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Hybridization levels of Tilapiine species in Lake Victoria basin, Kenya inferred from microsatellite genotyping based on next generation sequencing

Thesis submitted for the award of the title

“Master of Science”

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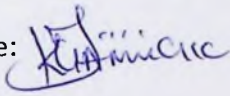
Declaration

I hereby declare that this thesis has been achieved by myself and is the result of my own investigations. It has neither been accepted nor submitted for any other degree. Except where indicated by specific reference in the text, the work is the candidate's own work. Any views expressed in the dissertation are those of the author.

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Dedication

In memory of my parents (Mr and Mrs. Magara Herbert). You left fingerprints of grace on my life. You shan't be forgotten.

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LIST OF ACRONYMS

μ l	Microliter
μ M	Micromolar
BP	Base Pair
CLUMPAK	Cluster Markov Packager Across K
EDTA	Ethylene Diamine Tetra-Acetic acid
EtOH	Ethanol
GenALEx	Genetic Analysis in Excel
HCl	Hydrochloric acid
He	Expected heterozygosity
Ho	Observed heterozygosity
MCMC	Markov Chain Monte Carlo
mM	Millimolar
NGS	Next Generation Sequencing
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
RAPD	Random Amplification of Polymorphic
DNA	Deoxynucleic acid
RFLP	Restriction Fragment Length Polymorphism
rpm	Revolutions Per Minute
SNP	Single Nucleotide Polymorphism
SSLP	Simple Sequence Length Polymorphism
SSR	Simple Sequence Repeat
TAE	Tris-Acetic acid EDTA
df	Degree of freedom
Ne	Number of effective alleles
Na	Number of different alleles
I	Shannon's Information Index
F	Fixation Index
uHe	Unbiased Expected Heterozygosity

Abstract

Despite the species richness of the tilapiines, the fish have been compromised by various factors like overfishing, climate change and un-controlled fish translocations. These challenges particularly fish translocations have negatively impacted on native tilapiines through competition, hybridization and introgression thus compromising genetic integrity of the native tilapiines. Despite the prevailing research interventions, insufficient information is available on the hybridization levels of different tilapiines in the Lake Victoria basin. The study utilized nuclear microsatellite markers to investigate hybridization signals and compare the genetic diversity of different tilapiines in Lake Victoria, Kenya, based on next-generation sequencing. Tilapiines were collected from different beaches using experimental seine nets. A fin clip/muscle tissue was extracted from the fish sample, preserved in 98% ethanol, for subsequent genotyping in Meimberg laboratory at BOKU, Austria. The genetic structure based on Bayesian clustering analysis using STRUCTURE program and Principal Coordinate Analysis generally revealed two clusters: one group of *O. niloticus* and the other congeneric species. Despite this, some alleles of *O. niloticus* were observed in the genetic structure of other congeneric tilapiines. This suggested some degree of admixture/introgression among the studied tilapiines. With *O. niloticus* populations, there was a strong genetic differentiation between Dunga, Usenge, Mbita, Siungu and Seka-Bay ($F_{ST} = 0.06, 0.05, 0.09$ and 0.06 respectively). The differences could be attributed to geographical isolation that has acted as a barrier to gene flow between those populations. The apparent admixture of the different populations might be attributed to uncontrolled fish translocations and escapees from fish farms. Therefore, the current study contributes to identifying conservation measures of tilapiines that may be threatened and require management interventions.

Key words: Tilapiines, Next generation sequencing, Hybridization, admixture, introgression, Conservation

CHAPTER 1

1.0 INTRODUCTION

1.1 Background of the study

The African cichlids, particularly the tilapiines have been widely distributed in the tropics, sub-tropics, and temperate regions. Most of the African lakes especially Tanganyika, Malawi and Victoria are depicted as centres of adaptive radiation for the cichlids (Salzburger, and Meyer, 2004; Meyer et al., 2015). Cichlids are considered as important model species for evolutionary biological research due to their morphological diversity as well as species richness with more than 1600 species and at least 20 genera (Dunz and Schliewen, 2013). Among the cichlids, the tilapiines which correspond to the species of the genera *Oreochromis niloticus*, *Oreochromis mossambicus* and *Oreochromis aureus* have gained significant scientific attention with most studies focussing on aquaculture, strain selection as well as genetic improvements (Eknath and Hulata, 2009; Weyl et al., 2010).

Globally, the farming of the tilapia species especially *Oreochromis niloticus* Linnaeus, 1758 has increased due to its good performance characteristics, like faster growth rates (Tidwell and Allan, 2001; FAO, 2016). The fast growth rates coupled with resistance to adverse environmental conditions have made *O. niloticus* the second most farmed fish to Grass carp (*Ctenopharyngodon idellus*) globally with more than 90 countries involved in aquaculture production (Bostock et al., 2010; FAO, 2020). Though most Tilapia aquaculture production is in Asian countries especially China and Thailand, its natural distribution covers some of the greater African lakes like Victoria, Tanganyika, and Malawi (Meyer et al., 2015).

Aquaculture production in Africa dates to the 1940s and 1950s, currently Egypt leads in production followed by Nigeria, Uganda, Ghana, and Kenya in that order (FAO, 2018). Tilapia production in Kenya contributes at least 60.21% of the total fish production with 50.99% from aquaculture production systems like ponds and cages while 9.21% is derived from natural water bodies mostly rivers and lakes (Opiyo et al., 2018). Despite the conservation measures developed for sustainable utilization of capture fisheries, the sector has kept at crossroads due to higher fishing pressure that comes along with catching immature fishes, climate change and un-controlled fish translocations, etc. (Eknath and Hulata, 2009; Njiru et al., 2010).

The translocations of tilapiines in East African freshwater bodies dates to the 1950s with Kenya being a hotspot for the introductions (Ogutu-Ohwayo and Hecky, 1991; Okwiri et

al., 2019). The introduced species were widely distributed across the country for different reasons like enhancing fish farming and boosting the wild fish stocks that had declined due to overfishing (Goudswaard et al., 2002; Keyombe et al., 2015). Nile tilapia being an invasive species has displaced many native species affecting their ecosystems. Many authors have stated that the invasion of congeneric tilapias often leads to hybridization with local allopatric species (Eknath and Hulata, 2009). For example, the invasion of *O. niloticus* in Lake Victoria is likely to have contributed to the decline of the native species mainly *Oreochromis variabilis* and *Oreochromis esculentus* (Goudswaard et al., 2002; Tibihika et al., 2020; Blackwell et al., 2020).

