



Genetic characterization and interspecies gene flow among the *Oreochromis* species in the Lake Victoria and Kyoga basins, Uganda: Implications for conservation

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ABSTRACT

Despite the significant fishery contribution of the Victoria and Kyoga basins, the ecosystems have continuously been threatened by overfishing and introduction of alien species, among others. These activities have contributed to a significant decline of the native tilapiines, the Singida tilapia (*Oreochromis esculentus*) and Victoria tilapia (*Oreochromis variabilis*). As a consequence, the native species have been relegated to smaller satellite lakes and replaced by non-native species comprising Nile tilapia (*O. niloticus*) and blue spotted tilapia (*O. leucostictus*). Therefore, to better understand the implications of these events, it is important to assess the status of both native and non-native species in the Victoria and Kyoga basins for sustainable science-based informed management options. Utilizing both microsatellite and mitochondrial (mt) DNA markers, we sequenced a total of 739 individuals from the Victoria and Kyoga basins comprising *O. niloticus* (333), *O. esculentus* (151), and *O. leucostictus* (258). Generally, all the species and their respective populations exhibited lower genetic diversity as well as genetic differentiation. The principal coordinate analysis showed three main distinct groups corresponding to the three species studied highlighting their genetic differentiation. However, some evidence of introgressive hybridization observed between *O. niloticus* and *O. esculentus* as well as *O. esculentus* and *O. leucostictus* suggests a need for a tailored management strategy to preserve the genetic integrity of all three *Oreochromis* species while minimizing the hybridization risks. The study recommends that the satellite waterbodies containing pure stocks of *O. esculentus* should be conserved separately with higher priority.

1. Introduction

The Eastern African inland aquatic ecosystems are distinguished on a global scale due to the species richness and endemism, particularly the freshwater bodies in the Lake Victoria and Kyoga basins (Balirwa et al.,

2003; Witte et al., 2013). The Victoria Basin covers the main Lake Victoria and its satellite lakes (e.g., lakes Nabugabo, Mburo, Kachera, and Kijanebalola) in Uganda, and the Kyoga basin includes Lake Kyoga and its satellite lakes (e.g., Nawampasa, Nyaguo, and Kwanja) (Mwanja et al., 2010). These basins have immense natural resources including

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fisheries, and wetlands that act as important spots for freshwater biodiversity and support the livelihoods of different communities (Natugonza et al., 2021). Besides being the second-largest freshwater body by surface area, Lake Victoria is described as ‘Darwin’s dream pond’ because it houses hundreds of species of cichlids that have displayed significant adaptive speciation (Fryer, 2004). The increasing pressures caused by the surging human population in the region continue to pose threats to biodiversity (Natugonza et al., 2021). Over the last 50 years, more than 200 haplochromines have gone extinct due to the introduction of invasive species, habitat destruction, overfishing, and increasing pollution levels (Outa et al., 2020; Witte et al., 2000).

Until the 1970 s, the native *O. esculentus* and *O. variabilis* were the main species supporting fisheries in lakes Victoria, and Kyoga (Goudswaard et al., 2002). For example, the catches of *O. esculentus* contributed at least 70 % by weight of the total fish catches in Lake Victoria, Uganda (Taabu-Munyaho et al., 2016). Beyond the 1970 s, there was a gradual decline in the fishery of *O. esculentus* and subsequent displacement and extinction in its native ranges, and the species is now listed as critically endangered on the IUCN Red List (Tibihika et al., 2022). The native *O. esculentus* and *O. variabilis* are now found in satellite lakes of Kyoga and Victoria where they survive as separate and isolated stocks (Mwanja et al., 2010).

On the other hand, multiple and deliberate introductions of *O. niloticus* and *O. leucostictus* into the Victoria and Kyoga basins have taken place since the 1950 s (Tibihika et al., 2022), with these species being native to lakes Albert, Edward, and George in Uganda. By the 1970 s, these species had flourished and dominated the main fishery in the Victoria and Kyoga basins (Tibihika et al., 2022). For example, being an aggressive and prolific breeder and its ability to withstand harsh limnological changes allows *O. niloticus* to outcompete other species in the same habitats (Barreto et al., 2011). All these factors have attracted a lot of genetic studies on the same species (Angienda et al., 2011; Robledo et al., 2024; Shechonge et al., 2018; Tibihika et al., 2020). For example, different studies have documented cases of hybridization between *O. niloticus* and its congeneric species including *O. esculentus* (Angienda et al., 2011; Mwanja et al., 2010) and among them (Kwikiriza et al., 2023). Most of the studies that have documented gene flow between *Oreochromis* species have taken place in the Victoria Basin, Kenya, with only one in Uganda (Mwanja et al., 2010).

In Uganda, most of the genetic studies have focused on *O. niloticus* (Robledo et al., 2024; Tibihika et al., 2020). Consequently, the occurrence of hybridization and introgression among the *Oreochromis* species in the Victoria and Kyoga basins is understudied (Mwanja et al., 2010). Also, the only study available characterizing *O. niloticus* and *O. esculentus* population genetics (Mwanja et al., 2010) is based on a few microsatellite markers (10); thus, statistical analyses may have lower statistical power in describing more subtle patterns. Therefore, this constitutes a knowledge gap in understanding the current diversity of these species in the Victoria and Kyoga basins. This is especially critical given the increasing fishing pressure that these species face, being necessary to enhance the conservation of native *O. esculentus* while preserving wild genetic resources of the remaining species for future aquaculture purposes (Angienda et al., 2011; Lind et al., 2019).

In this study, we focus on the extent of gene flow between native *O. esculentus* and introduced *Oreochromis* species (*O. niloticus* and *O. leucostictus*) in the Lake Victoria and Kyoga basins of Uganda while providing some additional information about their current genetic diversity patterns. This is done using microsatellite (single sequence repeats, SSR) and mitochondrial DNA (mtDNA) genotyping data. Microsatellites are highly polymorphic and codominantly inherited (Schlötterer, 2000). They also have the ability to recover multiple alleles per locus, resulting in a high statistical power with a low number of sequenced markers (Ellegren, 2004). On the other hand, mtDNA has uniparental inheritance (maternal) and does not undergo recombination (Ladoukakis and Zouros, 2017). Therefore, applying both methods in this study provides more statistical power and comprehensive

understanding of the population genetics among these species. While there is existing research on the genetic structure, diversity, and differentiation in East Africa, much of it focuses on tilapiines in the region using either microsatellite markers or mtDNA markers. Microsatellite genetic markers employed are recognized to exhibit notable weaknesses or limitations, including size homoplasy (Tibihika et al., 2020). We prevent this limitation by implementing a genotyping by amplicon sequencing approach (SSR-GBAS), where microsatellite markers are sequenced, and this information is used for genotype definition instead of relying on amplicon size variation (Curto et al., 2019; Tibihika et al., 2018). Amidst SSR-GBAS being a cheaper approach, it also provides for the reproducibility of the data (Curto et al., 2019).

The study utilized mtDNA and SSR based on next-generation sequencing (NGS) to quantify levels of genetic diversity and connectivity among refugial populations of *O. esculentus* and to contrast this with the invasive species *O. niloticus*, and *O. leucostictus*. Specifically, we intended to identify populations of *O. esculentus* that have not received genetic introgression from *O. niloticus* and *O. leucostictus* for future conservation and management efforts. The study also profiled the population diversity and structure of *O. esculentus*, *O. leucostictus*, and *O. niloticus* among the satellite lakes of Victoria and Kyoga basins. Lastly, we determined the possibility of new hybrids resulting from recent introductions of the *Oreochromis* species in the satellite lakes in Uganda. The study hypothesized that refugial populations of *O. esculentus*, due to their long-term isolation and adaptation in stable environments, preserve their genetic integrity despite the contact with the invasive *O. niloticus* and *O. leucostictus*. The results contribute to the knowledge of the genetic variation found within both *Oreochromis* species in their native range and help in defining future stocks for aquaculture.

2. Materials and methods

2.1. Description of the sampled water bodies

Kyoga basin is found in eastern Uganda downstream of Victoria Nile. It is one of the largest basins in Uganda, stretching from the central part of the country to most parts of the eastern region and extending up to the northeastern part (Ojara et al., 2020). Extensive mats of *Cyperus papyrus* dominate the basin in addition to the main Lake Kyoga and more than 10 satellite lakes (Mwanja et al., 2010). The catchment area is 59,680 km², with more than 95 % of the catchment located in Uganda and the rest in Kenya (Ojara et al., 2020). In this study, the sampled lakes in the Kyoga basin included Bisina, Nawampasa, and Nyaguo in addition to the following beaches: Kagwara, Dagala, Namasale, and Lwampanga, located on the main Lake Kyoga (Fig. 1).

The Lake Victoria Basin (LVB) is situated in the upper reaches of the Nile River basin and comprises various lakes, wetlands, and rivers with a catchment area of approximately 194,200 km² traversing through five East African Countries (Tanzania, Kenya, Uganda, Rwanda, and Burundi) (Twesigye et al., 2011). Within Victoria basin, samples were collected from the following beaches on Lake Victoria: Portbell, Ssenyi, and Gaba. Additionally, samples were collected from the following satellite lakes in the Victoria basin: Nakivale, Kachera, Mburo, and Kijanebalola (Fig. 1).

2.2. Sample collection

A total of 739 individuals from 29 populations were collected across 10 natural water bodies in Uganda between January to May 2023 (Table 1, Fig. 1). Sampling comprised *Oreochromis niloticus* (333), *Oreochromis esculentus* (151), and *Oreochromis leucostictus* (258). The samples were obtained from local fishermen on landing their catches following overnight fishing expeditions. Therefore, no special animal rights were observed while collecting all the samples. However, in situations when the fish was caught alive, individuals were promptly euthanized using an overdose of clove oil, and a fin clip was extracted.

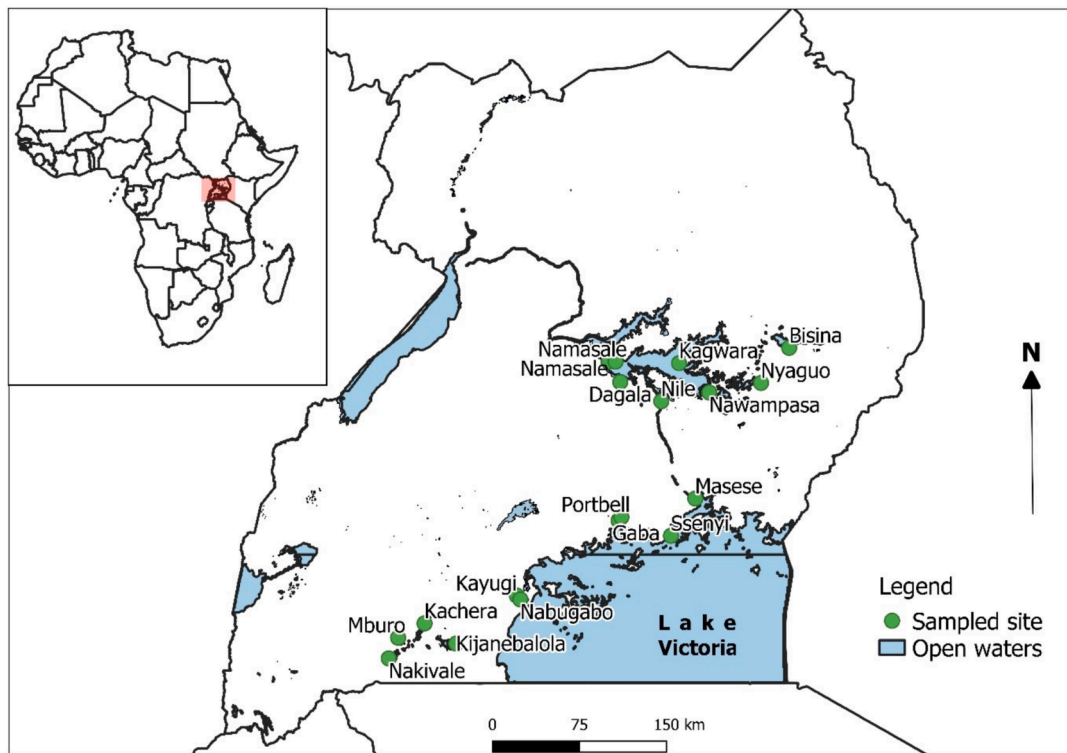


Fig. 1. Location of the sampling sites in different water bodies.

Table 1

Location of the sampling sites on different water bodies.

