2.7.2 package [30].

2.3.4. Runs of homozygosity identification

Finally, to investigate genomic regions that could potentially be undergoing natural or artificial selection in Ugandan Nile tilapia we performed runs of homozygosity (ROH) analysis with Plink version 1.9 [23] using the –homozyg flag. Following the recommendations from Meyermans et al., 2020 [33] on the best practices of performing ROH analysis in livestock species we considered the following parameters; scanning window of 40 SNPs (−-homozyg-window-snp 40), 0.05 as the threshold of the scanning window hit rate (−-homozyg-window-threshold 0.05), maximum of one heterozygous SNP allowed in the window (−-homozyg-window-het 1), and five SNPs were allowed as the maximum of markers in the with missing genotype information (−-homozyg-window-missing 5). We also considered 500 kb as the maximum gap/ distance between two homozygous SNPs (−-homozyg-gap 500), of one SNP per 60 kb as the minimum density in the homozygous segment (−-homozyg-density 60), minimum length of a ROH was 1000 kb (−-homozyg-kb 1000) and 100 SNPs as the minimum number of markers per the ROH (−-homozyg-snp 100). It is worth highlighting that before performing ROH analysis, genotype data was subjected to the same filtration as in the genomic analyses, however we did not filter genotypes for MAF in this stage as recommended by Meyermans et al., 2020 [33]. Candidate genes harboured within the most frequent ROH regions from the Nile tilapia genome (GCA_001858045.3) using the Ensembl BioMart [34] tool from the Ensembl genome browser (Ensembl genes 110).

3. Results

3.1. Marker genotype quality

We obtained high genotyping rates (average 97%), with only four samples of the 382 genotyped showing low genotyping rate (< 90%). A total of 47,288 out of 60,785 SNP markers were filtered from the analysis (10,136 missing in >5% of the samples, 2152 showed deviation from Hardy-Weinberg equilibrium, 32,851 had a MAF below 0.05, and 2149 with duplicated genomic positions). Consequently, a total of 13,497 SNP markers were retained for genomic analyses. A sizable number of markers had very low MAF across all sampled populations (Fig. 2), which probably reflects that Ugandan populations were not considered in the design of the SNP array used in this study (Fig. 2). The populations L. Albert, BUN1, KAM1 and L. Kyoga had the lowest average MAF (0.074), while HOM1 from Hoima district had the highest MAF (0.134 ± 0.146) (Fig. 2).

3.2. Population genetic structure and admixture

The DAPC results showed clear genetic substructure between the Ugandan Nile tilapia populations investigated (Fig. 3a). The linear discriminant axis 1 (LD1), which explained 44.7% of the total genomic variation, strongly separated one farmed population (BUG1) of all the other wild and farmed populations. The linear discriminant axis 2 (LD2), that explained 20.5% of the total genomic variation, moderately separated the populations into two main subgroups, with two (KAM1 and BUN1) farms clustering nearer to L. *Victoria* than L. Albert or L. Kyoga, and six farms (KUM1, KAS1, HOM1, SER1, BUG2, and BUG3) clustering together with L. Kyoga. Interestingly, when we added GIFT-derived strain samples (BMK) from Benchmark Genetics, LD1 explained 47.4% of genomic variation and strongly separated the fish into two major

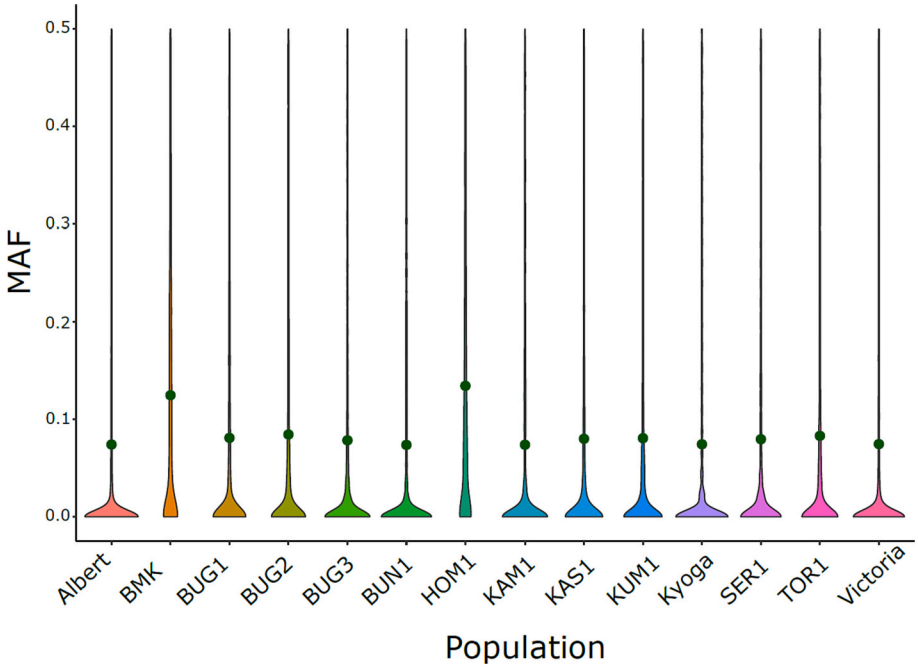

Fig. 2. Density plot summarizing minor allele frequency distribution of SNP markers in the Nile tilapia populations considered in this study.