Additionally, many intentional and un-intentional cases of the species introductions by fish farmers have been reported globally and Kenya as a country has also faced these scenarios as well (Aloo, 2003). Studies show that in 2013, many stocks of *Oreochromis niloticus* found their way into Lake Victoria from the nearby fish farms due to heavy floods (Nyingi and Agnèse, 2007; Nyingi et al., 2007; Ndiwa et al., 2014).

All these translocations of fish have fostered hybridization and led to admixtures between isolated populations (Shechonge et al., 2018). Despite the emerging concerns arising from the hybridization of *Oreochromis niloticus* with native species, there is still limited information on the presence of hybrids in Lake Victoria. Secondly, incidences of hybridization complicate the distinction of the *Oreochromis* species, and this has affected conservation measures developed by fisheries managers (Tibihika et al., 2020).

Morphological and molecular techniques have been used in the characterization of *Oreochromis* species. In comparison with morphological-based techniques, molecular genetic techniques have demonstrated superlative in identification and differentiation of *Oreochromis* species even in admixed populations (D'amato et al., 2007; Wu and Yang, 2012).

Earlier studies employed traditional genetic markers like allozymes and restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA) to differentiate the genetic diversity of the *Oreochromis* species in East African freshwater bodies (Mwanja et al., 1996; Agnèse et al., 1997). These traditional markers have low resolving power and thus cannot characterise the variations within and between populations causing a lot of contradictions to differentiate the fish species (Miah et al., 2013; Tibihika et al., 2019).

Application of microsatellite genotyping using next-generation sequencing has proven the best approach as it minimizes size homoplasy that is one of the constraints of traditional SSR fragment length (Tibihika et al., 2019). This technique has been applied to study the genetic diversity of *Oreochromis niloticus* populations but has not yet been applied to directly detect hybridization levels of tilapiines in a natural setting. Therefore, this study utilized nuclear microsatellite markers (SSR) to compare the genetic diversity of different tilapiines in Lake Victoria, Kenya based on next-generation sequencing (NGS). The information will help in the management and conservation of the fishery especially *Oreochromis esculentus* and *Oreochromis leucostictus* that are currently endangered in the lake.

1.2 Research questions

1. To what extent have the tilapiines in Lake Victoria been
 - I. differentiated genetically?
 - II. hybridized by the introduced *Oreochromis niloticus* in Lake Victoria?
2. Is there any signal of introgression in *Oreochromis niloticus* populations from Lake Victoria in comparison with the satellite lakes?

1.2 Research Objectives

1.2.1 Main Objectives

To determine the hybridization and introgression levels of tilapiines using microsatellite genotyping in Lake Victoria, Kenya for conservation and management purposes

1.2.2 Specific objectives

- To investigate the degree of hybridization between *Oreochromis niloticus* and other related tilapiines in Lake Victoria, Kenya
- To determine the genetic diversity of *O. niloticus* populations in Lake Victoria, Kenya

1.3 Description of the fish species used in the present study.

1.3.1 Taxonomy and distribution of Tilapia

Tilapias are representatives of large number of freshwater fish species within the family Cichlidae (Trewavas, 1982). The tilapias have been classified into three genera (*Oreochromis*, *Tilapia* and *Sarotherodon*). *Sarotherodon* are characterized by small mouth with very small teeth (Trewavas, 1983). They also have slender shafts and spoon-shaped crowns (Trewavas, 1982). The general colour is pale blue on lower flanks to orange or metallic

golden yellow on the back (Trewavas, 1983). In the genera *Tilapia*, teeth of the jaws and pharynx are typically coarser than in *Sarotherodon* (Trewavas, 1983). On the other hand, *Oreochromis* species are characterized by notched teeth throughout their life (Trewavas, 1966; Sodsuk et al., 1991).

Reproductively, *Tilapia* are known for being substrate spawners, *Sarotherodon* are biparental mouth brooders while *Oreochromis* species are maternal mouthbrooders (they hold their eggs in mouth and offspring for a certain period (Pullin et al., 1982; Mjoun and Rosentrater, 2010).

The genus *Oreochromis* is the largest, with approximately 79 species, followed by *Tilapia* with approximately 41 species while *Sarotherodon* has approximately 10 species (Kocher et al., 1998; Martins et al., 2004). *Oreochromis* is typical of the river and lakes of East and Central Africa (Ogutu-Ohwayo, 1990). Nile tilapia (*O. niloticus*), Mozambique tilapia (*O. mossambicus*) and blue tilapia (*O. aureus*) are the most commercially important species found in the genus *Oreochromis* (Eknath and Hulata, 2009).

1.3.2 Classification and distribution of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758)

Oreochromis niloticus belongs to the family Cichlidae, order Perciformes and class Actinopterygii (Trewavas, 1983; Nelson, 2004) (Figure 1). *O. niloticus* populations have established themselves in nearly all-natural water bodies in Africa including Lake Victoria and its satellite lakes (Aloo, 2002; Jembe et al., 2006). This is because of its ecological, physiological and genetical characteristics that make the fish have a competitive advantage over other fish (Canonico et al., 2005). For example, being an omnivorous and a prolific breeder makes *O. niloticus* outcompete other native species like *O. esculentus* on the breeding grounds (Goudswaard et al., 2002). The invasiveness nature of the *O. niloticus* with other native species makes the fish displace the endemic species in the natural environment (Canonico et al., 2005; Todesco et al., 2016).

Trewavas (1983), morphologically identified seven sub-species of *Oreochromis niloticus* from western and eastern Africa fresh waters: *Oreochromis niloticus niloticus* from West Africa (Lake Chad basin, river Niger, Benue, Volta, Gambia, and Senegal) and the Nile river system. *Oreochromis niloticus edwardianus* from the Lakes Edward, Albert, George (Uganda) Tanganyika (Tanzania and Burundi) and Kivu (Rwanda); *Oreochromis niloticus baringoensis* endemic to Lake Baringo (Kenya), *Oreochromis niloticus sugutae* endemic in

River Suguta (Kenya), *Oreochromis niloticus vulcani* from Lake Turkana, (Kenya) *Oreochromis niloticus cancellatus* from Lakes Tana, Zwai and Stefani as well as rivers Ergino and Awash in the Ethiopian rift valley; and *Oreochromis niloticus filoa* from the hot alkaline springs in the Awash system, Ethiopia. Nyingi et al. (2009) also discovered another endangered new sub species of *Oreochromis niloticus* from Bogoria hotel spring of the Lobo swamp, Kenya.