Basin	Water Body	Sampling site	Species	Code	No. Samples
Kyoga	Kyoga	Dagala	<i>O. niloticus</i>	KDN	23
			<i>O. leucostictus</i>	KDL	13
		Lwampanga	<i>O. leucostictus</i>	LWL	30
			<i>O. niloticus</i>	KNN	25
	Nawampasa	Namasale	<i>O. leucostictus</i>	KNL	19
			<i>O. niloticus</i>	KKL	24
		Kagwara	<i>O. niloticus</i>	NNN	34
			<i>O. leucostictus</i>	NNL	27
	Nyaguo	Nyaguo	<i>O. niloticus</i>	NYN	24
			<i>O. leucostictus</i>	NYL	33
	Bisina	Bisina	<i>O. niloticus</i>	BSN	27
			<i>O. leucostictus</i>	BSL	24
			<i>O. esculentus</i>	BSE	34
			<i>O. niloticus</i>	VSN	33
Victoria	Victoria	Ssenyi	<i>O. leucostictus</i>	VSL	12
			<i>O. niloticus</i>	GBL	18
		Gaba	<i>O. leucostictus</i>	GBL	18
			<i>O. niloticus</i>	GBN	18
	PortBell	PortBell	<i>O. leucostictus</i>	PtL	34
			<i>O. niloticus</i>	PtN	21
	Kijanebalola	Mikoni	<i>O. leucostictus</i>	KJL	21
			<i>O. niloticus</i>	KJN	17
		Mburu	<i>O. esculentus</i>	KJE	21
			<i>O. niloticus</i>	MRN	34
	Nabugabo	Rubare	<i>O. esculentus</i>	MRE	30
			<i>O. niloticus</i>	NAN	32
	Kachera	Nabugabo	<i>O. niloticus</i>	KRN	15
			<i>O. esculentus</i>	KRE	36
Nakivale	Kahirimbi	Nakivale	<i>O. esculentus</i>	KAE	30
			<i>O. niloticus</i>	KAN	30

The samples were then preserved in absolute ethanol (95 %) for subsequent genotyping at the Institute of Integrative Nature Conservation Research (INF), BOKU University.

2.3. Microsatellite and mtDNA genotyping

DNA extraction followed the protocol of Tibihika et al. (2019) using magnetic beads (MagSi-DNA beads-Magna Medics) and a magnetic separator SL-MagSep96 (Stein Brenner, Germany). DNA quality was verified using 1.5 % agarose gel and later visualized using a *trans*-illuminator system before amplification. Polymerase Chain Reaction (PCR) amplification was carried out using 43 previously developed microsatellites (SSR) for *O. niloticus* populations from East Africa (Tibihika et al., 2019). The selected primers were then divided into four multiplexes and used to prepare SSR genotyping by amplicon sequencing libraries (SSR-GBAS) based on the procedures by Kariuki et al. (2021) with a few modifications as indicated in Kwikiriza et al. (2023). The PCR products were pooled sample-wise, cleaned using AMPure magnetic beads, and subsequent indexing was performed following Tibihika et al. (2019). The individually indexed samples were pooled and used for a paired-end 300 bp sequencing run on an Illumina MiSeq at the Genomics Service Unit at Ludwig Maximilian Universität, München, Germany.

For mtDNA genotyping, primers specific for tilapia species were newly designed based on conserved regions among existing mitochondrial genomes of this group. One primer pair was constructed with Illumina-compatible overhangs targeting the cytochrome oxidase subunit I (COI) gene (Electronic Supplementary Material (ESM) Table S1). PCR reactions per sample contained a total volume of 5.0 µl including 2.0 µl of the genomic DNA, 0.25 µl of each primer (100 nM), 2.5 µl of Master mix (Qiagen Multiplex PCR Kit; Qiagen; Netherlands), and 0.25 µl of autoclaved water. The PCR conditions were as follows: initialization at 95 °C for 15 min, followed by denaturation for 30 cycles at 95 °C for 30 s, annealing at 55 °C for one minute, and elongation for one minute at 72 °C, and the last extension step at 72 °C for 10 min. The remaining amplicon library preparation and sequencing steps followed the same protocol of SSR-GBAS described above.

2.4. Sequence analysis, genotyping, and allele calling of microsatellite and mtDNA

Illumina sequencing data were quality-checked using FastQC version 0.11.9 and trimmed using Trimmomatic version 0.39 (Bolger et al., 2014) to remove adapters and poor-quality regions as described in Curto et al. (2019). Further steps including merging, demultiplexing, and allele calling were done using the Python scripts described in Curto et al. (2019). Codominant genotypes were used for microsatellite data, while mtDNA was further analyzed in a sequence form. Given the haploid nature of mtDNA, if more than one allele was found per individual, the one with the higher read count was kept. The sequences were then aligned with MAFFT (Kato and Standley, 2013) using the global alignment option and primers were removed manually.

2.5. Population genetics analysis with the microsatellite dataset

The SSR codominant matrix was mostly analyzed using GenAlEx v. 6.503 (Peakall and Smouse, 2006) to evaluate genetic diversity and differentiation patterns at both species and population levels. Genetic diversity was assessed based on the average number of alleles per locus (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and F-statistics (F).

Genetic differentiation was primarily evaluated using pairwise FST values across all populations and species. Nei genetic distance calculated in GenAlEx v. 6.503 was used to compute a principal coordinate analysis (PCoA) allowing the visualization of genetic structure patterns.

This was further explored in two approaches. Firstly, Nei's genetic distances among populations and species (Schneider et al., 2000) were visualized using an unweighted pair group method with an arithmetic mean (UPGMA) dendrogram generated by Populations v.1.2.32 (Langella, 1999). Support values were obtained by executing 1000 bootstrap replicates over 36 loci. Secondly, using STRUCTURE v. 2.3.4 (Hubisz et al., 2009), genetic clustering among samples was analyzed. The procedure involved 15 iterations with K set from 1 to 15 and 100,000 generations after a burn-in period of 10,000 using the admixture model and considering allele frequencies as independent. This was done for datasets consisting of all the species and their respective populations. Optimal K values were chosen based on the Evanno method as implemented in Structure Selector (Li et al., 2018).

2.6. MtDNA data analysis

The 687 generated COI sequences were aligned with the software BioEdit v.7.0 (Hall, 1999) and checked manually. To determine levels of extant genetic diversity, the number of polymorphic sites, haplotypes, haplotype diversity (H_d), and nucleotide diversity (π) were calculated using DnaSP Version: 6.12 (Librado and Rozas, 2009) for each species and population. The same program was used to test deviations from neutrality through the calculation of Tajima's D. PopArt was used to construct a haplotype network to visualize the relationship among *Oreochromis* species (Leigh and Bryant, 2015).

3. Results

3.1. Microsatellite markers and allele frequencies

In total, 279,662,004 paired reads were produced for genotyping, from which 246,291,333 passed the quality control steps that were later used for allele calling. Out of the 41 SSR loci used, five (TI29_TAAAA, TI44_GAAAA, TI50_ATGG, TI60_ATCC, and TI61_TGGA) were excluded since they showed more than 50 % missing data resulting in a final dataset of 36 markers.

3.2. Genetic diversity among *Oreochromis* species using microsatellite markers

The heterozygosity of *O. niloticus* populations was generally low, clustering in the lower quartile and indicating reduced genetic diversity, with median heterozygosity values below 0.5 (Fig. 2). In contrast, higher genetic diversity (> 0.5) was observed in the Victoria basin's satellite lakes, such as Lake Nakivale (0.56 ± 0.03), while Lake Kyoga's Dagala landing site exhibited the lowest diversity (0.44 ± 0.05) (Table 2). For *O. leucostictus*, Kyoga basin populations displayed greater heterozygosity (> 0.44), with Nawampasa having the highest value (0.46 ± 0.06) and Gaba the lowest (0.34 ± 0.05) on Lake Victoria (Table 2). Among *O. esculentus*, Bisina and Kijanebalola showed the highest diversity (> 0.6), while Lake Mburo had the lowest (0.29 ± 0.05) (Table 2).

3.3. Population differentiation and hierarchical clustering

Pairwise genetic differentiation (FST values) among the populations of the three *Oreochromis* species was investigated (Table 3).

Comparisons within the populations of *O. niloticus* showed the lowest FST values between Nawampasa and Namasale (0.01) and Senyi and PortBell (0.01), and the highest FST values were between Bisina and Kijanebalola (0.1) and Namasale and Mburo (0.1) (Table 3A). Comparisons involving Nakivale showed the highest FST values. Lower genetic differentiation levels were also observed among the populations of *O. esculentus*, with the least differentiation between Nakivale and Kachera (0.06) and the highest between Nakivale and Kijanebalola (0.13) as well as between Bisina and Mburo (0.14) (Table 3B).

Among the populations of *O. leucostictus*, a higher FST value was observed between Gaba and Kijanebalola (0.20) in Victoria basin, while the lowest value shown was between Kagwara and Lwampaga (0.08) in the Kyoga basin (ESM Table S2).

The UPGMA dendrogram showed three main clades corresponding to the three species under study (Fig. 3). The clade highlighted in black contains *O. niloticus* populations, while the purple and blue clades represent *O. leucostictus* and *O. esculentus* populations, respectively (Fig. 3). Among the *O. niloticus* populations, the UPGMA dendrogram showed that all the samples from the Kyoga basin were more similar to each other than to the Victoria basin. For example, populations from Bisina and Nyaguo located in the Kyoga basin were close to each other as were the populations of Kachera, Nakivale, Mburo, and Kijanebalola within Victoria basin. Similar closeness was observed among *O. niloticus* populations from Lake Victoria from Senyi, Gaba, and Portbell beaches (Fig. 3).

Among the *O. leucostictus* populations, samples from Nyaguo were divergent from other populations. Kagwara and Lwampaga were genetically similar to Nile and Portbell populations (Fig. 3).

Within *O. esculentus*, two main clades were observed corresponding to the Kyoga and Victoria basins. The first clade shows Nakivale, Mburo, and Kachera which are genetically similar and both from Victoria basin. Although the second one contains *O. esculentus* from Kijanebalola and Bisina, the length of the clade indicates that they are genetically different (Fig. 3). Kijanebalola is found in the Victoria basin while Bisina is in the Kyoga basin.

The Neighbor-Joining tree topology revealed three major divergences corresponding to the species (Fig. 4A). For example, in the case of *O. esculentus*, populations within Victoria basin were closer to each other and showed a higher divergence from populations in the Kyoga basin (Fig. 4A). All the populations of *O. leucostictus* were also divergent from other species (Fig. 4A). At the population level, *O. leucostictus* from Bisina and Nyaguo showed a slightly longer distance from other populations.

Samples of *O. niloticus* from Kachera, Kijanebalola, Mburo, and Nakivale, all found in the Victoria basin, were closer to each other, as were the Nyaguo and Bisina from Kyoga basin. A similar relationship was observed among the *O. niloticus* populations from Senyi, PortBell,

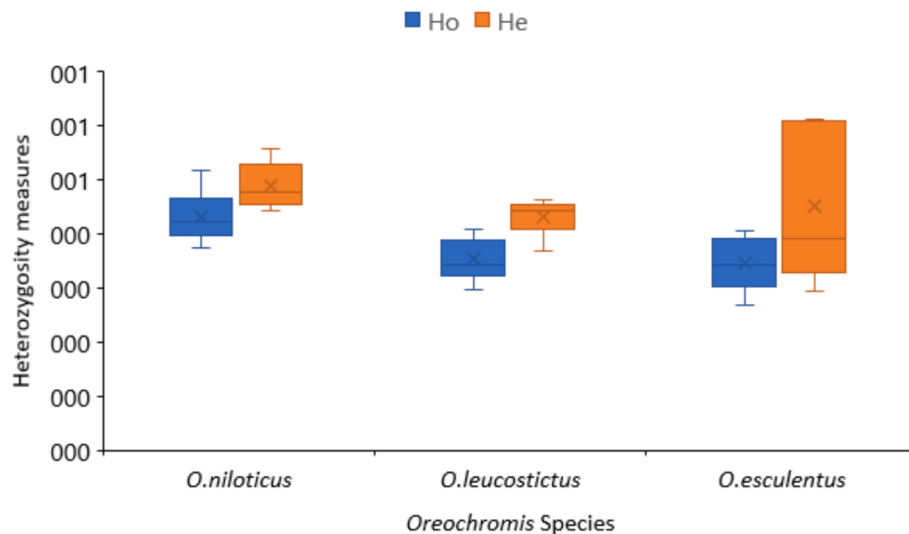


Fig. 2. Box plot showing the heterozygosity measures among the *Oreochromis* species.

Table 2

Heterozygosity by population for codominant data for tilapiines (Mean $\pm$ SE). N: Average number of samples, Na: Number of alleles, Ne: Number of effective population size, Ho: Observed heterozygosity, He: Expected heterozygosity, F: Fixation index.