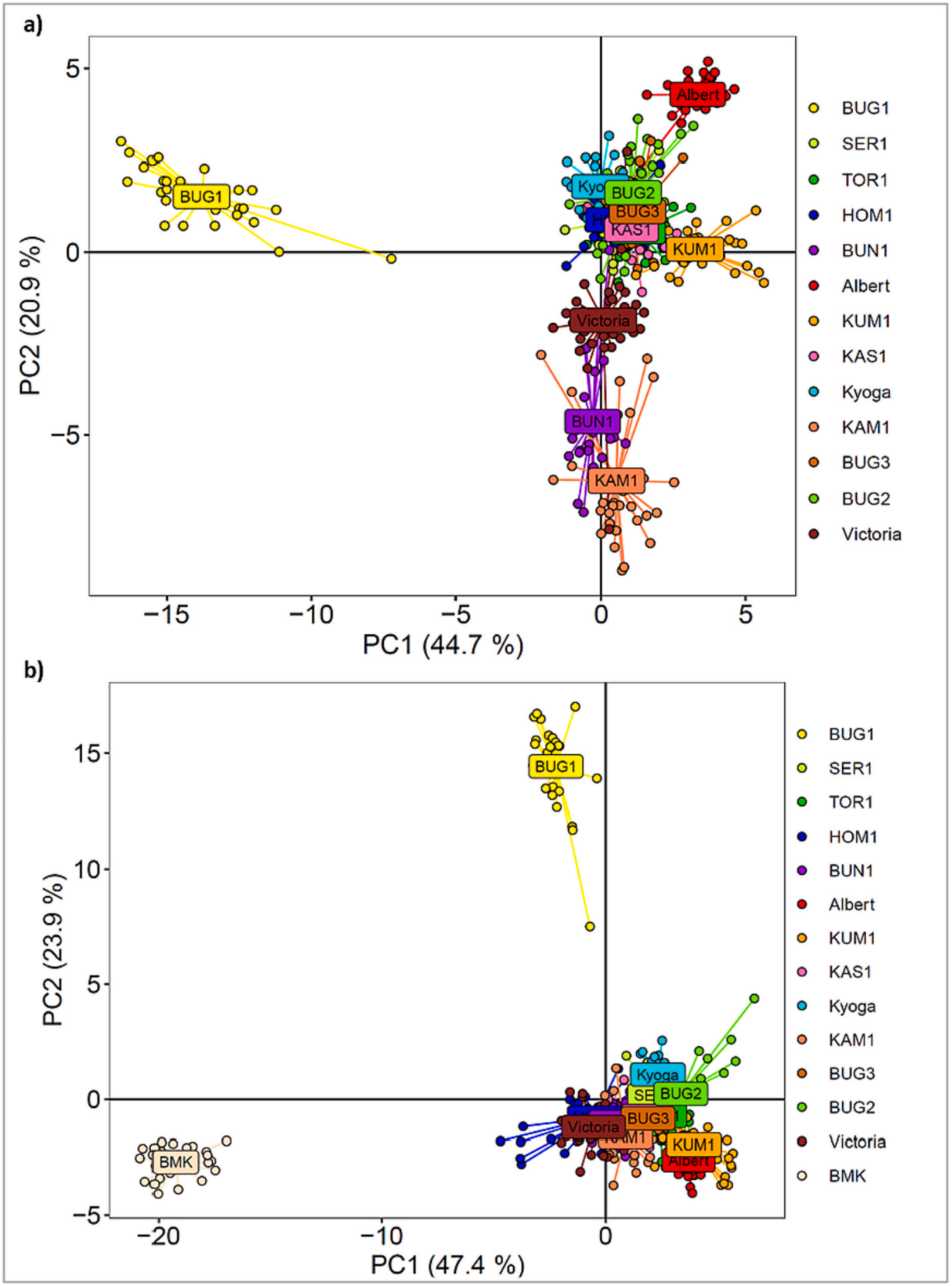

Fig. 3. Discriminant analysis of principal components scatter plots showing genetic structure within and between Ugandan Nile tilapia populations (a), and between the Ugandan populations in comparison to a GIFT-derived strain (b).

groups, with BMK fish distinctively separated from Ugandan populations (Fig. 3b). Moreover, LD2 explained 23.9% of the genomic variation and separated one BUG1 from the rest of the Ugandan fish, which clustered together. It is also worth noting that fish from the lakes formed three distinct clusters, with the population of each lake clearly separated from those of other lakes, and the fish within each lake showing high genetic similarity (Fig. 3a). For farmed fish, the individuals from the six farms (KUM1, KAS1, SER1, BUG1, BUG2 and BUG3) that clustered around L. Kyoga were genetically close to each other, and genetically distant of the fish from BUG1, BUN1 and KAM1, which were also divergent from each other.

Furthermore, genetic distance analysis results demonstrated closer genetic relationship between fish from L. Kyoga and L. Albert, while fish from L. *Victoria* were genetically very distinct from those of the other two lakes (Fig. 4a). Fish sampled from four farms (TOR1, SER1, BUG2 and BUG3) showed close genetic relationship with those sampled from both L. Albert and L. Kyoga. These results showed that fish from BUG1 and HOM1 are unique from the other studied populations with the later showing some genetic relationship with BMK animals than any of the other studied populations (Fig. 4b). Additionally, on average we obtained low pairwise fixation index estimates (0.01 to 0.12), between the Ugandan populations (Fig. 4c), although these estimates were significantly greater than zero (Table S1 in Supplementary file 1). These results demonstrate generally low genetic differentiation between the Ugandan fish populations investigated in this study. Nonetheless, two hatcheries, BUG1 and HOM1, showed moderate genetic differentiation from any of the lake or farmed populations, with Fst values between 0.07 and 0.12 and 0.06–0.11, respectively (Fig. 4c). In agreement with the genetic distance estimates, the Fst estimates showed strong genetic differentiation between the Ugandan populations and the GIFT-derived population, with Fst values ≥0.20. Again, the exception was the farmed population HOM1, which is genetically closer (Fst = 0.20) to GIFT (Fig. 4c).

From the admixture analysis, a K value of 11 had the lowest cross-validation error (Fig. S1 in Supplementary file 2), indicating 11 as the optimal number of ancestries among our studied populations. In general, fish from the lakes were largely of one ancestral lineage with little or no admixture with other ancestries (Fig. 5). L. Albert and *L. Victoria* Nile tilapia populations are largely pure stocks of distinct ancestry, while fish from Lake Kyoga showed some level of admixture and significant ancestry overlap with the fish from L. Albert (64.5 ± 5.4%). Fish from most hatcheries were greatly admixed with the majority of the fish showing genetic overlap with fish from L. Albert and L. Kyoga. For the hatcheries, admixture analysis results indicated that the genetic composition of fish from five (BUG2, BUG3, HOM1, KAS1 and TOR1) of the studied farms were predominantly admixed from multiple ancestries (Fig. 5). In addition to having admixed individuals, some of the hatchery farms had single ancestry individuals but with different lineages such as SER1, and BUN1 with individuals from two strains, and KAM1 which had individuals from three pure strains. Interestingly, one farm fish from BUG1 were predominantly of single ancestry which was distinct from all the investigated populations in the current study. It is worth highlighting that L. Albert/ L. Kyoga showed higher (2.55–52.75%) genetic contribution to farmed fish broodstocks in Uganda, compared to L. *Victoria* (2.12–28.02%). Admixture analysis also further revealed a strong genetic distinction between Ugandan Nile tilapia populations and GIFT-derived strain (Fig. S2 in Supplementary file 2). We observed low genetic composition of the GIFT strain ranging from 0.001 to 2.32% in the Ugandan populations, with the highest proportion (2.32%) observed in the HOM1 population.