Therefore, *O. niloticus* has been among the most studied fish in terms of aquaculture, ecology, and genetics globally (Canonico et al., 2005). Examples of genetics studies involved the use of the molecular markers like microsatellites and amplified fragment length polymorphism (AFLP), allozymes, Random Amplified Polymorphic DNA (RAPD), Restriction fragment length polymorphism (RFLP), Single-Nucleotide Polymorphism (SNPs) to generate the Nile tilapia genomic maps (Agnès et al., 1997; Moen et al., 2002; Hassanien et al., 2004; Hamilton et al., 2020).



Figure 1: *Oreochromis niloticus* (Source: Author photo)

1.3.3 Red-belly Tilapia *Coptodon zillii* (*Coptodon zillii*)

Coptodon zillii belongs to the genus *Coptodon*. Pioneer studies show that *C. zillii* is a native fish to Lake Nasser in Egypt and introductions have occurred into other African lakes like Victoria and Naivasha (Keyombe et al., 2020). It is one of the widely distributed tilapia species found throughout Kenyan waters (Keyombe et al., 2020). The fish introductions to various countries have mainly been due to aquaculture purposes (Soliman et al., 2017). The fish can tolerate a wide range of environmental conditions ranging from fresh water to brackish water though it favours shallow vegetated areas (Lung'Ayia et al., 2000). For example, *Coptodon zillii* can live in salinities as low as 0% up to 45% and it is able to feed on the macrophytes (Lung'Ayia et al., 2000). Climate change and overfishing have led to the decline in the populations of *Coptodon zillii* in Lake Victoria. Secondly, the occurrence of the fish in Lake Victoria has been linked to the decline of the native species due to the competition as well as hybridization with the native species like *Oreochromis esculentus* (Mwanja et al., 2001). The fish can be distinguished from other *Oreochromis* species by having a red belly on the ventral part (Figure 2).



Figure 2: Red belly *Coptodon zillii* (Source: Author photo)

1.3.4 Blue-spotted tilapia (*Oreochromis leucostictus*; Trewavas 1983)

Oreochromis leucostictus also known as blue-spotted tilapia belongs to the genus *Oreochromis*. The species was introduced from Lake Albert to Lake Victoria, Lake Naivasha and fishponds in catchments (Balirwa, 1992; Eccles, 1992; Keyombe et al., 2017). Morphologically, the fish can be distinguished from other species by having deep-bodied with a small mouth and a rounded head with a high back (Figure 3). Like other genera of the *Oreochromis*, it is also a maternal mouthbrooder and its reproduction is continuous throughout the year (Hickley et al., 2002).

The fish species is mostly preferred by the local communities because of having more fresh than bones (Laurent et al., 2020). Ecologically, the fish prefers papyrus fringes mostly towards the littoral parts of the lake and rivers (Keyombe et al., 2017). Studies by Jembe et al. (2006) show that the fish can live in a wide range of environments including high temperatures and salinity levels. Being an omnivorous fish, it can feed on a variety of feeds in its natural environment resulting in faster growth (Keyombe et al., 2017). Studies in Lake Baringo and Lake Naivasha have shown that the *O. leucostictus* can hybridize with native species (Britton et al., 2007). The disappearance of the endemic *Oreochromis spilurus* in Lake Naivasha has been attributed to the hybridization with *O. leucostictus* (Britton et al., 2007; Gherardi et al., 2011).



Figure 3: *Oreochromis leucostictus* (Source: Author photo)

1.3.5 Singida tilapia (*Oreochromis esculentus*)

Oreochromis esculentus also known as Singida tilapia dominated fisheries in Lake Victoria before the introductions of *Oreochromis niloticus* and other exotic species. *O. esculentus* has declined drastically from the main Lake Victoria and can only be found in the satellite lakes of the Lake Victoria basin (Balirwa, 1992; Aloo 2002). Examples of such satellite lakes include Kanyaboli and Sare in Kenya. These satellite lakes have acted as refugia for the species thus playing a significant role towards the conservation of the remnant species (Mwanja et al., 2001). *Oreochromis esculentus* has been considered an ecologically and critically endangered species and has suffered higher genetic modifications through hybridizations with other exotic *Oreochromis* species like *O. niloticus* (IUCN, 2014).

O. esculentus are easily differentiated from *O. niloticus* by having a smaller head with a whitish colour on the ventral parts as well as reddish colour on the dorsal parts (Figure 4).



Figure 4: *Oreochromis esculentus* (source: author photo)

1.4 Main threats to fish population in Lake Victoria, East Africa

Increased fishing pressures on Lake Victoria: Lake Victoria has been experiencing high fishing pressure leading to changes in the structure of the freshwater habitats, biodiversity, composition, and the productivity of the associated biota (Matsuishi et al., 2006; Kigano, 2016). This has altered the biodiversity of the lake and its associated productivity (Ojuok et al., 2007). The loss of the fishing stocks has led to the loss of the genetic diversity of some fish stocks like *O. esculentus* (Mwanja et al., 1996; Hemoiwa et al., 2013). This is because the increased fishing pressure results in phenotypic and genotypic changes in natural populations (Reznick and Ghalambor, 2001). In this context, molecular data can provide information of how natural fish populations respond to overfishing through information population structure, gene flow and effective population size (Hemoiwa et al., 2013). Genetic studies are recognized as an integral part of recognizing the biology of any organism by taking a long-term observation on species survival especially when the species environment changes (Reznick and Ghalambor, 2001). Therefore, fishing induces evolutionary changes in the life-

history traits of tilapiines due to the high selectivity of the fishing gear that targets and removes large species and large individuals (Stergiou and Tsikliras, 2011).