Category	Population	N	Na	Ne	Ho	He	F
Species	<i>O. niloticus</i>	280.69 $\pm$ 3.65	11.42 $\pm$ 1.09	3.37 $\pm$ 0.41	0.43 $\pm$ 0.04	0.54 $\pm$ 0.05	0.18 $\pm$ 0.03
	<i>O. leucostictus</i>	190.92 $\pm$ 12.75	9.11 $\pm$ 1.23	3.51 $\pm$ 0.48	0.36 $\pm$ 0.05	0.49 $\pm$ 0.06	0.18 $\pm$ 0.04
	<i>O. esculentus</i>	113.47 $\pm$ 6.99	11.28 $\pm$ 0.99	2.70 $\pm$ 0.27	0.36 $\pm$ 0.04	0.50 $\pm$ 0.04	0.39 $\pm$ 0.05
	Total	195.03 $\pm$ 8.25	10.60 $\pm$ 0.64	3.19 $\pm$ 0.23	0.38 $\pm$ 0.03	0.51 $\pm$ 0.03	0.25 $\pm$ 0.03
<i>O. niloticus</i>	Bisina_ON	25.39 $\pm$ 0.22	4.39 $\pm$ 0.43	2.67 $\pm$ 0.28	0.42 $\pm$ 0.05	0.46 $\pm$ 0.05	0.08 $\pm$ 0.04
	Nawampasa_ON	26.22 $\pm$ 0.24	5.25 $\pm$ 0.53	2.88 $\pm$ 0.32	0.43 $\pm$ 0.05	0.47 $\pm$ 0.05	0.07 $\pm$ 0.03
	Nyaguo_ON	24.58 $\pm$ 0.24	4.78 $\pm$ 0.53	2.89 $\pm$ 0.32	0.41 $\pm$ 0.05	0.48 $\pm$ 0.05	0.11 $\pm$ 0.04
	Dagala_ON	19.86 $\pm$ 0.39	4.03 $\pm$ 0.43	2.56 $\pm$ 0.26	0.40 $\pm$ 0.05	0.44 $\pm$ 0.05	0.06 $\pm$ 0.04
	Namasale_ON	19.56 $\pm$ 0.43	4.17 $\pm$ 0.47	2.69 $\pm$ 0.32	0.39 $\pm$ 0.05	0.45 $\pm$ 0.05	0.10 $\pm$ 0.05
	Gaba_ON	17.08 $\pm$ 0.44	5.22 $\pm$ 0.50	2.75 $\pm$ 0.27	0.44 $\pm$ 0.05	0.49 $\pm$ 0.05	0.07 $\pm$ 0.05
	PortBell_ON	20.36 $\pm$ 0.33	4.64 $\pm$ 0.41	2.66 $\pm$ 0.28	0.42 $\pm$ 0.04	0.48 $\pm$ 0.05	0.10 $\pm$ 0.04
	Senyi_ON	31.03 $\pm$ 0.84	5.06 $\pm$ 0.51	2.70 $\pm$ 0.29	0.38 $\pm$ 0.05	0.47 $\pm$ 0.05	0.17 $\pm$ 0.05
	Nakivale_ON	27.36 $\pm$ 0.27	5.67 $\pm$ 0.47	2.82 $\pm$ 0.25	0.52 $\pm$ 0.04	0.56 $\pm$ 0.03	0.05 $\pm$ 0.04
	Kijanebalola_ON	14.19 $\pm$ 0.31	4.78 $\pm$ 0.39	2.63 $\pm$ 0.22	0.47 $\pm$ 0.03	0.52 $\pm$ 0.04	0.07 $\pm$ 0.04
	Kachera_ON	11.33 $\pm$ 0.22	4.39 $\pm$ 0.31	2.72 $\pm$ 0.22	0.46 $\pm$ 0.04	0.53 $\pm$ 0.04	0.09 $\pm$ 0.05
	Mburo_ON	18.47 $\pm$ 0.27	5.06 $\pm$ 0.38	2.79 $\pm$ 0.25	0.49 $\pm$ 0.04	0.54 $\pm$ 0.04	0.07 $\pm$ 0.04
<i>O. leucostictus</i>	Nabugabo_ON	25.25 $\pm$ 0.35	4.56 $\pm$ 0.49	2.61 $\pm$ 0.28	0.37 $\pm$ 0.05	0.45 $\pm$ 0.05	0.14 $\pm$ 0.06
	Kagwara_LE	18.36 $\pm$ 1.13	4.39 $\pm$ 0.58	2.82 $\pm$ 0.36	0.41 $\pm$ 0.06	0.45 $\pm$ 0.06	0.07 $\pm$ 0.05
	Lwampanga_LE	20.36 $\pm$ 1.18	4.67 $\pm$ 0.62	3.04 $\pm$ 0.41	0.38 $\pm$ 0.06	0.45 $\pm$ 0.06	0.10 $\pm$ 0.06
	Bisina_LE	18.83 $\pm$ 1.28	4.39 $\pm$ 0.61	2.69 $\pm$ 0.34	0.39 $\pm$ 0.05	0.45 $\pm$ 0.05	0.08 $\pm$ 0.05
	Nawampasa_LE	24.06 $\pm$ 1.64	5.42 $\pm$ 0.72	3.15 $\pm$ 0.42	0.34 $\pm$ 0.05	0.46 $\pm$ 0.06	0.19 $\pm$ 0.06
	Nyaguo_LE	25.78 $\pm$ 1.86	4.31 $\pm$ 0.63	2.60 $\pm$ 0.34	0.34 $\pm$ 0.05	0.43 $\pm$ 0.05	0.20 $\pm$ 0.07
	Gaba_LE	11.61 $\pm$ 0.91	3.17 $\pm$ 0.44	2.07 $\pm$ 0.28	0.29 $\pm$ 0.05	0.34 $\pm$ 0.05	0.12 $\pm$ 0.06
	PortBell_LE	21.39 $\pm$ 1.58	4.28 $\pm$ 0.52	2.69 $\pm$ 0.32	0.38 $\pm$ 0.05	0.44 $\pm$ 0.05	0.08 $\pm$ 0.07
	Masese_LE	26.03 $\pm$ 2.00	3.69 $\pm$ 0.48	2.23 $\pm$ 0.27	0.30 $\pm$ 0.05	0.37 $\pm$ 0.05	0.14 $\pm$ 0.05
<i>O. esculentus</i>	Kijanebalola_LE	8.39 $\pm$ 0.52	3.11 $\pm$ 0.41	2.28 $\pm$ 0.28	0.31 $\pm$ 0.06	0.39 $\pm$ 0.05	0.23 $\pm$ 0.08
	Bisina_ES	23.36 $\pm$ 1.49	7.94 $\pm$ 0.76	3.79 $\pm$ 0.51	0.34 $\pm$ 0.04	0.61 $\pm$ 0.04	0.49 $\pm$ 0.04
	Nakivale_ES	24.97 $\pm$ 1.47	3.39 $\pm$ 0.39	1.93 $\pm$ 0.18	0.38 $\pm$ 0.06	0.36 $\pm$ 0.05	0.05 $\pm$ 0.06
	Kijanebalola_ES	11.72 $\pm$ 0.75	5.92 $\pm$ 0.54	3.44 $\pm$ 0.40	0.34 $\pm$ 0.04	0.60 $\pm$ 0.03	0.47 $\pm$ 0.04
	Kachera_ES	30.08 $\pm$ 1.94	3.86 $\pm$ 0.28	2.04 $\pm$ 0.17	0.41 $\pm$ 0.06	0.39 $\pm$ 0.04	0.22 $\pm$ 0.09
	Mburo_ES	23.33 $\pm$ 1.57	2.42 $\pm$ 0.26	1.72 $\pm$ 0.16	0.27 $\pm$ 0.05	0.29 $\pm$ 0.05	0.14 $\pm$ 0.07
Overall	Total	20.90 $\pm$ 0.27	4.53 $\pm$ 0.10	2.66 $\pm$ 0.06	0.39 $\pm$ 0.01	0.46 $\pm$ 0.01	0.14 $\pm$ 0.01

and Gaba beaches on Lake Victoria (Fig. 4B).

The PCoA analysis showed a similar segregation pattern as depicted from the UPGMA and NJ among the species where three main clusters were observed, with each cluster representing a species at both axes (Fig. 5A). A few samples from *O. esculentus* were observed in the cluster containing *O. leucostictus* (Fig. 5A). When the plots are labeled per population, it is possible to see that these individuals come from the Bisina sampling site (Fig. 5B). Similarly, one individual from *O. esculentus* Kachera clustered together with *O. niloticus*. Interestingly, several *O. esculentus* individuals from Bisina and Kijanebalola, as well as one *O. niloticus* individual from Nakivale, showed an intermediate

position among the three clusters.

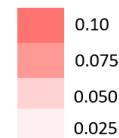
Among the *O. niloticus* populations, samples clustered according to the basin of origin (Fig. 5C). Victoria basin formed two clusters. The first contained Nakivale, Kijanebalola, Kachera, and Mburo, and the second contained Portbell, Senyi, and Gaba beaches on Lake Victoria. Although Nabugabo populations showed an independent clustering, they were closer to samples collected from Lake Victoria beaches than its satellite lakes (Fig. 5C). The populations from Kyoga basin generally formed a third cluster with a few samples from Namasale, Dagala, and Nawampasa closer to Victoria populations (Fig. 5C).

Populations of *O. leucostictus* formed two main clusters, with samples

Table 3The pairwise population FST values of the different populations A; *O. niloticus*, B; *O. esculentus*. NNN: Nawampasa, KNN: Namasale, KJN: Kijanebalola.

A												
Bisina	NNN	Nyaguo	Dagala	KNN	Gaba	Portbell	Senyi	Nakivale	KJN	Kachera	Mburo	Nabugabo
0.00												Bisina
0.04	0.00											NNN
0.02	0.03	0.00										Nyaguo
0.04	0.02	0.04	0.00									Dagala
0.05	0.01	0.04	0.02	0.00								KNN
0.05	0.03	0.05	0.04	0.04	0.00							Gaba
0.06	0.04	0.05	0.04	0.05	0.02	0.00						PortBell
0.06	0.04	0.06	0.04	0.04	0.02	0.01	0.00					Senyi
0.09	0.09	0.09	0.10	0.10	0.07	0.07	0.06	0.00				Nakivale
0.10	0.09	0.09	0.09	0.10	0.07	0.07	0.06	0.03	0.00			KJN
0.09	0.09	0.09	0.09	0.10	0.07	0.07	0.06	0.03	0.02	0.00		Kachera
0.08	0.06	0.07	0.07	0.07	0.04	0.04	0.04	0.02	0.04	0.04	0.00	Mburo
0.06	0.03	0.05	0.03	0.03	0.03	0.03	0.03	0.09	0.09	0.09	0.06	0.00 Nabugabo

B				
Bisina	Nakivale	Kijanebalola	Kachera	Mburo
0.00				
0.11	0.00			
0.06	0.14	0.00		
0.10	0.06	0.11	0.00	
0.13	0.09	0.14	0.07	0.00



from Nyaguo and a few individuals from Bisina clustering together. The second cluster contained samples from Masese, Portbell, Gaba, and Kijanebalola (Fig. 5D).

Natural populations of *O. esculentus* from Nakivale, Kachera, and Mburo closely clustered together. Bisina and Kijanebalola formed another cluster, with a few samples from Kachera forming a sub-cluster (Fig. 5E).

3.4. Genetic structure and signs of admixture among the *Oreochromis* species

Based on delta K (ΔK) values, $K = 3$ was considered the best fit for the data for both species and populations. Structure Selector results also suggested $K = 6$ and $K = 8$ as the second and third-best fit for the species' data (Fig. 6A). Generally, all the STRUCTURE outputs for different groups (Fig. 6) were congruent with the UPGMA (Fig. 3), Neighbor-Joining network (Fig. 4), and PCoA (Fig. 5) analyses. *Oreochromis*

species were assigned into three independent clusters, and admixture was observed for all K values (Fig. 6A). Intraspecific admixture was found for one individual of *O. niloticus* that showed assignment to the *O. esculentus* cluster and a few *O. esculentus* individuals partially assigned to the *O. leucostictus* and *O. niloticus* cluster. Some individuals from *O. esculentus* were completely assigned to the *O. esculentus* cluster.

Among the *O. niloticus* populations, $K = 2$ was considered the best fit for the data while $K = 7$ and $K = 10$ came in second and third, respectively (Fig. 6B). Samples from Bisina and Nyaguo were assigned together, as were Nawampasa, Dagala, and Namasale, all located in the Kyoga basin (Fig. 6B). Samples from the beaches of Lake Victoria (Gaba, PortBell, and Senyi) showed mixed assignments. The satellite lakes in the Victoria basin (Nakivale, Kijanebalola, Kachera, and Mburo) showed similar assignments (Fig. 6B). Nabugabo populations showed some degree of mixed assignment with Lake Mburo.