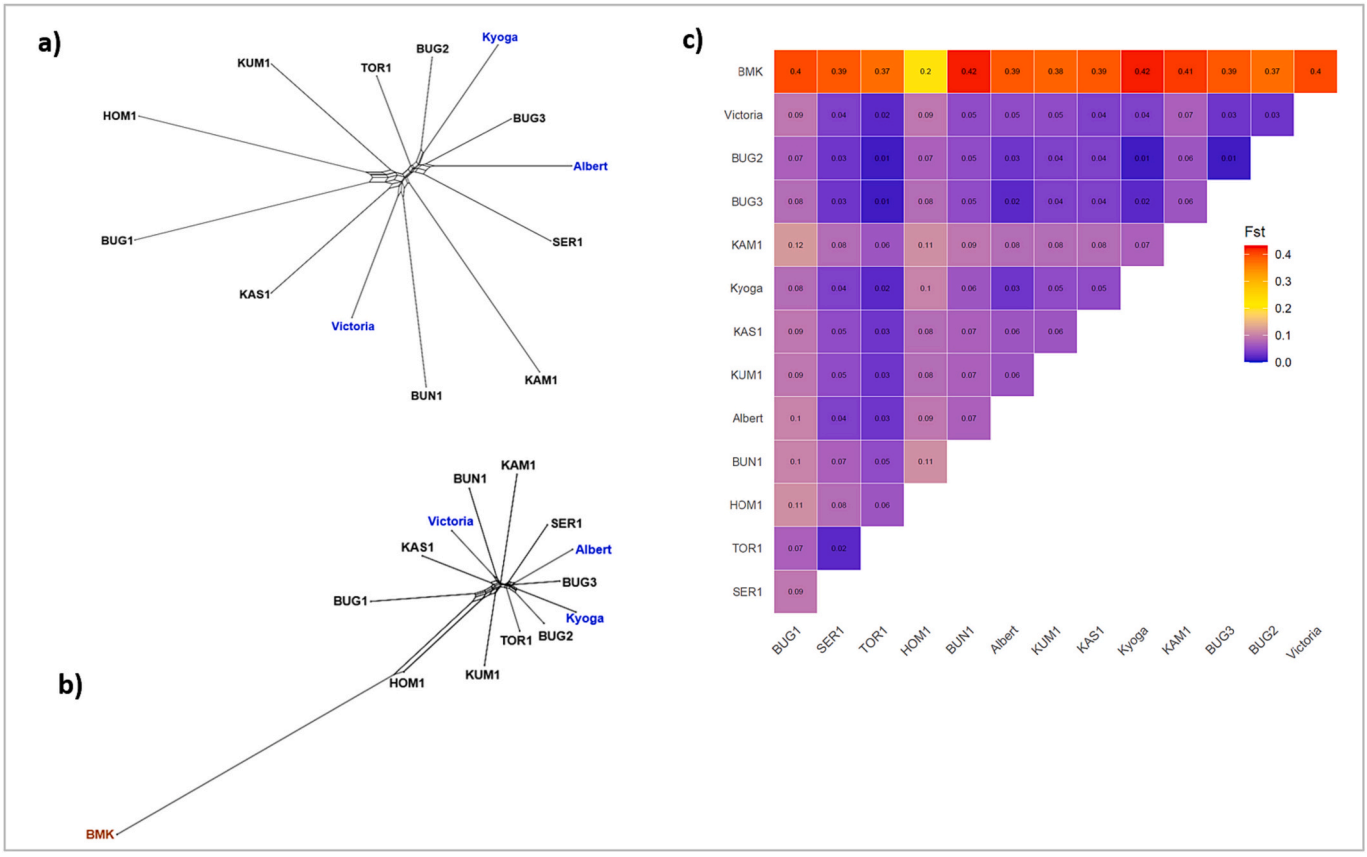

Fig. 4. Genetic distances between Ugandan Nile tilapia populations. A neighbour-net phylogenetic network summarizing genetic distances between the studies Ugandan Nile tilapia populations (a), a neighbour-net phylogenetic network summarizing genetic distances between the studies Ugandan Nile tilapia populations as compared to the Gift-derived strain (b), and a summary of the genome-wide Fst value estimates between the studied Nile tilapia populations (c).

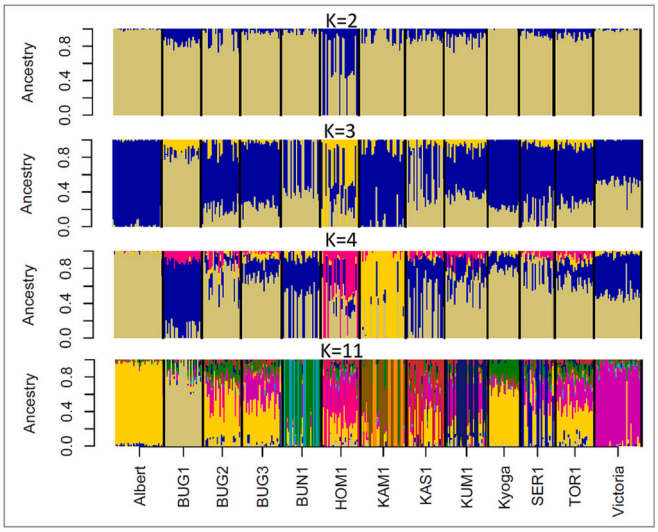

Fig. 5. Admixture plot showing genomic composition of the Ugandan Nile tilapia populations when we assumed two (K = 2), three (K = 3), four (K = 4) and twelve (K = 12, optimal K value) clusters for assignment of individuals.

3.3. Genetic diversity and inbreeding

Investigation of within-population genomic diversity of Ugandan Nile tilapia populations revealed moderate observed and expected heterozygosity estimates ranging from 0.291 to 0.354, and 0.284 to 0.368, respectively (Table 2). The fish from HOM1 had the highest observed heterozygosity (0.354) and expected heterozygosity (0.368), while BUN1 had the lowest observed heterozygosity (0.291) and expected heterozygosity (0.284). In general, observed heterozygosity was slightly higher than expected heterozygosity in most of the Ugandan studied populations, except for HOM1 where the observed was lower than the expected heterozygosity depicting some level of inbreeding in this population. Genome-wide inbreeding estimations revealed low inbreeding coefficient ($F_{is} < 0.1$) across all the Ugandan populations investigated in the current study (Fig. 6), however, we observed exceptionally high variability of inbreeding in the fish from HOM1, with a sizable number of individuals from this hatchery showing relatively high levels of inbreeding ($F_{is} > 0.1$).

3.4. Runs of homozygosity

We identified a total of 10,205 unique runs of homozygosity regions, and the frequency of these ROH regions within the studied fish varied between 0.265% and 37.3% (Fig. 5, and Table S2 in Supplementary file 3). The ROH regions were on average of 3585 ± 2233.28 kbp, and they contained on average 185 ± 117 SNPs with the proportion of homozygous sites ranging from 86 to 100%. The strongest ROH signal was observed on chromosome 5 and was detected in 141 Ugandan Nile tilapia in the current study. The region was detected in all the 13 populations in the current study, however, its frequency within the studied populations varied being most frequent (64% of the individuals) in the BUN1 farm, and least frequent (4% of the individuals) in BUG1 farm. This region harbours a total of 125 genes (Table S3 in Supplementary file 4). One of the most interesting candidate genes located within this region is the *BPI* gene, which encodes for the bactericidal permeability-increasing protein. Also, this region overlaps with previously identified quantitative trait loci for body weight gain with *GHRH* (growth hormone releasing hormone) among the candidate genes in the overlapping region. We also identified two other less frequent ROH regions on chromosome 2 and 12, which were present in 77 and 76 individuals, respectively. Additionally, we detected weaker signals (10 to 16% of samples) in chromosomes 1, 6, 13, 16, 19 and 22 (Fig. 7).

Table 2
Average genomic heterozygosity estimates within the studied populations.