Environmental Pollution: Lake Victoria is an enormous socio-ecological resource both nationally and internationally as it provides electricity through its hydro-power generation, tourism and a convenient disposal site for human, agricultural and industrial waste (Williams and Hecky, 2005). Different pollution sources either from industrial, domestic, or agricultural activities have affected the aquatic ecosystem of the lake (Figure 5). Most of the riverine nitrogen and phosphorous enters the Lake from agricultural waste, municipal and industrial sewage from the surrounding towns (Hong et al., 2012; Kayombo and Jorgensen, 2006). The discharge from the urban and agricultural drainage channels and raw sewage from villages and unauthorized settlements are dumped into the Lake (Kiwango Wolanski, 2008). Land use changes like deforestation, soil erosion and atmospheric pollution have increased the introduction of both organic and inorganic wastes into the lake (Beeton, 2002). Nutrient input from the catchment's triggers eutrophication through release of fertilizers that contain Nitrogen and Phosphorus. The sewage coming from the industries plus domestic wastes encourages algal bloom that instigate anaerobic conditions leading to toxic tides and associated mass mortality of the fish species (Awange and Ong'ang'a, 2006). Eutrophication has substantially reduced water clarity into Lake Victoria, and this has narrowed light spectrum in the Lake (Muli and Mavuti, 2001; Ormerod et al., 2010). Notably, limited light penetration affects the vision of the fish, colouration of its integuments, migration and movement, reproduction which later causes genetic and ecological differentiation among species (Balirwa et al., 2003; Njiru et al., 2005).

Besides, Lake Victoria mixes completely owing to its water movements this physical instability tends to trap fish in hypoxic regions killing local fish (Kaufman and Victoria, 1992). Unpolluted water contains saturated oxygen for its given temperature unfortunately use of oxygen by the highly organic bottom deposit or in organic wastes from domestic sewage has resulted in hypoxia or reduced dissolved oxygen required for respiration (Njiru et al., 2005).

The presence of the thick fringe and floating mat of the water hyacinth has decreased light and oxygen levels of the lake causing dense phytoplankton production and anoxic conditions, this has resulted to tremendous fish kills (Williams and Hecky, 2005). Water hyacinth is native to the northern tropics of South America and depicted as one of the world's

worst aquatic weed (Wilson et al., 2007). Water hyacinth was first spotted on Lake Kyoga and Lake Victoria, Uganda in 1988 and 1989 respectively (Twongo 1991; Bwathondi and Mahika 1994), Lake Victoria, Kenya, in 1990 (Mailu et al., 1998) (Figure 6).

The effects of invasive water hyacinth in the region and worldwide are serious, varied, and well documented (Gallagher and Haller 1990; Villamagna and Murphy, 2010; Rezanian et al., 2015). In the Lake Victoria area, these have included impeding shore access for fishing, hindering ferry transportation, interfering with hydroelectric power generation, blocking water intake for water supply and industry, and disrupting native aquatic plant communities (Mailu et al., 1998; Gichuki et al., 2001; Williams et al., 2005; Kateregga and Sterner, 2009).



Figure 5: Effects of water pollution. Adapted from <https://www.independent.co.ug/video-lake-victoria-pollution-threatens-lives/>



Figure 6: Water hyacinth on Lake Victoria (Source: Author photo)

1.5 Fish introductions and Translocations in Lake Victoria Basin

Many studies state that there has been changes in the species composition of the catches in Lake Victoria compared to the species which existed before the 20th century when the fisheries development started (Njiru et al., 2005; Ogutu-Ohwayo et al., 2013). Before 1970s, the most important commercial tilapiine species in Lake Victoria comprised of *Oreochromis esculentus* (Graham) and *Oreochromis variabilis* (Boulenger) (Ogutu-Ohwayo, 1990; Goudswaard et al., 2002). On the other hand, other species of commercial importance included *Protopterus aethiopicus*, *Bagrus docmak*, *Clarias gariepinus* and *Brabus* species. The rivers of Lake Victoria basin were mostly composed of *Labeo victorianus* (Ogutu-Ohwayo, 1990). Four tilapiine species, *Oreochromis niloticus* (L), *Oreochromis leucostictus* (Trewavas), *Tilapia zillii* (Gervais) and *Tilapia rendalii* (Boulenger), were introduced into Lake Victoria in the 1950s and 1960s to increase catches, which had declined due to overfishing (Ogutu-Ohwayo 1990; Goudswaard et al., 2002; Njiru et al., 2005;). Introductions of the Nile perch occurred around the same time (1950s) and was purposely to transform the bony and small, but abundant, haplochromines to suitable table fish (Ogutu-Ohwayo 1990). This is because the contribution of haplochromines to fish biomass decreased rapidly, from 83% during the 1970s to < 1% by the mid-1980s (Ogutu-Ohwayo 1990; Witte

et al., 2007). Currently, Lake Victoria is dominated by *L. niloticus*, *R. argentea* and *O. niloticus* (Cowx et al. 2003; Njiru et al., 2005).

In addition to exotic fish introductions, water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Lauc, invaded Lake Victoria in 1988 (Muli et al., 2000). The rapid expansion of densely compacted mats covered the lake with severe economic, social, health and environmental impacts. However, the aquatic plant also facilitated the recovery of some indigenous species, such as mud fish (*Clarias* spp.) and lungfish (*P. aethiopicus*) (Njiru et al., 2002).

Kenya's freshwater bodies have so far had at least 14 fish introductions described and documented (Hickley et al., 2002). Among these introductions, six fish introductions have taken place in Lake Victoria, seven in Lake Naivasha while one has occurred in Lake Baringo (Ogutu-Ohwayo, and Hecky, 1990; Hickley et al., 2002). All these introductions have comprised *Lates niloticus*, *Cyprinus carpio*, *Coptodon zillii*, *Oreochromis niloticus*, *Oreochromis leucostictus* and *Oreochromis melanopleura* (Ogutu-Ohwayo, and Hecky, 1990).

The introductions of *C. zillii* was purposely to fill the niche of macrophytophage while *Oreochromis niloticus* and *Oreochromis leucostictus* were introduced to boost fisheries of the native tilapiines that had reduced because of overfishing (Njiru et al., 2006).

Introduction of fish species in Lake Victoria basin, like elsewhere accounts for positive and negative implications. Contrary, fish introductions have led to the disappearance of the native species in the natural water bodies due to competition for resources and hybridization levels (Hickley et al., 2002; Njiru et al., 2006). On the other hand, fish introductions have boosted the fishery stocks thus increasing the landing catches in the lakes.

Other cases of introductions have come because of increased aquaculture farms near the water bodies where a lot of fingerlings have escaped into the natural water bodies and end up mixing with the wild species affecting the genetic pool of natural environments (Munguti et al., 2014; Ndiwa et al., 2014). Nevertheless, the magnitude of effects of the fish introductions depends on the fish species and its relationship with the environment in which it is introduced.