Within *O. leucostictus* populations, $K = 2$, $K = 4$, and $K = 8$ were considered the first, second, and third best fit for the data (Fig. 6C). At K

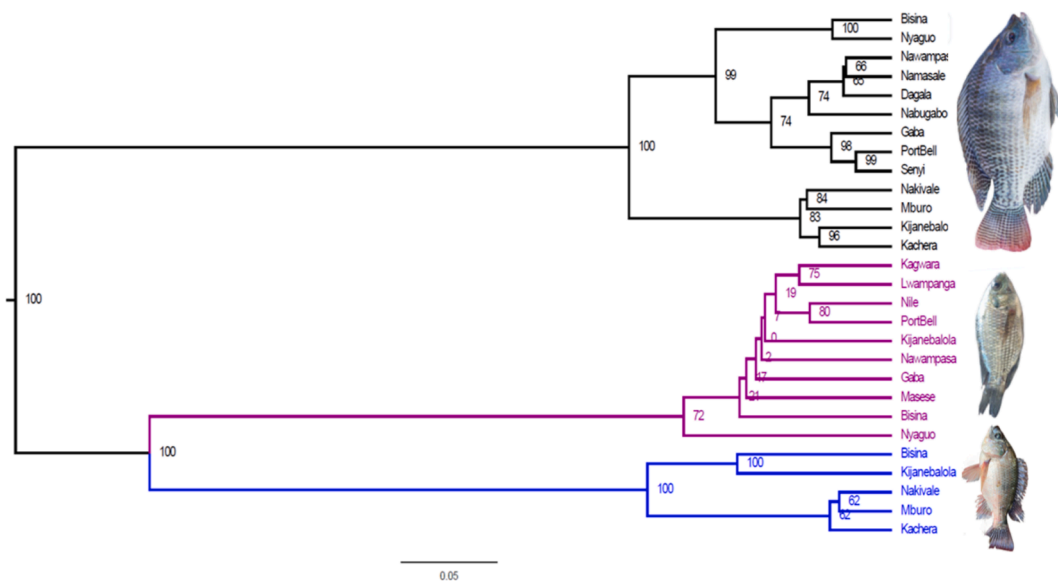


Fig. 3. UPGMA dendrogram of the different populations of *Oreochromis* species constructed using Nei's genetic distance. Support is given by bootstrap values. Black: *O. niloticus*, Purple: *Oreochromis leucostictus*, Blue: *Oreochromis esculentus*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

= 2, two main assignments were observed, with populations from Bisina, Nawampasa, and Nyaguo clustering together (Fig. 6C). At $K = 4$ and $K = 8$, apart from Nyaguo and Masese which showed independent assignments, the remaining populations demonstrated mixed genetic clusters (Fig. 6C).

Among the *O. esculentus* populations, all $K = 2$, $K = 4$, and $K = 8$ were considered the first, second, and third best, respectively, and showed similar assignments among the *O. esculentus* populations (Fig. 6D). Populations from Nakivale, Kachera, and Mburo were assigned to one genetic cluster, while Bisina and Kijanebalola showed mixed genetic clustering (Fig. 6D).

3.5. Genetic diversity and population differentiation using mtDNA markers

The dataset comprised a total of 61 and 58 polymorphic and parsimony informative sites, respectively. Invasive *O. niloticus* showed the highest value of polymorphic and parsimony informative sites ($S = 47$ and $PIS = 45$, respectively), followed by *O. esculentus*; the lowest values were observed in *O. leucostictus* (Table 4).

Generally, the dataset showed 26 total haplotypes with *O. niloticus* showing the highest haplotype diversity values (number of haplotypes = 14, $Hd = 0.78$), followed by *O. esculentus* (haplotypes = 11, $Hd = 0.41$) (Table 4). The lowest was found in *O. leucostictus* with only four haplotypes observed. Additionally, both *O. niloticus* and *O. esculentus* had a similar nucleotide diversity (π) of 0.01, while *O. leucostictus* had a value of 0.00 (Table 4).

Among the *O. niloticus* populations, samples from Lake Nawampasa (haplotypes = 7, $Hd = 0.74$) and Lake Nakivale (haplotypes = 7, $Hd = 0.84$) showed the highest number of haplotypes, followed by Kijanebalola (haplotypes = 6, $Hd = 0.66$) and Mburo (haplotypes = 6, $Hd = 0.72$), both located in Victoria basin, and the least in Namasale (haplotypes = 1, $Hd = 0.00$) (Fig. 7A). Generally, all the populations from the Kyoga basin exhibited lower haplotypes than Victoria basin populations (Fig. 7A). All the samples from beaches on Lake Victoria (Gaba, Portbell, and Senyi) showed a positive Tajima's D . Similar positive value was observed in samples from the Kyoga basin (Dagala, Bisina, and Nyaguo). Samples from the satellite lakes of the Victoria basin showed a negative Tajima's D indicating an excess of rare alleles compared to neutral expectations (Table 4).

With *O. leucostictus* populations, samples from Gaba (haplotypes = 4, $Hd = 0.63$) had the highest haplotypes followed by Portbell (haplotypes = 3, $Hd = 0.39$), while Lwampanga, Kagwara, Nile and Masese had the lowest value (haplotypes = 1, $Hd = 0.00$) (Fig. 7B). Apart from Kijanebalola, all other populations had a negative Tajima's D (ESM Table S3B).

For *O. esculentus*, Bisina samples (haplotypes = 9, $Hd = 0.77$) showed the highest number of haplotypes followed by Kijanebalola (haplotypes = 8, $Hd = 0.82$), while Nakivale had the smallest (haplotypes = 1, $Hd = 0.00$) (Fig. 7C). All the *O. esculentus* populations had a negative Tajima's D , indicating an excess of rare alleles compared to neutral expectations (ESM Table S3C).

3.6. Population differentiation of the *Oreochromis* species

Generally, 26 haplotypes were observed with *O. niloticus* having a higher number of singleton (monomorphic) sites (12) followed by *O. esculentus* (8), while *O. leucostictus* had the fewest sites (2) (Fig. 8A). Within *O. niloticus*, six haplotypes (H14, H15, H19, H20, H22, and H23) were more frequent than the others. Among *O. esculentus* and *O. leucostictus* individuals, H2 and H26, respectively, were the most abundant (Fig. 8A). Seven out of the eleven haplotypes for *O. esculentus* were private or singleton haplotypes, which generally indicated the different levels of genetic variability in the entire dataset. The most widespread haplotypes were H22 and H15, with H22 being the most widely distributed across the *O. niloticus* dataset (Fig. 8A).

Generally, three groups of haplotypes are found corresponding to the three *Oreochromis* species. Nevertheless, some haplotypes were shared among species. H2, the most abundant haplotype among *O. esculentus* populations, was found in three individuals of *O. niloticus*. Similarly, H22 was predominantly associated with *O. niloticus* and shared with only six *O. leucostictus* (Fig. 6). Finally, H26, mostly found in *O. leucostictus*, was found in seven *O. esculentus* (ESM Table S4).

Among the *O. esculentus* populations, H3 was the most abundant and shared by all the populations (Fig. 8B). Within this haplotype, samples from Nakivale, Kachera, and Mburo showed the highest contribution. Populations from Kijanebalola and Bisina lakes shared some haplotypes (H7, H12, and H4). Populations from Bisina showed the highest contribution of individuals with H7 (Fig. 8B). Samples from Bisina located in the Kyoga basin had five private or singleton haplotypes (H6,

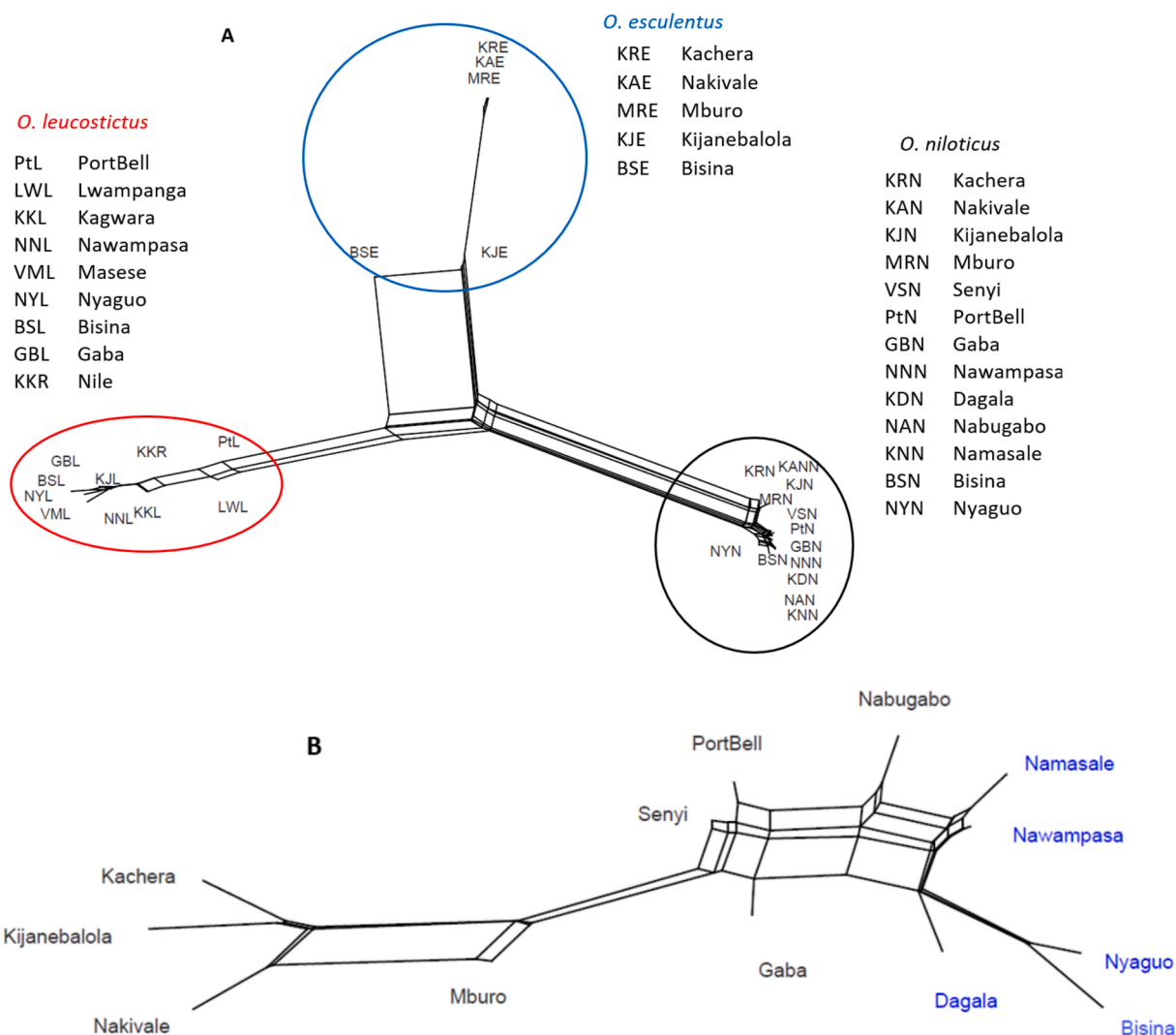


Fig. 4. Genetic structure based on unrooted network tree illustrating population relationships that are depicted on genetic distance. **A:** All populations, **B:** *Oreochromis niloticus* populations. In Fig. 3B, the sites labeled blue are located in the Kyoga basin while black is in the Victoria basin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

H9, H10, H13, and H15). Similarly, Kijanebalola had some private haplotypes (H5, H11, H14, and H15) (Fig. 8B). Kachera and Mburo lakes showed two haplotypes (H1 and H16), and Kooki Lakes showed one haplotype (H2).

4. Discussion

Lake Victoria and Lake Kyoga basins are vital aquatic ecosystems, playing important ecological and socioeconomic roles, including providing a livelihood for surrounding communities through fisheries production (Natugonza et al., 2021). Amidst the importance of these basins, anthropogenic activities like overfishing, unregulated fish transfers, and the introduction of non-native species have threatened the genetic diversity of *Oreochromis* species (Bilirwa et al., 2003). Therefore, for the efficient management of these species, understanding the patterns and extent of genetic divergence is essential within these basins.