Population	Number of individuals	Ho (SD)	He (SD)
Albert	35	0.299 (0.183)	0.295 (0.171)
BMK	30	0.23 (0.195)	0.231 (0.183)
BUG1	28	0.306 (0.191)	0.292 (0.167)
BUG2	28	0.317 (0.169)	0.315 (0.153)
BUG3	29	0.314 (0.176)	0.307 (0.159)
BUN1	28	0.291 (0.198)	0.284 (0.174)
HOM1	28	0.354 (0.139)	0.368 (0.126)
KAM1	33	0.303 (0.199)	0.284 (0.172)
KAS1	28	0.308 (0.177)	0.306 (0.158)
KUM1	31	0.311 (0.173)	0.309 (0.155)
Kyoga	23	0.294 (0.194)	0.287 (0.176)
SER1	25	0.324 (0.195)	0.303 (0.161)
TOR1	28	0.327 (0.168)	0.317 (0.150)
Victoria	34	0.302 (0.180)	0.298 (0.168)

4. Discussion

In the current study we utilized genome-wide markers to perform genetic characterization of wild and farmed Nile tilapia populations in Uganda. We obtained high genotyping rates across the investigated fish (97%), however, a significant number of the markers from the panel (32,851, 54%) were not polymorphic or not segregating in the Ugandan populations in the current study. The low number of polymorphic markers encountered in this study could be attributed to the population used in SNP discovery and selection during the panel designing process. The SNP panel used in the current study was designed based on Benchmark genetics' GIFT-like strain population, thus explaining the relatively higher average MAF (0.125) of the BMK population as compared to the Ugandan populations, except HOM1. Similar results were observed in a study that developed a publicly available Nile tilapia genotyping platform based on WorldFish's GIFT population, where a significant number of markers on the panel were fixed in African Nile Tilapia strain populations [35]. This highlights the need to develop genomic resources adapted to the African indigenous tilapia strains to support Nile tilapia breeding and conservation programs on the continent. The GIFT strain is a unique commercial tilapia strain developed through an international collaborative project genetic improvement of farmed tilapia at the International Center for Living Aquatic Resources Management, Philippines, (currently WorldFish) that involved the crossing of wild stocks (from Ghana, Kenya, Egypt, and Senegal) and farmed strains (within The Philippines) followed by generations of selective breeding [36,37]. Despite the low MAF realised in the Ugandan populations in the current study, we retained a substantial number (13,489) of polymorphic genome-wide distributed markers that we are confident are sufficient the genetic characterization analyses of the investigated Ugandan Nile tilapia populations. Interestingly, it has been demonstrated that as low as 65 random SNP markers in the genome are sufficient for genetic characterized populations, and increasing the SNP markers to over 100 SNPs only increased the probability of assigning individuals to the correct ancestry groups [38].

With respect to the three wild populations studied in the current study, our results from population structure analysis demonstrated that each of these populations was comparably distinct. However, fish from L. Kyoga's are genetically closer to those from L. Albert, while the fish from L. *Victoria* are genetically distant from the fish from either L. Kyoga or L. Albert. These results were concordant with the results from genetic distance and population differentiation estimates, both showing closer genetic relationship between L. Kyoga and L. Albert fish. Similarly, admixture results showed that the L. *Victoria* population was predominantly a pure stock, while fish from L. Kyoga are admixed and share significant genetic composition with the pure stock fish from L. Albert. These results highly correlate with a recent study by Tibihika et al. *2020* using 40 microsatellite markers, which showed that fish sampled from L.

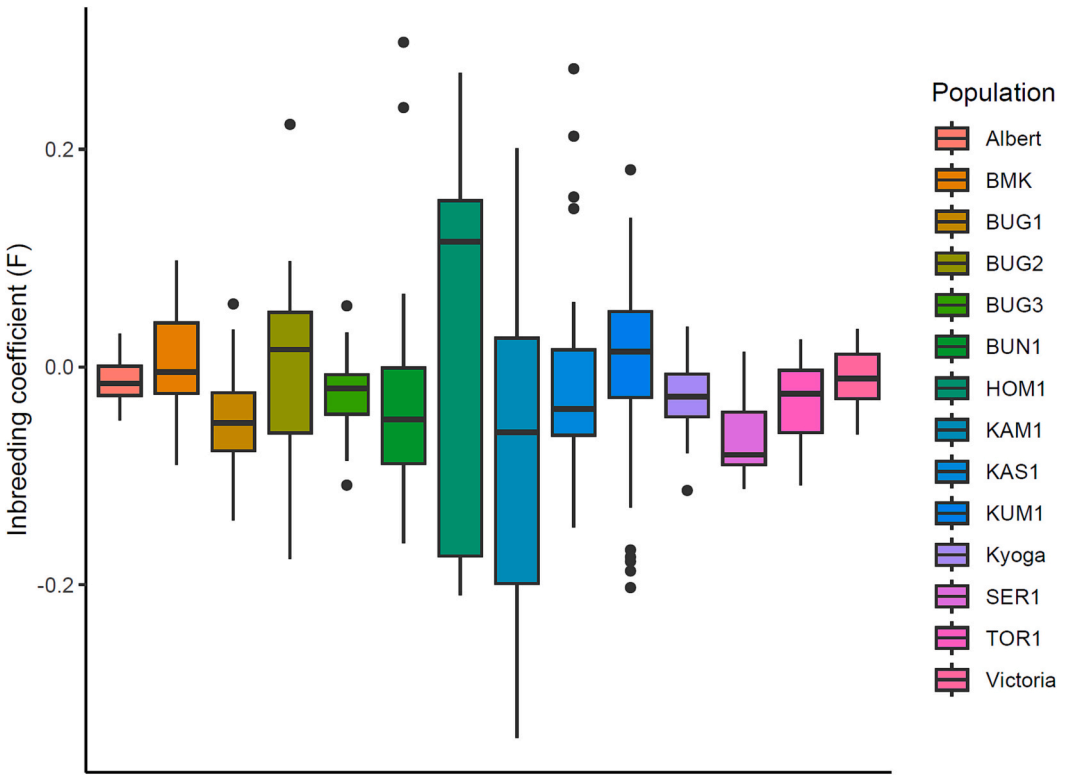

Fig. 6. Box plot showing genomic inbreeding coefficient (Fis) estimations within the studied populations.