1.6 Molecular characterization and population genetics of *Oreochromis* species

The morphological identification of cichlid species based on differences in body characters such as colour, number of spines, number of scales and number of rays has proven

to be subtle in identification of tilapia species especially hybrids (Moralee et al., 2000; Nagl et al., 2001). Compared to traditional morphometric based techniques, DNA based methods have been proven to be ideal in identification of tilapia strains even in mixed populations (D'amato et al., 2007; Wu and Yang, 2012).

Genetic markers offer a steadfast method for understanding the genetic structure both among and within populations of different organisms. Notwithstanding they help in species identification of unknown samples allowing the authorities in monitoring protected nature reserved areas (Kajungiro et al., 2019). As such, knowledge of population genetic structure and genetic diversity of tilapiines is very useful for conservation practices and breeding of the fishes. Earlier studies explored the genetic structure and diversity between populations of different tilapiines especially *O. niloticus* with most studies using either on phenotypic traits (Trewavas, 1983), allozymes (Sodsuk and McAndrew, 1991), mitochondrial DNA (Romana-Eguia et al., 2004), randomly amplified polymorphic DNA (Hassanien et al., 2004) or microsatellites (Bhassu et al., 2004; Hassanien and Gilbey, 2005; Mireku et al., 2017). However, the genetic markers used to date have limitations regarding their maximal resolution in detecting the complex genetic structure typically encountered in Nile tilapia populations. Therefore, microsatellites being nuclear indicates that there is recombination between loci since different loci can show ancestry from different species and that is why they are great to study hybridization. Moreover, they have a mutation rate higher than mtDNA being possible to detect small scale genetic variation patterns (He et al., 2010; Firmat et al., 2013), species identification (Wu and Yang, 2012) and Phylogenetics (Nagl et al., 2001). In comparison with mtDNA markers, MtDNA has this characteristics of no recombination, high mutation rate and small size. The fact that mtDNA is maternal inherited and there is no recombination makes them not good to study hybridization since they will only show the ancestry from the maternal part (Ali et al., 2005).

1.7 Molecular markers used during the study.

1.7.1 Microsatellite/Simple Sequence Repeats (SSR)

This is a class of repetitive DNA sequence having 1–6 bp repeat length found in any region of the genome of an organism (Duran et al., 2009). They are abundant and most diverse in nature with higher polymorphism (Duran et al., 2009). The shortness of the markers makes them easier to amplify the loci using the PCR. They consist of tandem repeating units of one

to six nucleotides (mono-, di-, tri-, tetra-, penta- and hexanucleotides) (Fungtammasan et al., 2015). Only small amounts of tissue are required for typing microsatellites and these markers can be assayed using non-lethal fin clips and archived scale samples, facilitating retrospective analyses and the study of depleted populations (Fungtammasan et al., 2015). Due to their high discriminatory power, they are used in the identification and differentiation of species including *Oreochromis* sp. as well as performance traits in population genetics studies (Basiita et al., 2015; Tibihika et al., 2019). Studies show that nearly 1–4% of the genome consists of microsatellites, and at least one microsatellite appears around every 10 kilobases in fishes. To identify microsatellites, there is a need to design specific markers that are complementary to the flanking sequence of either side of the repeat unit array. This is then subjected to Polymerase Chain Reaction (PCR) to target and amplify the highly conserved regions in the genome (Shen et al., 2015). The easiest technique used to screen for microsatellite is by developing flanking primer pairs directly from already published sequences of the species of interest or those that have been deposited in National Centre for Biotechnology Information (NCBI) (Medlin et al., 2006).

1.7.2 Next generation sequencing (NGS) marker technique tool

The continued demand for microsatellite loci has made NGS a better alternative for marker development compared to traditional markers. This is because traditional markers depend on construction of genomic libraries that are enriched for certain SSRs (Curto et al 2013) thus causing biases related to the use of probes. Therefore, use of NGS allows to get markers with lower effort, costs, and biases. In genotyping, the technique helps to lower homoplasy because the microsatellite sequences are used to define alleles (Curto et al., 2019).

Among the two NGS technologies, illumina sequencing is commonly used in the discovery of SSRs compared to 454 because the 454 has been discontinued. Roche's 454 pyrosequencing technique utilizes fragmented nucleic acid template of between 300 – 800 base pairs fitted with two different adaptor sequences at both ends. These are used as priming sites for emulsion Polymerase Chain Reaction (ePCR) and later sequencing reactions. This technique is rarely applied in most genetics' studies and has been discontinued (Shendure and Ji, 2008). Illumina technology on the other hand relies on bridge amplification of fragmented DNA in a PCR reaction. Four fluorescently labelled nucleotides are used to perform sequencing by

synthesis whereby the four nucleotides are characterized by their reversible terminators which permit single base incorporation (Turcatti et al., 2008). The labelled nucleotides are identified by imaging in cyclic reactions (50 to 300 cycles) which results in reads of up to 300 nucleotides.

NGS technologies have enabled the discovery of large numbers of genetic markers for different organisms at an affordable cost allowing the investigation of genetic diversity within and between populations (Candy et al., 2015). Restriction-site associated DNA (RAD) and double-digest RAD (ddRAD) sequencing are NGS-based techniques providing a reduced representation of the genome in question (Baird et al., 2008; Peterson et al., 2012). ddRAD-seq and similar genotyping by sequencing techniques depend on digestion of the genomic DNA with restriction enzyme(s), and subsequent high-depth sequencing of the flanking regions of the cut site. Such genotyping by sequencing techniques have been widely applied in aquaculture species (Robledo et al., 2018). Many studies have applied ddRAD-seq sequencing to generate high-density linkage maps (Manousaki et al., 2016) and estimate genetic diversity (Antoniou et al., 2017; Hosoya et al., 2018). Additionally, ddRAD-seq has been used in several tilapia studies for evaluating the suitability of DNA from skin mucus swabs (Taslima et al., 2017), identification of sex determining regions (Wessels et al., 2017), and quantitative trait loci (QTL) analysis (Li et al., 2017), hybridization levels as well as stock assessment (Reuter et al. 2015).