4.1. Genetic structure and diversity of endangered *O. esculentus*

The native critically endangered *O. esculentus* exhibited lower genetic diversity but did not show substantial differences among the populations, which aligns with previous studies (Kariuki et al., 2021;

Mwanja et al., 2010). However, genetic diversity was generally lower than in the non-native species, which may reflect the bottleneck that this species went through as a result of its extirpation from the main lakes. The higher genetic diversity exhibited in Bisina could be attributed to factors like the larger size of the lake as well as minimal levels of exploitation of the fishery. Among the studied satellite lakes, Bisina is the largest with a surface area of 192 km² (Barasa and Wanyama, 2020), which could mean that the lake supports larger fish populations leading to higher genetic diversity (Lind et al., 2019). Additionally, larger water bodies provide different habitats and ecological niches leading to the adaptation of different genetic lineages to specific environmental conditions (Ndiwa et al., 2014). Contrary, lower genetic diversity observed in populations of Nakivale, Kachera, and Mburo found in the Victoria basin could be attributed to factors such as smaller size, inbreeding, and over-exploitation. These smaller lakes—Nakivale (26 km²), Kachera (42 km²), and Mburo (11 km²) (Adonia, 2013)—may contain lower carrying capacity and consequently lower effective population sizes. Additionally, overfishing can also contribute to lower genetic diversity through a fast decrease of the effective population size (Angienda et al., 2010). This is unlikely to be the case for Lake Mburo, since it is part of the Lake Mburo National Park, being protected by both park and fisheries authorities. However, this is not the case for some of the satellite lakes where heavy fishing pressures coupled with habitat destruction,

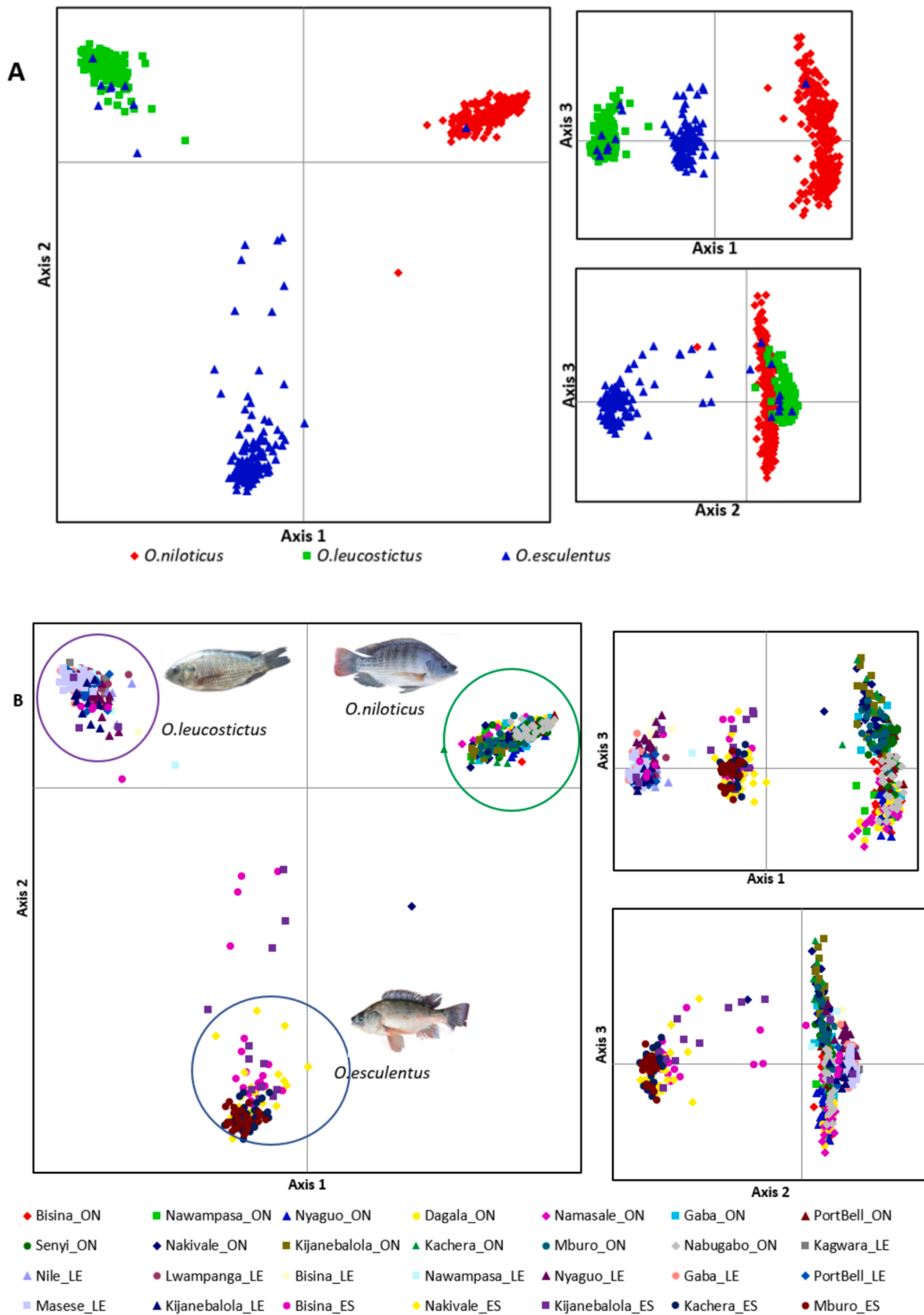


Fig. 5. Genetic structure as visualized through scatter plots based on PCoA for the tilapiines. **A:** Species, **B:** All populations, **C:** *O. niloticus* populations, **D:** *O. leucostictus* populations, **E:** *O. esculentus* populations.

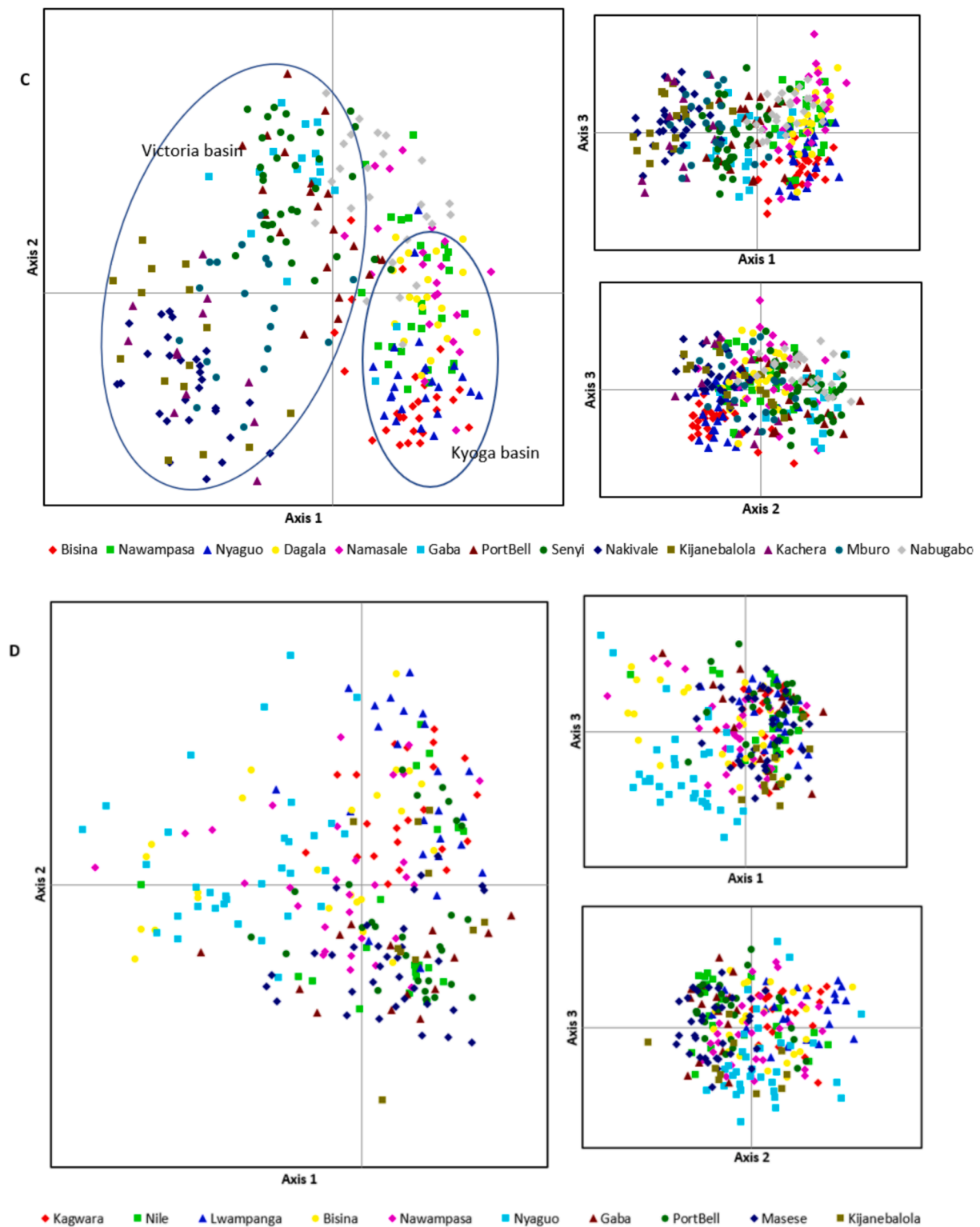


Fig. 5. (continued).

especially in the lakes of Kachera and Kijanebalola (Musinguzi et al., 2020; Mwanja et al., 2010), may be contributing to a loss of genetic diversity.

Different approaches including UPGMA-dendrogram, Neighbor-Joining tree (NJ), Principal Coordinates Analysis (PCoA), and Bayesian clustering algorithms (STRUCTURE) observed two main *O. esculentus* population groups: Victoria basin and Kyoga basin. Therefore, our results affirm that *O. esculentus* populations from Uganda can be delimited

into two stocks and therefore managed as such. Nevertheless, some degree of admixture was observed among them, which could be a result of uncontrolled movement/transfer of fish from one location to another by fishermen. Additionally, the close connectivity of these water bodies provided by the swamps and rivers could contribute to the observed pattern (Angienda et al., 2010; Kwikiriza et al., 2023; Mwanja et al., 2012). Studies by Mwanja et al. (2012) observed that populations from the Koki lakes showed a higher genetic similarity, and this aligns with

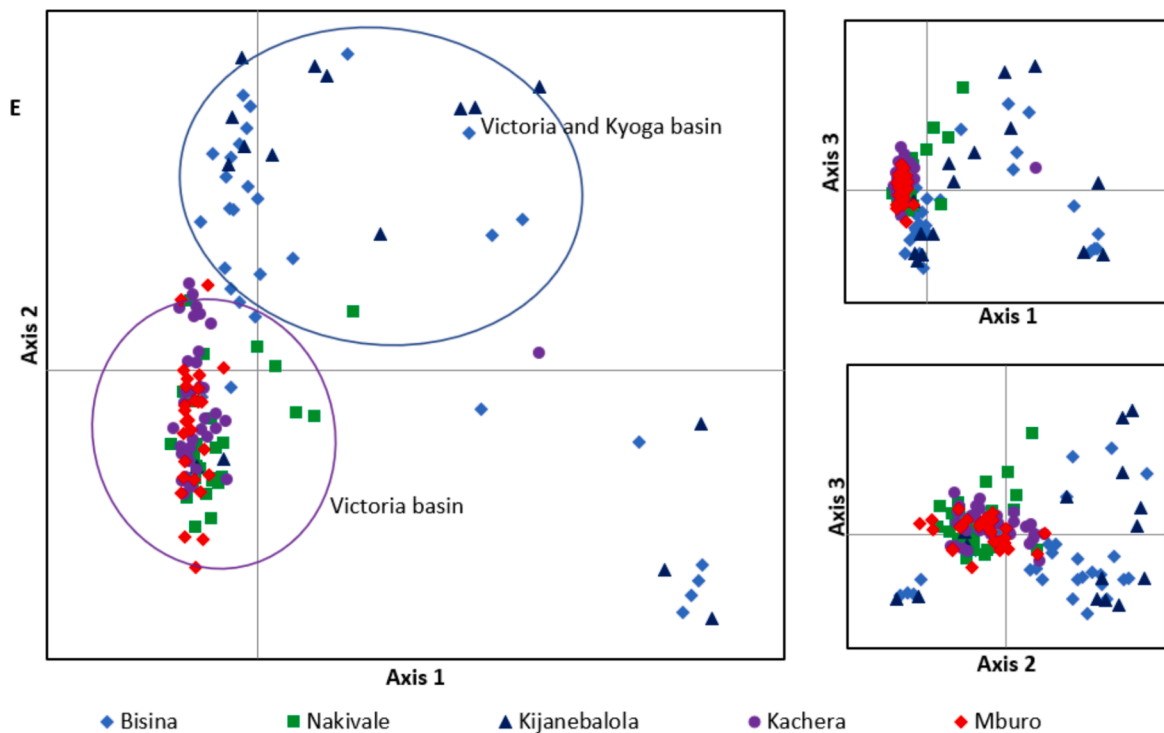


Fig. 5. (continued).

the current results. Lakes Nakivale, Mburo, and Kachera are known as Koki lakes and connected through an extensive wetland with one inflow, River Rwizi (Mwanja et al., 2012). Among them, the river changes its name to Kibale through a series of papyrus wetlands as it drains to Lake Kijanebalola, with subsequent outflow into Sango Bay on Lake Victoria (Kamukasa and Bintoor, 2014). This connectivity between these water bodies suggests gene flow between the *O. esculentus* populations, as indicated by the lower F_{ST} values between Mburo and Kachera showing the importance of these types of habitats to the conservation of this species. Therefore, populations from Kachera, Mburo, and Nakivale can be considered the same, thus allowing for the development of similar conservation measures. To further explore the dynamics of admixture within these populations, all the haplotypes of *O. esculentus* from the Victoria basin are close to each other with less than three mutational changes, indicating the higher levels of admixtures. All these confirm that there is gene flow between *O. esculentus* populations in the sampled satellite lakes.

4.2. Genetic diversity and differentiation of translocated *O. niloticus* populations

Populations of *O. niloticus* from satellite lakes Kachera and Mburo had slightly lower genetic diversity than the main Lake Victoria landing beaches, which aligns with previous studies (Angienda et al., 2011; Kariuki et al., 2021; Robledo et al., 2024; Tibihika et al., 2020). Notably, the population of Lake Nakivale exhibited the highest genetic diversity, likely attributed to the various management strategies implemented on the lake aimed at mitigating overfishing and promoting conservation efforts. As Lake Nakivale is situated within a refugee settlement area, various interventions have been implemented by both governmental and international entities to bolster food security in the region (Mwangu, 2022). Among them is the continuous restocking of the lake with *O. niloticus* from major lakes like Victoria to boost capture fisheries and support refuge communities (Mwangu, 2022). The continuous restocking of this lake has introduced individuals that may carry genetic variation not previously present in the lake's population, thereby

increasing genetic diversity (Shechonge et al., 2018).