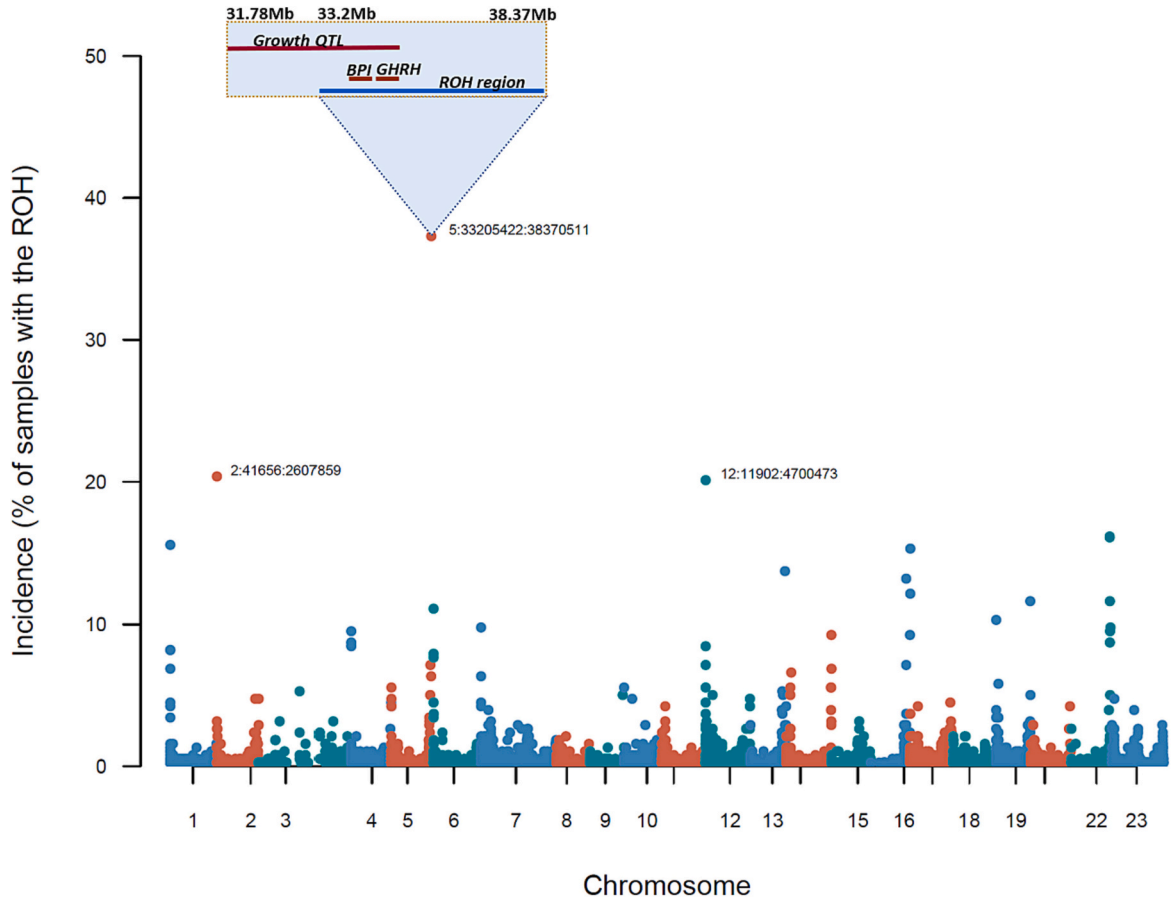

Fig. 7. Manhattan plot showing the genomic distribution and frequency of the identified runs of homozygosity in the Ugandan Nile tilapia populations.

Albert and L. Kyoga were genetically close, while fish from L. *Victoria* were distant from fish from the two latter lakes [18]. Moreover, in the same study, genetic composition results also demonstrated genetic overlap between L. Kyoga and L. Albert populations, while L. *Victoria* population was genetically distinctively unique from either of the two lakes [18]. Interestingly, substantial genetic similarity between fish from L. Albert and those of L. Kyoga and its basin has been reported by earlier genetic characterization studies that utilized Randomly Amplified Polymorphic DNA (RAPD) [20] and microsatellite markers [21]. All these results strongly suggest that L. Albert fish were among the stocks that were used to introduce Nile tilapia in L. Kyoga. Indeed, Nile tilapia is not native to either L. Kyoga or L. *Victoria*, but it was introduced into these lakes from the 1950s and 1960s by deliberate human translocation of fish from native habitats such as L. Albert, L. Edward, L. Turkana and River Nile as a means of improving fisheries output of the two lakes [39–41]. However, the origin of the stocks that were stocked into each of the lakes was never recorded. The high genetic dissimilarity of L. *Victoria* fish and fish from L. Albert or L. Kyoga, observed in the current study as well as from Tibihika et al. 2020, suggests that L. Albert was not the source of the stock that was used for introducing Nile tilapia into L. *Victoria*.

Regarding the farmed Nile tilapia populations, clustering and genetic distance analyses revealed some genetic structure between fish from the hatcheries, with fish from the majority of these hatchery farms showing close similarity with the fish from L. Kyoga and L. Albert, demonstrating the importance of these two lakes as sources of broodstock for farmed fish in Uganda. This was further supported by the results from our genetic/genomic composition estimation results, which showed higher L. Kyoga/Albert genetic proportions (2.55–52.75%) as compared to L. *Victoria* composition proportions (2.12–28.02%). We observed weak genetic differentiation between the hatchery populations, as well as between the lakes' populations and the hatchery populations. This observed lack of structure is the result of genetic mixing, likely driven by the management of hatchery broodstock in Uganda. Indeed, it has been demonstrated by Mwanja et al. 2015 that many hatchery owners source their broodstock from their fellow farmers, while the remaining farmers source their animals from the Aquaculture Research and Development Centre and or directly from the wild [14]. Similarly, weak differentiation between farmed Nile tilapia populations, as well as between farmed and wild populations, has been reported by a similar study in the Democratic Republic of Congo (borders Uganda in the West), where the methods of sourcing broodstock for farmed Nile tilapia are largely similar to those in Uganda [17]. It is worth highlighting that the GIFT strain included in the current was remarkably different from the Uganda Nile tilapia populations with very traces of GIFT in these populations. These results demonstrate that both wild and farmed Nile tilapia populations are largely free of the GIFT introgression. However, fish from one of the hatcheries investigated in the current study showed more genetic similarity to GIFT than any of the other populations in the study, indicating possiple introgression of the GIFT strain into Uganda farmed populations. Although there is no documentation of importation of GIFT into Uganda since use of such imported strains is restricted to protect the native strains and stocks, this strain is largely used by Nile tilapia farmers in the neighbouring countries such as the Democratic Republic of Congo [17,42], Tanzania [43,44] and Kenya [45,46]. Therefore, given the minimal restrictions on border crossings between these countries and Uganda, it is not unexpected that this strain could be circulating in Uganda's Nile tilapia aquaculture industry.

Regarding genetic diversity, except HOM1, all investigated populations had observed heterozygosity estimates higher than the expected estimates, implying good genetic health of these populations. Indeed, our inbreeding rate estimations revealed low rates of inbreeding in most of the investigated populations. We speculate that, considering the unmonitored mating at most of the hatchery farms in Uganda, the high levels of genomic heterozygosity and low rates of inbreeding observed are mainly a result of the constant introduction of new fish (either from the wild or other hatchery farms) into their broodstock as demonstrated by our admixture results.

Our runs of homozygosity analysis detected an interesting genomic region that is potentially under selection in the Uganda tilapia strains and stocks on chromosome 5. This region harbours several genes of interest, such as the *BPI* gene that encodes for bactericidal permeability-increasing protein, an important component of the innate immune system animals [47]. The bactericidal permeability-increasing protein is mainly expressed by neutrophils and has strong anti-microbial activity against gram negative bacteria through bacteria opsonization to promote intracellular and extracellular bacteria killing by phagocytes, bacterial lipopolysaccharide neutralization and clearance [48–50]. Also, the bactericidal permeability-increasing protein delivers gram negative bacteria membrane antigens to dendritic cells, inducing both innate and adaptive lymphocyte antimicrobial responses against the invading bacteria [48]. In teleost fish, recombinant bactericidal permeability-increasing protein from multiple species has been demonstrated to have bactericidal effects against different gram-negative pathogenic bacteria such as; *Aeromonas hydrophila* and *Escherichia coli* in blunt snout bream (*Megalobrama amblycephala*) [51], *Pseudomonas fluorescens* and *Edwardsiella tarda* in tongue sole (*Cyanoglossus semilaevis*) [52], as well as *Vibrio alginolyticus* and *Edwardsiella tarda* in golden pompano (*Trachinotus ovatus*) [53]. Therefore, given this demonstrated importance of BPI in the host response against gram negative bacteria, the identified region might be of significant importance to the aquaculture sector, especially to breed for disease-resilient strains of Nile tilapia.

Furthermore, the ROH region identified on chromosome 5 overlapped with a growth rate QTL previously identified in the GIFT strain by Barria et al. 2021 and was proximal (1.86 Mb downstream) to the feed intake QTL identified in the same study [54]. This demonstrates that this region might not only be limited to host pathogen resistance but also could be important regarding feed efficiency traits, hence being of great interest for Nile tilapia production efficiency. *GHRH* is one of the candidate genes in the overlapping region between the growth rate QTL from Barria et al. 2021 and the strongest ROH region identified in the current study. *GHRH* encodes for the growth hormone-releasing hormone, which is a neuroendocrine factor that stimulates growth hormone (GH) synthesis and release from the anterior pituitary gland [55]. *GH* regulates fish growth through modulation of processes such as feed intake, nutrient absorption and assimilation processes including protein deposition and lipid mobilization via the GH/IGF system [56–59], and is a major determinant of fish growth.