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Sampling design

A total of 322 fish samples were captured using experimental seine nets with the help of fishermen from Lake Victoria and its satellite lakes. The samples included *Oreochromis esculentus*, *Oreochromis leucostictus*, *Oreochromis niloticus* and *Coptodon zillii*. The sampling sites included Mbita (0°25'36.76872"S, 34°12'55.24632"E), Dunga (0°08'41"S, 34°44'12"E), Luanda Nyamasaria (0°28'23.81326"S, 34°16'59.64076"E), Usenge(0° 4'20.526"S, 34°3'34.722"E), Usoma(0°6'16.878"S, 34° 43'8.382"E), Siungu(0°3'2.652"S, 34°2'27.882"E), Seka Bay (0°21'20.724"S, 34°40'29.67"E) and Lake Sare (0°3'7.836"S, 34°2'28.098"E) (Table 1). All these sites are located on Lake Victoria, Kenya (Figure 7).

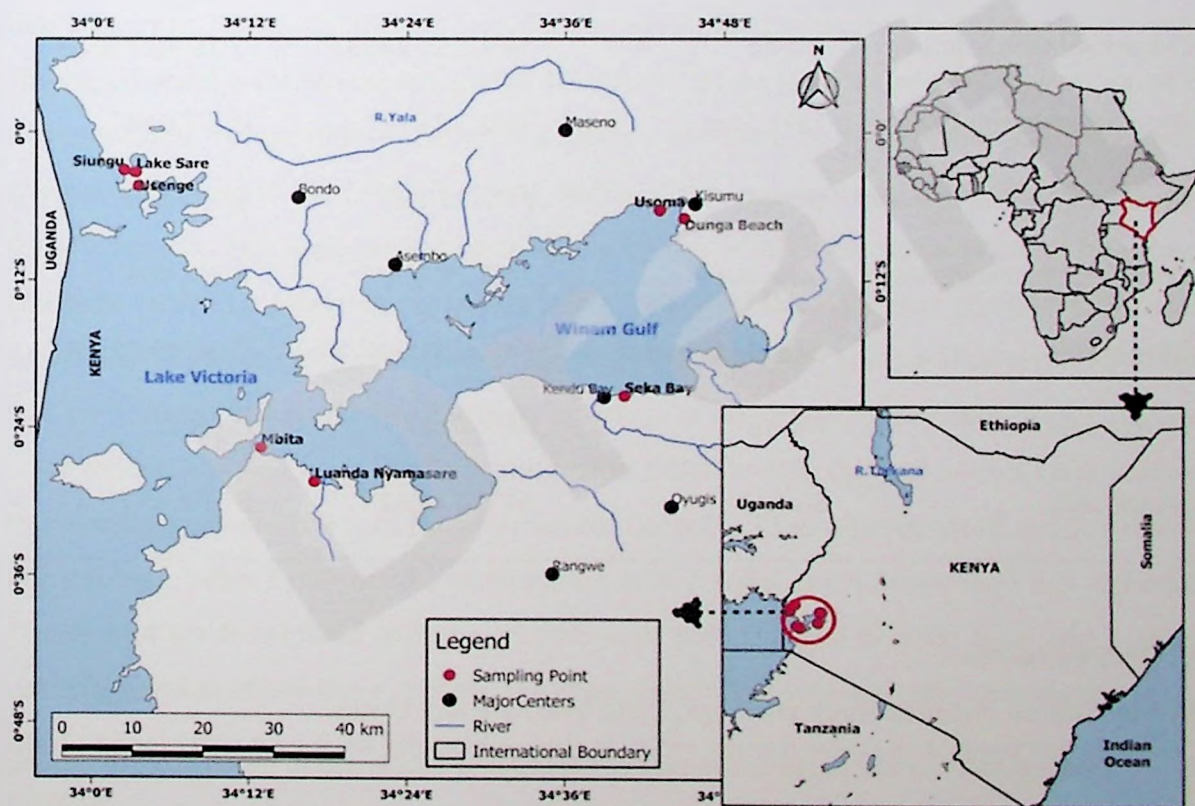


Figure 7: Map showing the sampling sites on Lake Victoria, Kenya

Table 1: Source and number of the fish samples collected in different sites.

Species	Source of samples	Location		No. sample
		Longitude (E)	Latitude (S)	
<i>Oreochromis niloticus</i>	Dunga	34°44'12''	0°08'41''	30
<i>Oreochromis niloticus</i>	Usenge	34° 3'34.722''	0° 4'20.526''	22
<i>Oreochromis niloticus</i>	Usoma	34° 43'8.382''	0°6'16.878''	32
<i>Oreochromis niloticus</i>	Siungu	34°2'27.882''	0° 3'2.652''	18
<i>Oreochromis niloticus</i>	Seka Bay	34°40'29.67''	0°21'20.724''	25
<i>Oreochromis niloticus</i>	Luanda Nyamasaria	34°16'59.64076''	0°28'23.81326''	23
<i>Oreochromis niloticus</i>	Mbita	34°12'55.24632''	0°25'36.76872''	25
<i>Oreochromis leucostictus</i>	Lake Sare	34°2'28.098''	0°3'7.836''	30
<i>Oreochromis leucostictus</i>	Siungu	34°2'27.882''	0° 3'2.652''	30
<i>Oreochromis esculentus</i>	Lake Sare	34°2'28.098''	0°3'7.836''	30
<i>Tilapia zillii</i>	Luanda Nyamasaria	34°16'59.64076''	0°28'23.81326''	14
<i>Tilapia zillii</i>	Mbita	34°44'12''	0°08'41''	30
<i>Tilapia zillii</i>	Siungu	34°2'27.882''	0° 3'2.652''	13

2.2 DNA extraction

DNA extraction involved three steps i.e., Lysis, Washing and Elution

Lysis: A fish muscle or fin tissue was collected and preserved in 98% absolute ethanol, while in the field. The samples fixed in ethanol were later airlifted to Vienna, Austria in Meimberg Laboratory at BOKU for subsequent genotyping. Prior to DNA extraction, the tissue was removed from a 1.5ml Eppendorf tube and placed on an aluminium foil to allow ethanol to dry off from the tissue before processing.

The dried tissue was then placed in a 2.0ml new Eppendorf tube, 180µl of pre-lysis buffer T1 (Macherey Nagel) was added into the sample and vortexed for at least five seconds so that the buffer can fully mix with the tissue. The buffer provides favourable pH for Proteinase K enzyme to work as well as preventing the degradation of DNA by inactivating all other enzymes in the cytosol. Then 10µl of Proteinase K (10mg/ml) enzyme was added to the sample for digestion purposes and vortexed shortly. The resultant mixture was then put on a mixing block (PEQLAB) that was calibrated at 56°C, 300 rpm and incubated overnight. This was done to provide optimum conditions for full digestion of the sample (Appendix 1).