The moderately higher genetic diversity among the populations from Lake Victoria and Kyoga beaches could be attributed to multiple introductions, uncontrolled fish escapes from aquaculture farms, and the larger size of the respective water bodies (Kwikiriza et al., 2023; Tibihika et al., 2020). Similarly, the current mtDNA results show a relatively higher haplotype diversity in *O. niloticus* compared to other *Oreochromis* species, aligning to the previous studies (Angienda et al., 2011; Kwikiriza et al., 2023). Given that the introduction of *O. niloticus* to Lake Victoria occurred as far back as the 1950 s, this extended period has allowed for the establishment of genetic divergence within the introduced population, influenced by factors such as ecological tolerance and biological fecundity (Angienda et al., 2011; Firmat et al., 2013; Tibihika et al., 2020). Therefore, the higher haplotype and genetic diversity of *O. niloticus* populations could be attributed to the widespread introductions and distributions of *O. niloticus* to different geographical areas within Ugandan water bodies. Moreover, this stoking through time was likely sourced from multiple native stocks boosting the genetic diversity even further (Tibihika et al., 2020).

The UPGMA dendrogram categorized *O. niloticus* populations into four distinct groups corresponding to the geographical source of the samples. As observed in *O. esculentus*, the genetic structuring of the introduced *O. niloticus* is most likely attributed to the chances/instances of uncontrolled movement/transfer of fish from one location to another since most of the sampled beaches are close to each other. The fact that all the haplotypes of *O. niloticus* are close to each other with less than five mutational changes is an indication of high levels of gene flow among these populations. This can also be attributed to the use of common source of stocking material when Nile tilapia was first introduced to these lakes, especially since there is some level of isolation that is barrier to gene flow (Firmat et al., 2013).

4.3. Translocated *O. leucostictus* populations from Victoria and Kyoga basins

The genetic clustering observed between the *O. leucostictus*

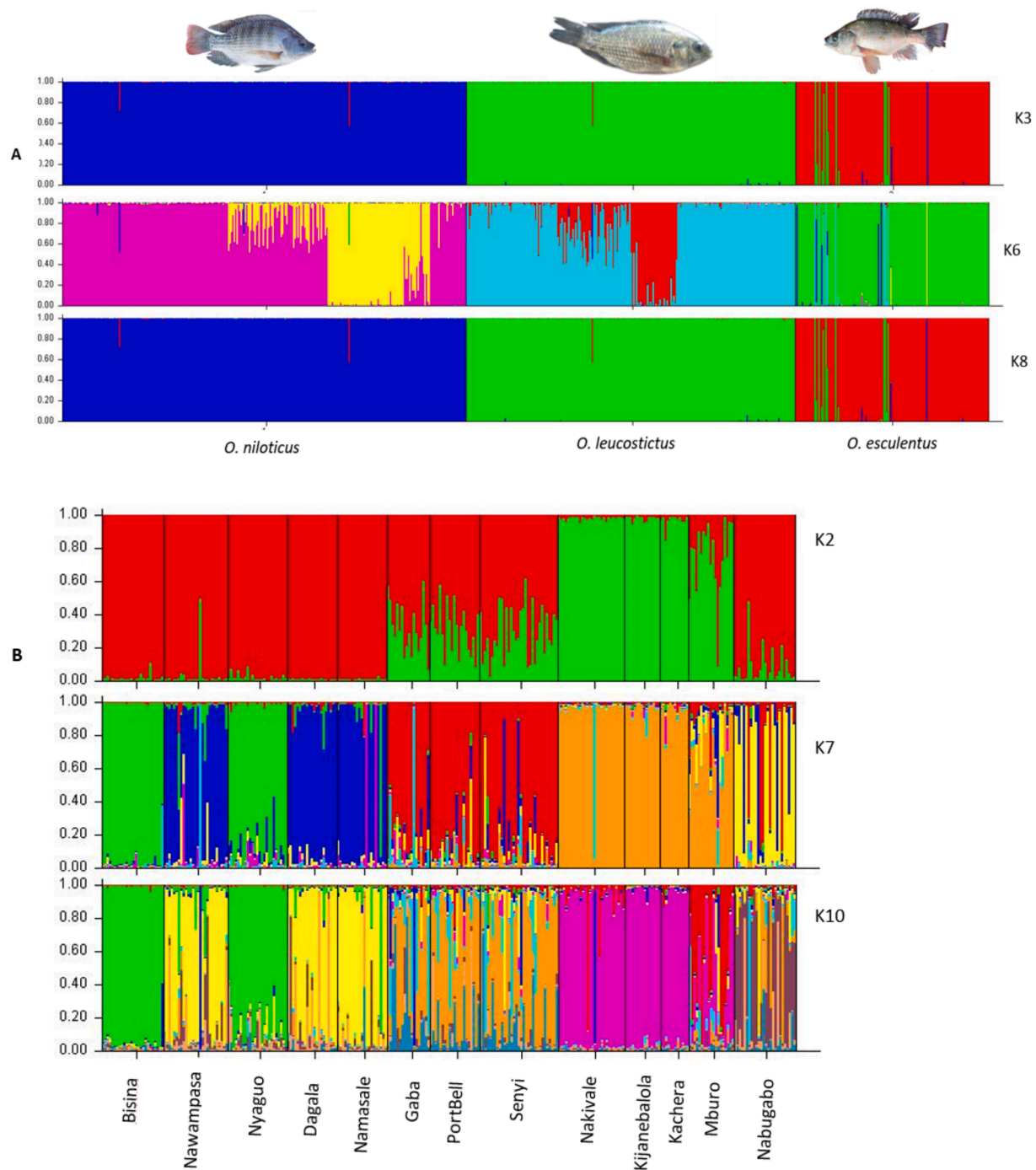


Fig. 6. Bayesian structure analysis showing the clustering of different populations. **A:** species, **B:** *O. niloticus* populations, **C:** *O. leucostictus* populations, **D:** *O. esculentus* populations.

populations from the main Lake Kyoga and Nawampasa is facilitated by the shoreline vegetation, mainly *Vossia cuspidata* and some *Cyperus papyrus* swamps, through which a water channel connects to the main Lake Kyoga (Ogutu-Ohwayo et al., 2013). The lower genetic differentiation between Lwampanga and Nawampasa as well as Gaba and Portbell could be attributed to the geographical proximity and connectivity of multiple stockings and unintentional fish transfers by fishermen (Tibihika et al., 2020).

The UPGMA, NJ, and PCoA indicate that samples from Nyaguo are divergent from other populations within Lake Kyoga basin. The divergence of the Nyaguo population could be attributed to the physical

barriers that prevent gene flow. Lake Nyaguo is mainly dominated by open waters with lots of sudds (Ogutu-Ohwayo et al., 2013). The shoreline vegetation is dominated by *Cyperus papyrus* and other swamp vegetation (Ssegawa and Kalema, 2008). All these have isolated the lake and kept its aquatic diversity intact.

4.4. Admixture, introgressive hybridization between the *Oreochromis* species

In the current study, mitochondrial and microsatellite markers indicated that the three *Oreochromis* species remain highly, though not

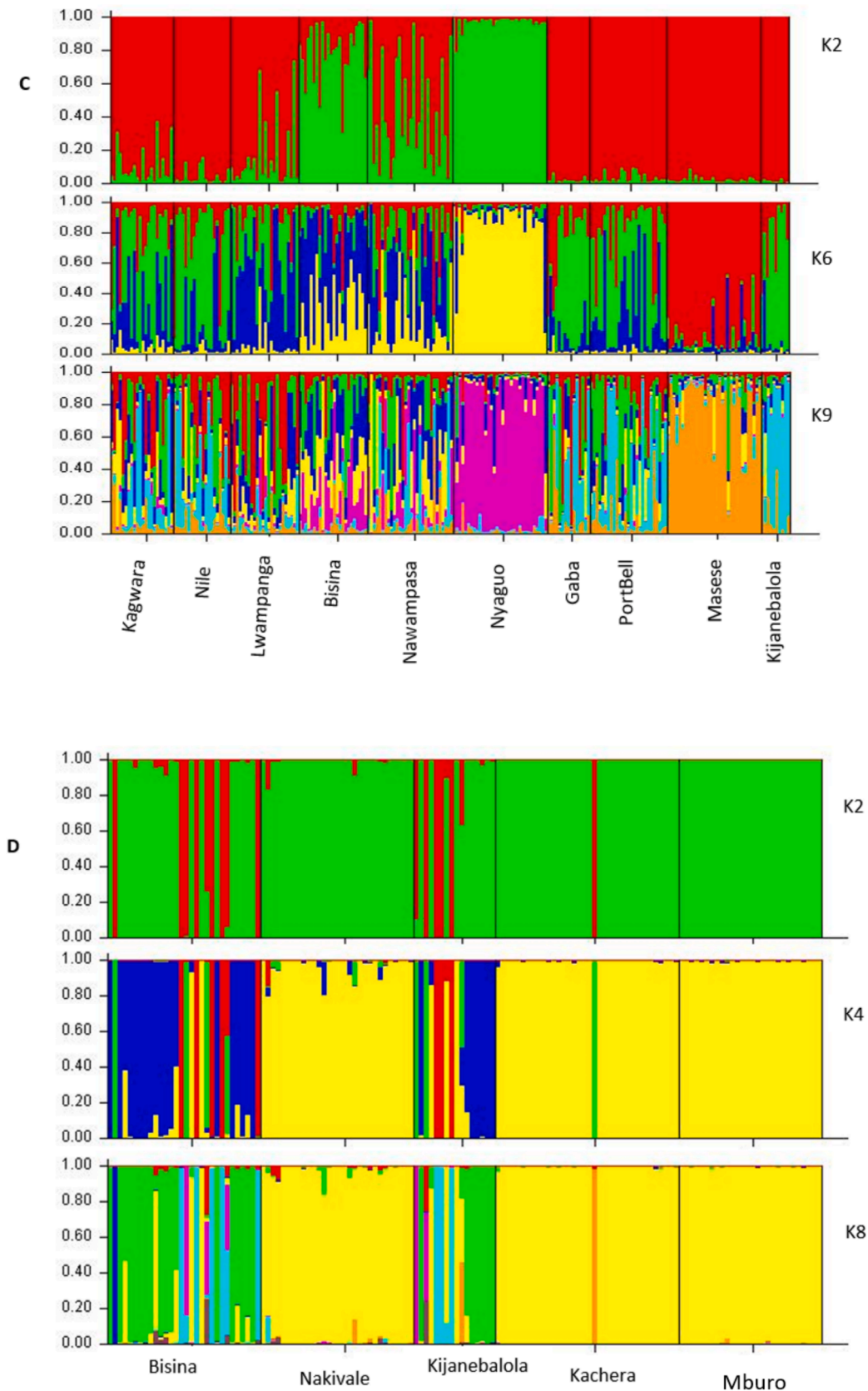


Fig. 6. (continued).

completely, genetically distinct. This was revealed by admixed cluster assignment in PCoA, STRUCTURE analysis, and mtDNA haplotype sharing. Studies by [Angienda et al. \(2011\)](#) showed that *O. esculentus* and

O. niloticus exhibited independent clustering, with a small degree of admixture between the species, which aligns with the findings of the current study. However, there was an indication of low-level

Table 4

Genetic diversity measures as indicated by mtDNA control region for *Oreochromis* species.

Statistic	All	<i>O. esculentus</i>	<i>O. leucostictus</i>	<i>O. niloticus</i>
No. sequences	687	139	252	296
No. of sites	395	395	395	395
Polymorphic sites, S	61	45	28	47
Parsimony informative sites	58	43	4	45
Total number of mutations	65	46	28	48
Number of haplotypes	26	11	4	14
Haplotype diversity (Hd)	0.809	0.414	0.127	0.782
Nucleotide diversity (π)	0.054	0.013	0.002	0.010
Theta-W	0.024	0.021	0.012	0.020
Tajima's D	3.61	-1.19	-2.35	-1.38
*Significant $p < 0.05$	$P < 0.01$	$P > 0.10$	$P < 0.01$	$P > 0.10$

asymmetric nuclear introgression from the invasive *O. leucostictus* to the native *O. esculentus* as revealed by the assignment of individuals of this species to *O. leucostictus* clusters but not the other way around. Moreover, multiple individuals were completely assigned to *O. leucostictus* clusters. This is mostly observed in Lake Bisina where both species coexist. This suggests that even in the refugial lakes, *O. esculentus* is subject to low-level hybridization with *O. leucostictus* which might eventually lower genetic integrity. This was additionally confirmed by the presence of shared H26 which had a great dominance of *O. leucostictus* with a few *O. esculentus*. Studies show that *O. leucostictus* and *O. esculentus* prefer habitats near papyrus fringes in littoral, shallow muddy bays, and lake inlets (Kariuki et al., 2021). Therefore, sharing similar habitats might enhance their ability to randomly mate. This pattern of phylogenetically intermediate 'mixed' populations observed in the study suggests the occurrence of introgressive hybridization. Among the *O. esculentus* populations from Victoria basin, the current results did not show any levels of introgression with other *Oreochromis* species. This could mean that the satellite lakes of Victoria basin still harbor pure stocks of *O. esculentus*.