5. Conclusions

In this study, we investigated the genetic structure, diversity and putative regions under selection in wild and farmed Nile tilapia populations in Uganda. A significant number of markers on the panel used in the current study were not segregating in the Ugandan populations, indicating the need to develop genomic tools adapted for these populations that could be utilized to support improvement of local strains. Our results demonstrated clear genetic substructure between wild populations, with L. Kyoga fish showing high genetic similarity with fish from L. Albert, suggesting L. Kyoga was originally stocked with fish from L. Albert. We observed some genetically unique populations among the studied that could be further studied for phenotypic characterization in terms of production traits. We also observed high genetic linkage between most of the farmed populations and the stock from L. Albert / L. Kyoga which might be related to some phenotypic differences or superiority of the fish from these sources when compared to other wild stock sources, however, this remains to be experimentally studied. On average, we observed high genetic diversity and low inbreeding rate across the studied populations in widely genetically healthy breeding populations, although some of the farms had high numbers of highly inbreed individuals, which could potentially have negative impacts on the reproductive and survivability as well production efficiency of these

population. Interestingly, we identified a genomic region putatively under selection in Ugandan Nile tilapia that overlaps with a previously identified growth rate QTL and harbours a key innate immune gene, implying that this region could be exploited to support the development of more production efficient strains that are also resilient to the prevailing pathogens in the region. Indeed, this region could be harbouring genetic markers that could support marker-assisted selection.

Ethics declarations

All procedures and protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Makerere University (SVAR_IACUC/133/2022, 20th, February 2023) and registered by the Uganda National Council for Science and Technology (A269ES, 31st March 2023).

Consent to participate

Farmers whose fish was sampled as part of this study voluntarily consented (by written consent) to participating in the study after the aims and procedures of the project were explained to them. Every farmer received monitory compensation for each of the sampled fish included in this study.

Consent to publish

Not applicable.

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Authors' contributions

RM lead the designing and execution of the study; Conceptualization and methodology: RM, DR, JO, EB, BKK, PB, CF; Formal analysis: RM, JNS, SS, CF; DR; Project administration: RM, DR, EB, PB; Result visualization and interpretation: RM, JNS, DR, SS, CF, JO, EB, KKB, CM; Field sample collection and processing: EB, JO, KKB, RM, MS, JM; Writing – original draft: RM, DR, JO, JNS; Data resources: RH; Writing – review & editing: All authors.

CRediT authorship contribution statement

Diego Robledo: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Joel Ogwang:** Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. **Ezra Byakora:** Methodology, Resources, Writing – review & editing, Conceptualization, Data curation, Funding acquisition, Investigation. **Jennifer C Nascimento-Schulze:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Katali Kirungi Benda:** Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing, Investigation. **Clemence Fraslin:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Writing – review & editing. **Sarah Salisbury:** Investigation, Methodology, Resources, Writing – review & editing. **Moses Solimo:** Data curation, Methodology, Writing – review & editing. **Johnson Francis Mayega:** Data curation, Investigation, Methodology, Writing – review & editing. **Beine Peter:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing. **Charles Masembe:** Investigation, Methodology, Validation, Writing – review & editing. **Ross Houston:** Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. **Robert Mukiibi:** Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

Authors declare no competing interests.

Data availability

We utilized a Benchmark Genetics' customized SNP panel shared to us in kind to genotype samples in the current study, genotype data will be available for sharing upon request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2024.110781.

References

[1] A.G. Tacon, M. Metian, Food matters: fish, income, and food supply—a comparative analysis, Rev. Fish. Sci. Aquac. 26 (1) (2018) 15–28, https://doi.org/10.1080/23308249.2017.1328659.
[2] C. Béné, M. Barange, R. Subasinghe, P. Pinstrup-Andersen, G. Merino, G.I. Hemre, M. Williams, Feeding 9 billion by 2050–putting fish back on the menu, Food Secur. 7 (2015) 261–274, https://doi.org/10.1007/s12571-015-0427-z.
[3] FAO, The State of World Fisheries and Aquaculture 2022, Towards Blue Transformation, Rome, FAO, 2022, https://doi.org/10.4060/cc0461en.
[4] UBOS, The Statistical Abstract, Uganda Bureau of Statistics: Kampala, Uganda, 2022, p. 128. https://www.ubos.org/wp-content/uploads/publications/05_2023 2022_Statistical_Abstract.pdf.
[5] B. Mapfumo, Market Assessment for Farmed Fish in Uganda (with a Focus on Practical Distribution Requirements & Buyer Relationships) PESCA MAOPE Activity 3.1. 1.1 UGANDA, 2019.
[6] S. Namatovu, G. Atukunda, L. Obeti, R. Walozi, A. Candia, S. Onep, M. Bwambale, A. Izaara, Profitability and viability analysis of aquaculture production in Central Uganda: a case of urban and peri-urban areas, Asian J. Agric. Ext. Econ. Sociol 22 (4) (2018) 1–11, https://doi.org/10.9734/AJAEES/2018/37721.
[7] R.E. Brummett, J. Lazard, J. Moehl, African aquaculture: realizing the potential, Food Policy 33 (5) (2008) 371–385, https://doi.org/10.1016/j.foodpol.2008.01.005.
[8] UNEP, Integrating Natural Capital into Sustainable Development Decision Making in Uganda, United Nations Environment Programme, 2018. https://wedocs.unep.org/20.500.11822/27186.
[9] M. Magunda, Situational analysis study of the agriculture sector in Uganda, in: CCAFS Report, CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), Wageningen, the Netherlands, 2020. https://hdl.handle.net/10568/111685.
[10] S.P.K. Sserwambala, Viability of aquaculture in Uganda, UNU-FTP, 2017. https://www.grocentre.is/static/gro/publication/498/document/Simon%20Peter_final%20project_2018.pdf (Accessed on 14th–09–2023).
[11] Netherlands Enterprise Agency (RVO), Aquaculture Road Map Uganda Opportunities in the Aquaculture Value Chain. https://www.agroberichtenbuitenland.nl/documenten/rapporten/2022/04/01/aquaculture-road-map-uganda, 2022. Accessed on 14th–09–2023.
[12] B. Keizire, Sustainability Impact Assessment of Proposed WTO Negotiations: The Fisheries Sector, Country Case Study: Uganda, 2006.
[13] B. Adeleke, D. Robertson-Andersson, G. Moodley, S. Taylor, Aquaculture in Africa: a comparative review of Egypt, Nigeria, and Uganda vis-a-vis South Africa, Rev.