The digested solution was then removed from the mixing block the following morning, 10µl of RNase (10 mg/ml) was added and vortexed shortly. The solution was then placed in a mixing block that was calibrated at 37°C, 300 rpm for 15 minutes. After 15 minutes, 180 µl lysis buffer B3 (Macherey Nagel) was added to the solution and placed in the mixing block that was set at 70°C with 300 rpm and allowed to run for 10 minutes (Appendix 1).

The supernatant was then centrifuged at 4000 rpm for two minutes and this was repeated for a second time. These different centrifugations were done to discard solid particles and to prevent damaging of the DNA. Carefully, 360µl of the supernatant/ lysat was pipetted and transferred into 2ml deep-well-plate followed by the addition of 180µl of absolute ethanol and then mixed by pipetting ten times (10X). 540 µl of the lysat was transferred into the EconoSpin™ Columns with silica membrane (Epoch Life Science, USA) and centrifuged at 2000 rpm for 15 seconds followed by another centrifuge of 4000 rpm for one minute.

Washing: The first wash involved addition of 600µl of 80% ethanol in each tube followed by centrifugation at 4000 rpm for one minute. Centrifugation was repeated at 6000 rpm for two minutes. After 1 minute of centrifugation at 8000 rpm, the flow-through was discarded. The second wash involved addition of 300 µl of 80% ethanol in each tube and a similar centrifugation as above done. Finally, the EconoSpin™ Columns with silica membrane (Epoch Life Science, USA) was then allowed to dry at room temperature in a hood for at least 15 minutes.

Elution: The first elution involved addition of 50µl of the elution buffer (10 mM Tris, pH8 [µl]) to the dried EconoSpin sample plate and centrifuged at 6000 rpm for five minutes. The second elution then involved the addition of 100 µl of the elution buffer (10 mM Tris, pH8 [µl]) to the dried sample and centrifugation in first elution repeated.

2.2.1 Quality check for DNA using Gel Electrophoresis.

The quality of DNA was verified using 1.5% agarose gel (5.25 g agarose powder in 350 ml 1X TAE, pH 7.5. The mixture containing 5.25 g agarose powder and 350 ml of 1X TAE was then heated under a laboratory microwave machine and regular shaking done until the powder dissolved. This was later allowed to cool and subsequently stained with 4.67µl HDGreen dye. 5.0µl of DNA sample was mixed with 4.0µl of loading dye and loaded onto individual wells on the gel. The solution was then mixed by pipetting at least five times (5X). An empty well was used as a reference in which 4.0µl of the λ ladder (=250 ng) was added. The gel was then run at 80 volts for 30 minutes and later visualized and documented using a trans-illuminator system (Intas GEL IX IMAGER, Germany) (Appendix 2). The visualized DNA samples (Appendix 3) were then labelled and stored in a freezer set at -20°C awaiting PCR amplification and purification.

2.3.0 PCR Amplification

Microsatellite markers were used for amplification in both 96 well and 384 PCR plates. A total of 43 microsatellite loci that were developed at the Institute for Integrative Nature Conservation Research, Boku were utilized to genotype the DNA of the fish samples (Tibihika et al., 2019). The primers were mixed and pooled into four combinations.

Preparation of the PCR master mix took place on ice and for each reaction, a 96 PCR plate individual well contained 2.5µl of the Master mix (Qiagen Multiplex PCR Kit), 1.0µl of the primer mix (containing forward and reverse; PM_Ti2a, PM_Ti2b, PM_Ti4 and PM_Ti5), 0.5µl of the autoclaved water and 1.0µl of the DNA sample (Table 1). The Master Mix, Primer mix and Autoclaved water were pipetted into a 1.5ml tube for all samples except the DNA, which was later added to the PCR plate. DNA from the different samples was added into the wells as the last step with the help of the Liquid Handling Station robot with a data pool version 2.1.14 (Appendix 4). A silicone mat was then placed on the PCR plate to avoid evaporation.

Table 2: Reaction composition for the PCR amplification

S/No.	Content	Qty(μ l)	X96 plates Considering 15% pipetting errors (110 Reaction
1	Master Mix	2.5	275
2	Primer Mix	1.0	110
3	Autoclaved water	0.5	55
4	DNA Sample	1.0	110
Total		5.0	550

For a 384 PCR plate per well, 2.5 μ l of Master Mix was mixed with 1.0 μ l of Primer Mix, 0.5 μ l water and 1.0 μ l of DNA added using a single pipetting procedure with a multi-channel pipette and replenishing the pipette tips to avoid cross-contamination.

The 96-well plate was centrifuged shortly at 12000 rpm to ensure that all reagents are on the bottom of the wells. Then, the plate was placed into a T100 Thermocycler (Bio-Rad Laboratories, USA), initialized at 95°C for 15 minutes, followed by denaturation for 30 cycles at 95°C for 30 seconds, annealing at 55°C for 1 minute and elongation for 1 minute at 72°C and the last extension steps at 72°C and incubation at 10°C for 10 minutes (Appendix 5).

2.3.1 Quality check for PCR product using Gel electrophoresis.

The quality of PCR product was verified using 1.5% agarose gel (5.25 g agarose powder in 350 ml 1X TAE, pH 7.5. This was then heated under a laboratory microwave and regularly stirring until the powder dissolved, it could cool and while warm, it was stained with 4,67 μ l HDGreen). 2.0 μ l of PCR product was mixed with 4.0 μ l of loading dye and loaded onto individual wells on the gel plate. The solution was then mixed by pipetting at least five times (5X). An empty well was used as a reference in which 4.0 μ l of the ladder (=250 ng) per lane by using λ ladder mix for DNA. The gel was run at 80 volts for 30 minutes and later visualized and documented using a Trans illuminator system (Intas GEL IX IMAGER, Germany). The DNA samples was then labelled and stored in a freezer set at -20°C awaiting purification.

2.4.0 PCR Purification

To achieve a pure PCR product that is free from unwanted components like PCR artefacts, 2.86 μ l of Magnetic bead AMPure XPB was added in the PCR plates followed by 4.0 μ l of the DNA sample. The solution was mixed by pipetting ten times (10X) in the PCR plate. The

supernatant was incubated for five (5) minutes at room temperatures. A magnetic separator was inserted into the sample plate and circular movements done by hand for two minutes until the solution turned clear.