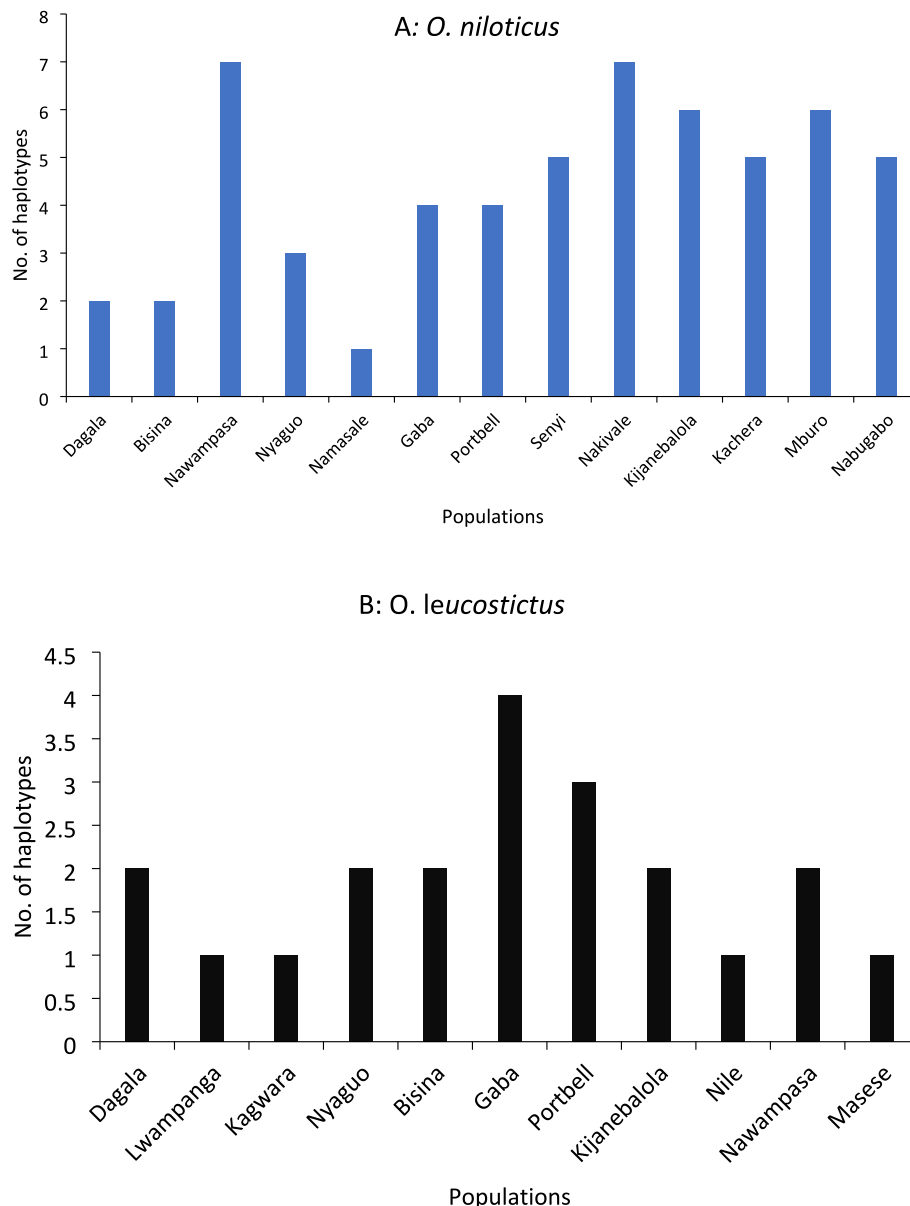


Fig. 7. Number of haplotypes for different populations. **A:** *O. niloticus*, **B:** *O. leucostictus*, **C:** *O. esculentus*.

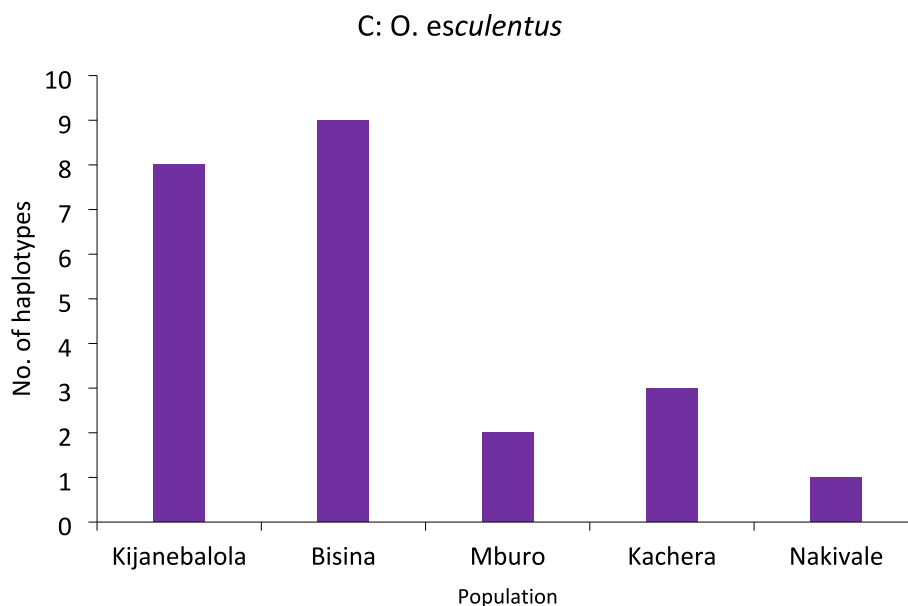


Fig. 7. (continued).

The current study also indicates that H22 is shared between *O. niloticus* and *O. leucostictus* with greater contribution by *O. niloticus* suggesting mtDNA introgression (Angienda et al., 2011; Diedericks et al., 2021; Kwikiriza et al., 2023; Mwanja et al., 2010; Ndiwa et al., 2014; Shechonge et al., 2018). The study by Ndiwa et al. (2014) and Kwikiriza et al. (2023) observed low hybridization levels between *O. niloticus* and *O. leucostictus* in Lake Naivasha and Lakes Victoria basin, respectively, and attributed this to shared haplotypes. Recent study in the Lake Edward-George system indicates that hybridization between *O. niloticus* and *O. leucostictus* is happening, though rarely (Diedericks et al., 2021).

The current study also revealed a very low degree of asymmetric nuclear introgression from the native species *O. esculentus* to the invasive species *O. niloticus* as these species shared H2. Similar studies were documented by Mwanja et al. (2012), who found genetic signals of asymmetric introgression in the Lake Victoria region, with more genetic contribution from *O. niloticus* into *O. esculentus* using random amplified polymorphic DNA (RAPD) markers. Contrary to the findings by Angienda et al. (2011), who did not observe shared mtDNA haplotypes between *O. esculentus* and *O. niloticus*, the current study revealed shared haplotypes between the two species. Such differences could be attributed to the type of markers as well as the sample size (Tibihika et al., 2019). The current study used 140 samples of *O. esculentus* and 292 samples of *O. niloticus*, a significant increase from the 90 samples of each species used by Angienda et al. (2011). Additionally, our study incorporated a larger number of water bodies compared to the three examined in Angienda et al. (2011). In addition to using both mtDNA and 36 microsatellite markers, the current study employed a next-generation sequencing technique that is highly accurate (Salk et al., 2018).

For all species comparison, some individuals showed both mtDNA and SSR assignment to other species (e.g., some *O. esculentus* to *O. leucostictus*), suggesting signs of backcrosses and introgression given the fact that these species are morphologically distinct. Thus, all the factors in the present study are key to providing robust information. Therefore, the present results contribute further evidence to the hypothesis that there is a low level of hybridization between non-native *Oreochromis* species and the native *O. esculentus*. Although this happens in low prevalence, this introgression can have an impact, especially in a scenario of continuously decreasing population sizes or even a bottleneck where non-native alleles can rapidly increase in frequency in the native species gene pool. This may have further compromised the

survival of the native species if this results in maladaptive populations decreasing the fitness of admixed *O. esculentus* populations.

5. Conclusion

We found differences in genetic diversity among *Oreochromis* species and populations, with *O. esculentus* population being generally the least diverse, which likely reflects the bottleneck that this species went through. Nevertheless, conservation measures need to be put in place to protect the remnant gene pool, which the results of this study can help develop. Differences in genetic diversity and differentiation could be attributed to the size of the water bodies, conservation measures, and uncontrolled and multiple fish transfers. This is evidenced in Lake Nakivale which had a higher genetic diversity as a result of multiple restocking of the lake with *O. niloticus*, as well as the lakes of Kachera and Kijanebalola for *O. esculentus* where habitat destruction and overfishing may have led to lower levels of genetic diversity. Moreover, since *O. esculentus* populations from Kyoga and Victoria basins are genetically distinct, the study recommends management of these populations as distinct conservation units given the species' extremely limited contemporary geographic distribution.

The study also recommends future genetic studies include wider coverage of the Victoria basin to include those satellite lakes in Tanzania and Kenya while applying whole genome sequencing. Lakes Nakivale, Mburo, and Kachera may be important to maintain gene flow among the *O. esculentus* populations of these water bodies. Much of the conservation of this species has focused on stock management; however, these results also show that habitat preservation is also important.

The study confirms the probable existence of low levels of introgressive hybridization among the three species of *Oreochromis* where they co-exist, but that pure stocks of *O. esculentus* are still available in the satellite lakes. Nevertheless, the populations where hybridization was detected should be monitored to ensure that this does not become a more prevalent phenomenon. This may be especially important for the populations contacting *O. leucostictus* since it seems to hybridize more frequently.

CRedit authorship contribution statement

Gerald Kwikiriza: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration,

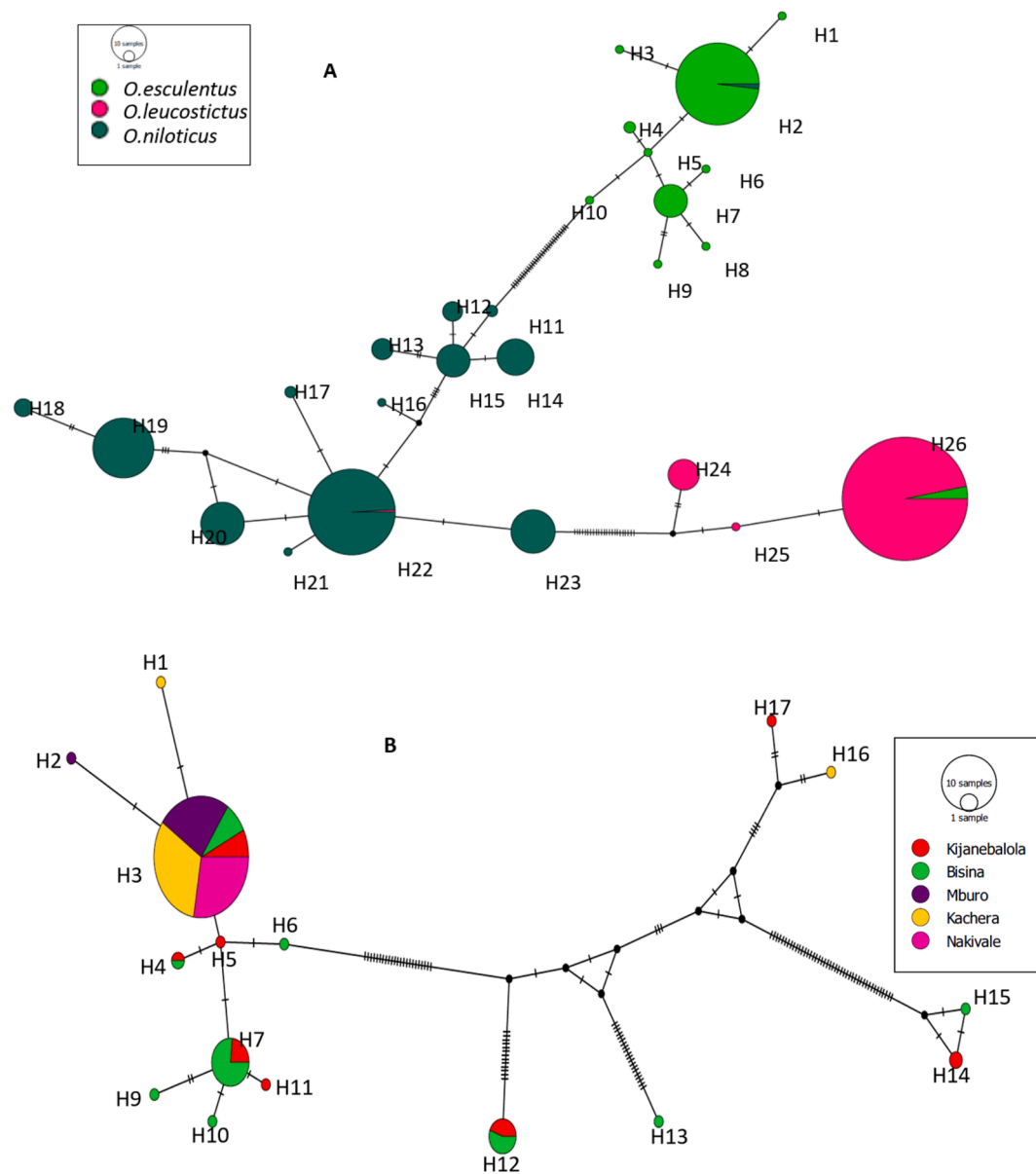


Fig. 8. Median-joining haplotype network inferred from mtDNA sequences of *Oreochromis* species from different sampling sites. Each circle represents a different haplotype. The size of a circle is proportional to the frequency of each haplotype. H: Haplotype. **A:** *Oreochromis* species, **B:** *O. esculentus* populations.