Fish. Sci. Aquac. 29 (2) (2020) 167–197, https://doi.org/10.1080/23308249.2020.1795615.
[14] M. Mwanja, J. Rutaisire, C. Ondhoro, R. Ddungu, C. Aruho, Current fish hatchery practises in Uganda: the potential for future investment, Int. J. Fish. Aquat. Stud. 2 (4) (2015) 224–232. https://www.fisheriesjournal.com/archives/2015/vol2issue4/PartE/2-4-58.pdf.
[15] C.E. Lind, S.K. Agyakwah, F.Y. Attipoe, C. Nugent, R.P. Crooijmans, A. Toguyeni, Genetic diversity of Nile tilapia (*Oreochromis niloticus*) throughout West Africa, Sci. Rep. 9 (1) (2019) 16767, https://doi.org/10.1038/s41598-019-53295-y.
[16] A.J. Ropp, K.S. Reece, R.A. Snyder, J. Song, E.E. Biesack, J.R. McDowell, Fine-scale population structure of the northern hard clam (*Mercenaria mercenaria*) revealed by genome-wide SNP markers, Evol. Appl. 16 (2023) 1422–1437, https://doi.org/10.1111/eva.13577.
[17] M. Geraerts, C. Vangestel, T. Artois, J.M.D.O. Fernandes, M.W. Jorissen, A. Chocha Manda, C. Danadu Mizani, K. Smeets, J. Snoeks, G. Sonet, Y. Tingbao, Population genomics of introduced Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) in the Democratic Republic of the Congo: repeated introductions since colonial times with multiple sources, Mol. Ecol. 31 (12) (2022) 3304–3322, https://doi.org/10.1111/mec.16479.
[18] P.D. Tibihika, M. Curto, E. Alemayehu, H. Waidbacher, C. Masembe, P. Akoll, H. Meimberg, Molecular genetic diversity and differentiation of Nile tilapia (*Oreochromis niloticus*, L. 1758) in East African natural and stocked populations, BMC Evol. Biol. 20 (2020) 1–20, https://doi.org/10.1186/s12862-020-1583-0.
[19] P.D. Tibihika, M. Curto, E. Dornstauder-Schrammel, S. Winter, E. Alemayehu, H. Waidbacher, H. Meimberg, Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African Oreochromis niloticus, Conserv. Genet. 20 (2019) 357–372, https://doi.org/10.1007/s10592-018-1136-x.
[20] W.W. Mwanja, G.C. Booton, L. Kaufman, P.A. Fuerst, A profile of the introduced *Oreochromis niloticus* (Pisces: Teleostei) populations in Lake Victoria Region in relation to its putative origin of Lakes Edward and Albert (Uganda-E. Africa) based on random amplified polymorphic DNA analysis, Afr. J. Biotechnol. 7 (11) (2008) 1769–1773, https://doi.org/10.5897/AJB08.813.
[21] P. Fuerst, W. Mwanja, L. Kaufman, The genetic history of the introduced Nile tilapia of Lake Victoria (Uganda–E. Africa): the population structure of *Oreochromis niloticus* (Pisces: Cichlidae) revealed by DNA microsatellite markers, in: Tilapia Aquaculture in the 21st Century Proceedings from the Fifth International Symposium on Tilapia in Aquaculture, Panorama da Aquicultura Magazine Rio de Janeiro, Brazil, 2000, pp. 30–40.
[22] S. Berensmeier, Magnetic particles for the separation and purification of nucleic acids, Appl. Microbiol. Biotechnol. 73 (2006) 495–504, https://doi.org/10.1007/s00253-006-0675-0.
[23] S. Purcell, B. Neale, K. Todd-Brown, L. Thomas, M.A. Ferreira, D. Bender, J. Maller, P. Sklar, P.I. De Bakker, M.J. Daly, P.C. Sham, PLINK: a tool set for whole-genome association and population-based linkage analyses, Am. J. Hum. Genet. 81 (3) (2007) 559–575, https://doi.org/10.1086/2F519795.
[24] T. Jombart, S. Devillard, F. Balloux, Discriminant analysis of principal components: a new method for the analysis of genetically structured populations, BMC Genet. 11 (1) (2010) 1–15, https://doi.org/10.1186/1471-2156-11-94.
[25] R Core Team, R: A Language and Environment for Statistical Computing , R Foundation for Statistical Computing, Vienna, Austria, 2023. https://www.R-project.org/.
[26] T. Jombart, I., Ahmed, adegenet 1.3-1: new tools for the analysis of genome-wide SNP data, Bioinformatics 27 (21) (2011) 3070–3071, https://doi.org/10.1093/bioinformatics/btr521.
[27] B.J. Knaus, N.J. Grünwald, vcfr: a package to manipulate and visualize variant call format data in R, Mol. Ecol. Resour. 17 (1) (2017) 44–53, https://doi.org/10.1111/1755-0998.12549.
[28] J.A. Thia, Guidelines for standardizing the application of discriminant analysis of principal components to genotype data, Mol. Ecol. Resour. 23 (3) (2022) 523–538, https://doi.org/10.1111/1755-0998.13706.
[29] H. Wickham, ggplot2, Wiley Interdisc. Rev. Comput. Stat. 3 (2) (2011) 180–185, https://doi.org/10.1002/wics.147.
[30] B. Gruber, P.J. Unmack, O.F. Berry, A. Georges, dartr: an r package to facilitate analysis of SNP data generated from reduced representation genome sequencing, Mol. Ecol. Resour. 18 (3) (2018) 691–699, https://doi.org/10.1111/1755-0998.12745.
[31] D.H. Huson, SplitsTree: analyzing and visualizing evolutionary data, Bioinformatics 14 (1) (1998) 68–73, https://doi.org/10.1093/bioinformatics/14.1.68.
[32] D.H. Alexander, K. Lange, Enhancements to the ADMIXTURE algorithm for individual ancestry estimation, BMC Bioinform. 12 (2011) 1–6, https://doi.org/10.1186/1471-2105-12-246.
[33] R. Meyermans, W. Gorssen, N. Buys, S. Janssens, How to study runs of homozygosity using PLINK? A guide for analyzing medium density SNP data in livestock and pet species, BMC Genomics 21 (1) (2020) 1–14, https://doi.org/10.1186/s12864-020-6463-x.
[34] R.J. Kinsella, A. Kähäri, S. Haider, J. Zamora, G. Proctor, G. Spudich, J. Almeida-King, D. Staines, P. Derwent, A. Kerhornou, P. Kersey, Ensembl BioMarts: a hub for data retrieval across taxonomic space, Database (2011), https://doi.org/10.1093/database/bar030 p.bar030.
[35] C. Peñaloza, D. Robledo, A. Barría, T.Q. Trịnh, M. Mahmuddin, P. Wiener, J. A. Benzie, R.D. Houston, Development and validation of an open access SNP array for Nile tilapia (*Oreochromis niloticus*), G3-Genes Genom. Genet. 10 (8) (2020) 2777–2785, https://doi.org/10.1534/g3.120.401343.
[36] ADB, An Impact Evaluation of the Development of Genetically Improved Farmed Tilapia and their Dissemination in Selected Countries, The Asian Development Bank, Manila, Philippines 137, Asian Development Bank, 2005, https://www.adb.org/sites/default/files/publication/29623/ies-tilapia-dissemination.pdf.