Investigation, Formal analysis, Conceptualization. **Papius Dias Tibihika:** Writing – review & editing, Supervision, Formal analysis. **Ivan Abaho:** Writing – review & editing, Conceptualization. **Juliet Kigongo Nattabi:** Writing – original draft, Supervision. **Thapasya Vijayan:** Software, Writing – review & editing, Formal analysis. **Christina Rupprecht:** Software, Writing – review & editing, Formal analysis. **Manuel Curto:** Software, Supervision, Writing – review & editing, Formal analysis. **Andreas Melcher:** Software, Supervision, Writing – review & editing, Formal analysis, Project administration. **Harald Meimberg:** Supervision, Project administration, Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jglr.2025.102535>.

References

- Adonia, B.K.K., 2013. The cost of poor land use practices in Lake Nakivale wetland in Isingiro district, Uganda. *Afr. J. Environ. Sci. Technol.* 7 (6), 448–456.
- Angienda, P.O., Lee, H.J., Elmer, K.R., Abila, R., Waindi, E.N., Meyer, A., 2011. Genetic structure and gene flow in an endangered native tilapia fish (*Oreochromis esculentus*) compared to invasive Nile tilapia (*Oreochromis niloticus*) in Yala swamp, East Africa. *Conserv. Genet.* 12, 243–255.
- Balirwa, J.S., Chapman, C.A., Chapman, L.J., Cowx, I.G., Geheb, K., Kaufman, L.E.S., Lowe-McConnell, R.H., Seehausen, O., Wanink, J.H., Welcomme, R.L., Witte, F., 2003. Biodiversity and fishery sustainability in the Lake Victoria basin: an unexpected marriage? *Bioscience* 53 (8), 703–715.
- Barasa, B., Wanyama, J., 2020. Freshwater lake inundation monitoring using Sentinel-1 SAR imagery in Eastern Uganda. *Ann. GIS* 26 (2), 191–200.
- Barreto, R.E., Carvalho, G.G.A., Volpato, G.L., 2011. The aggressive behavior of Nile tilapia introduced into novel environments with variation in enrichment. *Zoology* 114 (1), 53–57.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30 (15), 2114–2120.
- Curto, M., Winter, S., Seiter, A., Schmid, L., Scheicher, K., Barthel, L.M., Plass, J., Meimberg, H., 2019. Application of a SSR-GBS marker system on investigation of European Hedgehog species and their hybrid zone dynamics. *Ecol. Evol.* 9 (5), 2814–2832.
- Diedericks, G., Maetens, H., Van Steenberge, M., Snoeks, J., 2021. Testing for hybridization between Nile tilapia (*Oreochromis niloticus*) and blue spotted tilapia (*Oreochromis leucostictus*) in the Lake Edward system. *J. Great Lakes Res.* 47 (5), 1446–1452.
- Ellegren, H., 2004. Microsatellites: Sample sequences with complex evolution. *Nat. Rev. Genet.* 5, 435–445.
- Firmat, C., Alibert, P., Losseau, M., Baroiller, J.F., Schliwen, U.K., 2013. Successive invasion-mediated interspecific hybridizations and population structure in the endangered cichlid *Oreochromis mossambicus*. *PLoS One* 8 (5), e63880.
- Fryer, G., 2004. Speciation rates in lakes and the enigma of Lake Victoria. *Hydrobiologia* 519, 167–183.
- Goudswaard, P.C., Witte, F., Katunzi, E.F., 2002. The tilapia fish stock of Lake Victoria before and after the Nile perch upsurge. *J. Fish Biol.* 60 (4), 838–856.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41 (41), 95–98.
- Hubisz, M.J., Falush, D., Stephens, M., Pritchard, J.K., 2009. Inferring weak population structure with the assistance of sample group information. *Mol. Ecol. Resour.* 9 (5), 1322–1332.
- Kamukasa, A., Bintooro, K., 2014. Assessment of the effects of changing land use from pastoralism to crop farming on Lake Nakivale Wetland System in Isingiro District, Uganda. *J. Afr. Stud. Dev.* 6 (4), 56–66.
- Kariuki, J., Tibihika, P.D., Curto, M., Alemayehu, E., Winkler, G., Meimberg, H., 2021. Application of microsatellite genotyping by amplicon sequencing for delimitation of African tilapia species relevant for aquaculture. *Aquaculture* 537, 736501.
- Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30 (4), 772–780.
- Kwikiriza, G., Vijayan, T., Tibihika, P.D., Curto, M., Winkler, G., Nattabi, J.K., Kariuki, J., Meimberg, H., 2023. Introgressive hybridization levels of Tilapia species in Lake Victoria basin, Kenya inferred from microsatellite and mitochondrial DNA genotyping based on next-generation sequencing. *Conserv. Genet.* 25, 305–318.
- Ladoukakis, E.D., Zouros, E., 2017. Evolution and inheritance of animal mitochondrial DNA: rules and exceptions. *J. Biol. Res. Thessalon.* 24, 1–7.
- Langella, O., 1999. Populations 1.2. 28: a population genetic software. <http://www.pge.cnrs-gif.fr/bioinfo/populations/index.php>.
- Leigh, J.W., Bryant, D., 2015. POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6 (9), 1110–1116.
- Li, Y.L., Liu, J.X., 2018. StructureSelector: a web-based software to select and visualize the optimal number of clusters using multiple methods. *Mol. Ecol. Resour.* 18 (1), 176–177.
- Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25 (11), 1451–1452.
- Lind, C.E., Agyakwah, S.K., Attipoe, F.Y., Nugent, C., Crooijmans, R.P., Toguyeni, A., 2019. Genetic diversity of Nile tilapia (*Oreochromis niloticus*) throughout West Africa. *Sci. Rep.* 9 (1), 16767.
- Musinguzi, L., Olokotum, M., Natugonza, V., 2020. Status and targets for rebuilding the three major fish stocks in Lake Victoria. *BioRxiv*. <https://doi.org/10.1101/2020.10.26.354639>.
- Mwangu, R.A., 2022. An assessment of economic and environmental impacts of refugees in Nakivale, Uganda. *Migr. Dev.* 11 (3), 433–449.
- Mwanja, W.W., Kaufman, L., Fuerst, P.A., 2010. Comparison of the genetic and ecological diversity of the native to the introduced tilapiines (Pisces: Cichlidae), and their population structures in the Lake Victoria region. *East Africa. Aquat. Ecosyst. Health Manag.* 13 (4), 442–450.
- Mwanja, W.W., Fuerst, P.A., Kaufman, L., 2012. Reduction of the ngege, *Oreochromis esculentus* (Teleostei: Cichlidae) populations, and resultant population genetic status in the Lake Victoria region, Uganda. *J. Agric. Sci.* 13 (2), 65–82.
- Natugonza, V., Musinguzi, L., Kishe, M.A., van Rijssel, J.C., Seehausen, O., Ogutu-Ohwayo, R., 2021. The consequences of anthropogenic stressors on cichlid fish communities: revisiting Lakes Victoria, Kyoga, and Nabugabo. In: Abate, M.E., Noakes, D.L. (Eds.), *The behavior, ecology, and evolution of cichlid fishes*, Springer, Dordrecht, pp. 217–246.
- Ndiwa, T.C., Nyirigi, D.W., Agnese, J.F., 2014. An important natural genetic resource of *Oreochromis niloticus* (Linnaeus, 1758) threatened by aquaculture activities in Loboi drainage, Kenya. *PLoS One* 9 (9), e106972.
- Ogutu-Ohwayo, R., Odongkara, K., Okello, W., Mbabazi, D., Wandera, S.B., Ndawula, L. M., Natugonza, V., 2013. Variations and changes in habitat, productivity, composition of aquatic biota and fisheries of the Kyoga Lake system: lessons for management. *Afr. J. Aquat. Sci.* 38 (1), 1–14.
- Ojara, M.A., Lou, Y., Aribi, L., Namumbya, S., Uddin, M.J., 2020. Dry spells and probability of rainfall occurrence for Lake Kyoga Basin in Uganda, East Africa. *Nat. Hazards* 100 (2), 493–514.
- Outa, N.O., Yongo, E.O., Keyombe, J.L.A., Ogello, E.O., Namwaya Wanjala, D., 2020. A review on the status of some major fish species in Lake Victoria and possible conservation strategies. *Lakes Reserv. Res. Manag.* 25 (1), 105–111.
- Peakall, R.O.D., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* 6 (1), 288–295.
- Robledo, D., Ogowang, J., Byakora, E., Nascimento-Schulze, J.C., Benda, K.K., Frasin, C., Salisbury, S., Solimo, M., Mayega, J.F., Peter, B., Masembe, C., 2024. Genetic diversity and population structure of farmed and wild Nile tilapia in Uganda: the potential for aquaculture selection and breeding programs. *Genomics* 116 (1), 110781.
- Salk, J.J., Schmitt, M.W., Loeb, L.A., 2018. Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations. *Nat. Rev. Genet.* 19 (5), 269–285.
- Schlötterer, C., 2000. Evolutionary dynamics of microsatellite DNA. *Chromosoma* 109 (6), 365–371.
- Schneider, S., Roessli, D., Excoffier, L., 2000. Arlequin: a software for population genetics data analysis. Ver. 2.000. Genetics and Biometry Laboratory, Department of Anthropology and Ecology, University of Geneva, Switzerland.
- Sheehong, A., Ngatunga, B.P., Tamatamah, R., Bradbeer, S.J., Harrington, J., Ford, A.G., Turner, G.F., Genner, M.J., 2018. Losing cichlid fish biodiversity: genetic and morphological homogenization of tilapia following colonization by introduced species. *Conserv. Genet.* 19, 1199–1209.
- Segawa, P., Kalema, J., 2008. Species communities and associations formed by the family Cyperaceae Juss. in some of Uganda's wetlands. *Afr. J. Ecol.* 46, 98–108.
- Taabu-Munyaho, A., Marshall, B.E., Tomasson, T., Marteinsdottir, G., 2016. Nile perch and the transformation of Lake Victoria. *Afr. J. Aquat. Sci.* 41 (2), 127–142.
- Tibihika, P.D., Curto, M., Dornstauder-Schrammel, E., Winter, S., Alemayehu, E., Waidbacher, H., Meimberg, H., 2019. Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African *Oreochromis niloticus*. *Conserv. Genet.* 20, 357–372.
- Tibihika, P.D., Curto, M., Alemayehu, E., Waidbacher, H., Masembe, C., Akoll, P., Meimberg, H., 2020. Molecular genetic diversity and differentiation of Nile tilapia (*Oreochromis niloticus*, L. 1758) in East African natural and stocked populations. *BMC Evol. Biol.* 20, 16.
- Tibihika, P.D., Meimberg, H., Curto, M., 2022. Understanding the translocation dynamics of Nile tilapia (*Oreochromis niloticus*) and its ecological consequences in East Africa. *Afr. Zool.* 57 (4), 171–179.
- Twesigye, C.K., Onywere, S.M., Getenga, Z.M., Mwakilila, S.S., Nakiranda, J.K., 2011. The impact of land use activities on vegetation cover and water quality in the Lake Victoria watershed. *Open Environ. Eng. J.* 4 (1), 66–77.
- Witte, F., Msuku, B.S., Wanink, J.H., Seehausen, O., Katunzi, E.F.B., Goudswaard, P.C., Goldschmidt, T., 2000. Recovery of cichlid species in Lake Victoria: an examination of factors leading to differential extinction. *Rev. Fish Biol. Fish.* 10 (2), 233–241.
- Witte, F., Seehausen, O., Wanink, J.H., Kishe-Machumu, M.A., Rensing, M., Goldschmidt, T., 2013. Cichlid species diversity in naturally and anthropogenically turbid habitats of Lake Victoria, East Africa. *Aquat. Sci.* 75, 169–183.