[37] G.J. Etherington, W. Nash, A. Ciezarek, T.K. Mehta, A. Barria, C. Penaloza, M. G. Khan, A. Durrant, N. Forrester, F. Fraser, N. Irish, Chromosome-level genome sequence of the genetically improved farmed Tilapia (GIFT, *Oreochromis niloticus*) highlights regions of introgression with *O. mossambicus*, BMC Genomics 23 (1) (2022) 832, https://doi.org/10.1186/s12864-022-09065-8.
[38] R. Turakulov, S. Easteal, Number of SNPS loci needed to detect population structure, Hum. Hered. 55 (1) (2003) 37–45, https://doi.org/10.1159/000071808.
[39] R. Ogutu-Ohwayo, The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*, Environ. Biol. Fish 27 (1990) 81–96, https://doi.org/10.1007/BF00001938.
[40] R. Ogutu-Ohwayo, The purpose, costs and benefits of fish introductions: with specific reference to the Great Lakes of Africa, Int. Verein. Theor. Angew. Limnol. Mitteil. 23 (1) (1992) 37–44, https://doi.org/10.1080/05384680.1992.11904006.
[41] T. Twongo, The Fisheries of the Kyoga Lakes and the Likely Impact on them by Human Activities in the Catchment. http://hdl.handle.net/1834/35599, 1991.
[42] R. Yossa, R.K. Basiita, J.N. Mushagalusa, T.Q. Trinh, D. Matempa, P. Manzwanzi, L. Bwamayama, S.M. Cole, P. Matungulu, P.M. Dontsop Nguezet, B. Vanlauwe, Performance evaluation of available strains of Nile Tilapia (*Oreochromis niloticus*) fed commercial and locally-made feeds in the Democratic Republic of the Congo, Appl. Sci. 13 (1) (2022) 424, https://doi.org/10.3390/app13010424.
[43] M. Moses, L.J. Chauka, D.J. de Koning, C. Palaiokostas, M.S. Mtolera, Growth performance of five different strains of Nile tilapia (*Oreochromis niloticus*) introduced to Tanzania reared in fresh and brackish waters, Sci. Rep. 11 (1) (2021) 11147, https://doi.org/10.1038/s41598-021-90505-y.
[44] M. Moses, M.S. Mtolera, L.J. Chauka, F.A. Lopes, D.J. de Koning, R.D. Houston, C. Palaiokostas, Characterizing the genetic structure of introduced Nile tilapia (*Oreochromis niloticus*) strains in Tanzania using double digest RAD sequencing, Aquac. Int. 28 (2020) 477–492, https://doi.org/10.1007/s10499-019-00472-5.
[45] S. Genschick, W. Mekkawy, C. Rossignoli, J.A. Benzie, Growth performance of three strains of Nile tilapia (*Oreochromis niloticus*) on four different feeds in Western and Central Kenya, Aquac. Rep. 20 (2021) 100701, https://doi.org/10.1016/j.aqrep.2021.100701.
[46] Y.B. Ansah, E.A. Frimpong, E.M. Hallerman, Genetically-improved tilapia strains in Africa: potential benefits and negative impacts, Sustainability 6 (6) (2014) 3697–3721, https://doi.org/10.3390/su6063697.
[47] B.C. Krasity, J.V. Troll, J.P. Weiss, M.J. McFall-Ngai, LBP/BPI proteins and their relatives: conservation over evolution and roles in mutualism, Biochem. Soc. Trans. 39 (4) (2011) 1039–1044, https://doi.org/10.1042/BST0391039.
[48] A. Balakrishnan, S.A. Marathe, M. Joglekar, D. Chakravortty, Bactericidal/permeability increasing protein: a multifaceted protein with functions beyond LPS neutralization, Innate Immun. 19 (4) (2013) 339–347, https://doi.org/10.1177/1753425912465098.
[49] N.M. Iovine, P. Elsbach, J. Weiss, An opsonic function of the neutrophil bactericidal/permeability-increasing protein depends on both its N-and C-terminal domains, Proc. Natl. Acad. Sci. U. S. A. 94 (20) (1997) 10973–10978, https://doi.org/10.1073/pnas.94.20.10973.
[50] M. Gonzalez, Y. Gueguen, D. Destoumieux-Garzón, B. Romestand, J. Fievet, M. Pugnière, F. Roquet, J.M. Escoubas, F. Vandenbulcke, O. Levy, L. Sauné, Evidence of a bactericidal permeability increasing protein in an invertebrate, the *Crassostrea gigas* Cg-BPI, Proc. Natl. Acad. Sci. U. S. A. 104 (45) (2007) 17759–17764, https://doi.org/10.1073/pnas.0702281104.
[51] L. Tang, Y. Liang, Y. Jiang, S. Liu, F. Zhang, X. He, T. Wang, Y. Zhou, H. Zhong, J. Yan, Identification and expression analysis on bactericidal permeability-increasing protein/lipopolysaccharide-binding protein of blunt snout bream, *Megalobrama amblycephala*, Fish Shellfish Immunol. 45 (2) (2015) 630–640, https://doi.org/10.1016/j.fsi.2015.05.013.
[52] Y.Y. Sun, L. Sun, A teleost bactericidal permeability-increasing protein kills gram-negative bacteria, modulates innate immune response, and enhances resistance against bacterial and viral infection, PLoS One 11 (4) (2016) e0154045, https://doi.org/10.1371/journal.pone.0154045.
[53] Y. Wu, H. Du, L. Zhu, N. Zhao, S. Zhang, Z. Cao, Y. Zhou, Y. Sun, Bactericidal permeability-increasing protein/LPS-binding protein (BPI/LBP) enhances resistance of golden pompano *Trachinotus ovatus* against bacterial infection, Fish Shellfish Immunol. 131 (2022) 872–880, https://doi.org/10.1016/j.fsi.2022.10.065.
[54] A. Barría, J.A. Benzie, R.D. Houston, D.J. De Koning, H. De Verdal, Genomic selection and genome-wide association study for feed-efficiency traits in a farmed Nile Tilapia (*Oreochromis niloticus*) population, Front. Genet. 12 (2021) 737906, https://doi.org/10.3389/fgene.2021.737906.
[55] B.H. Nam, J.Y. Moon, Y.O. Kim, H.J. Kong, W.J. Kim, K.K. Kim, S.J. Lee, Molecular and functional analyses of growth hormone-releasing hormone (GHRH) from olive flounder (*Paralichthys olivaceus*), Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 159 (2) (2011) 84–91, https://doi.org/10.1016/j.cbpb.2011.02.006.
[56] E. JÖnsson, B.T. BjÖrnsson, Physiological functions of growth hormone in fish with special reference to its influence on behaviour, Fish. Sci. 68 (sup1) (2002) 742–748, https://doi.org/10.2331/fishsci.68.sup1_742.
[57] R. Company, A. Astola, C. Pendón, M.M. Valdivia, J. Pérez-Sánchez, Somatotropic regulation of fish growth and adiposity: growth hormone (GH) and somatolactin

(SL) relationship, Comp. Biochem. Physiol. Part - C: Toxicol. Pharmacol. 130 (4) (2001) 435–445, https://doi.org/10.1016/s1532-0456(01)00269-1.

[58] C. Petro-Sakuma, F.T. Celino-Brady, J.P. Breves, A.P. Seale, Growth hormone regulates intestinal gene expression of nutrient transporters in tilapia (*Oreochromis mossambicus*), Gen. Comp. Endocrinol. 292 (2020) 113464, https://doi.org/10.1016/j.ygcen.2020.113464.

[59] R.E. Peter, J.P. Chang, Brain regulation of growth hormone secretion and food intake in fish, in: P.D. Prasada Rao, R.E. Peter (Eds.), Neural Regulation in the Vertebrate Endocrine System, Springer, Boston, MA, 1999, pp. 55–67, https://doi.org/10.1007/978-1-4615-4805-4.