Washing: The sample plate was then transferred into a first wash source plate that contained 200 μ l of 80% ethanol and then mixed for 45 seconds. The sample plate was transferred to the second wash plate that also contained 200 μ l of 80% ethanol and mixed for 45 seconds. The magnetic beads were collected by the magnet and air dried for five minutes. The magnetic particles could air dry for at least five minutes.

Elution: A new plate was prepared and labelled with the sample codes, 20 μ l of the elution buffer 10mM Tris pH8 was added to the sample plate followed by the DNA sample plate. Two minutes of mixing were done. Then a magnetic separator removed the beads from the sample plate through circular movements for more two minutes and the DNA remained in the solution (Appendix 6).

2.5.0 PCR Indexing

This involved addition of 5.0 μ l of master mix into the Econospin plate followed by 2.0 μ l of P5 with the help of a Liquid Handling Station robot with a data pool version 2.1.14 that was calibrated to pipette the aliquots. 2.0 μ l of P7 was added into the plate followed by the addition of 1.0 μ l of the PCR sample. The mixture was centrifuged shortly less than 1.0minutes and then put in a Thermal cycler PCR machine and run for 30 cycles as previously described in 2.3.0 section above.

After indexing the PCR products, all the samples marked using 43 microsatellite markers were pooled into a single 1.5ml tube. The indexed PCR product samples in the two tubes were then sent for Illumina MiSeq (PE300) sequencing at the Genomics Service Unit at the Ludwig-Maximilians-Universität München (LMU) Bio-center in Germany.

2.6.0 Bioinformatics: Sequence analysis, genotyping and allele calling of microsatellites (SSRs)

Standard Laboratory procedures were used during the sequence analysis. After receiving the reads from illumina sequencing, they were subjected to quality check using FastQC version 0.11.9 and trimmed to remove artefact adapters and poor-quality regions using Trimmomatic version 0.39 (Bolger et al., 2014). The python script from Curto et al.

(2019) was used to search for the mismatches between the motif adapters in the forward and reverse primer sequences at the beginning and end of the sequences, respectively.

In allele calling, two steps were assumed specifically 'genotype calling' to define the genotype of an individual at a given amplicon length and 'SNP calling' to identify sites that vary within the alleles having the same length. After running the python script for the first allele call using the program "SSR-GBS-pipeline_Allele-Length-Call_win_0.12.py", a codominant matrix was produced as one of the output files showing that incorporated length variation at each repeated motif and flanking regions, and histograms based the length of the sequence.

2.7.0 Statistical analysis

To assess the genetic structure between species and populations of the samples collected, a codominant matrix with alleles coded as numbers was generated. The matrix was used as an input for population genetic analysis with GenALEx v. 6.503 (Peakall and Smouse, 2006) program to obtain variability measures like number of alleles, private alleles, mean allelic patterns, heterozygosity (Observed and expected), Shannon information index, number of effective alleles and divergence values such as F_{st} . For example, heterozygosity describes the genetic diversity of a population, and it varies from 0 to 1, low values indicate a low genetic diversity and vice-versa.

The F_{st} value is the proportion of the genetic variance contained in a subpopulation compared to the total genetic distance over all populations, it also varies from 0 to 1. High F_{st} value indicates differentiation between populations while low F_{st} shows genetic similarity.

Test for deviations from Hardy–Weinberg Equilibrium of the samples was also achieved using GenALEx.

Absolute genetic distances among the tilapines were analysed and visualized in a Principal Coordinate Analysis (PCoA) using GenALEx, for multiple subsets of data. All the tilapiines combined were analysed first followed by *Oreochromis* species (*O. niloticus*, *O. esculentus* and *O. leucostictus*), other tilapiines excluding *O. niloticus*, *Oreochromis* species from Lake Sare populations of *O. niloticus*, *O. leucostictus* from different beaches. PCoA helps to explore and visualize the divergence between the different individuals in a dataset. It is achieved by grouping samples based on genetic distance and plotting all possible plots using the three best coordinates (1 vs 2, 1 vs 3 and 2 vs 3) computed by the software.

STRUCTURE v. 2.3.4 (Hubisz et al., 2009) program was used to analyse the genetic clustering of samples. It involved 13 iterations with K set from 1-10 and 10,000 generations after a burn-

in period of 10, 000. This was done for datasets consisting of all tilapiines, *Oreochromis* species and their respective populations. STRUCTURE classifies populations by genetically assigning them into groups whose individuals share similar patterns of variation (Porrás-Hurtado et al., 2013).

Structure Harvester, an online program (<http://taylor0.biology.ucla.edu/structureHarvester/>) was used to detect the best optimal K value. CLUMPAK - Clustering Markov Packager Across K, an online main pipeline (<http://clumpak.tau.ac.il/>) (Kopelman et al., 2015) was used to summarize various iterations per K. Each bar in the plot computed by Structure and Clumpak represents one sample, the different colours of the bars show the likelihood that the specimen belongs to a certain genetic cluster.

CHAPTER 3

3.0 RESULTS

3.1 Distribution of the tilapiines in the sampling sites

In the present study, a total of 322 fish samples were collected using seine nets from Lake Victoria and its satellite lakes. Fewer samples of tilapiines were collected from Usenge, Siungu and Luanda beaches (Table 3). The samples composed of *Oreochromis esculentus*, *Oreochromis leucostictus*, *Oreochromis niloticus* and *Coptodon zillii* (Table 2). During genotyping, 86 samples were removed from the final dataset due to genotyping failure leaving only 254 samples that were used in the final analysis.

Table 3: Distribution of the tilapiines in the sampling beaches

Species	Source of samples	samples collected	Sequenced samples
<i>Oreochromis niloticus</i>	Dunga	30	25
<i>Oreochromis niloticus</i>	Usenge	22	22
<i>Oreochromis niloticus</i>	Usoma	32	20
<i>Oreochromis niloticus</i>	Siungu	18	7
<i>Oreochromis niloticus</i>	Seka Bay	25	16
<i>Oreochromis niloticus</i>	Luanda Nyamasaria	23	13
<i>Oreochromis niloticus</i>	Mbita	25	23
<i>Oreochromis leucostictus</i>	Lake Sare	30	28
<i>Oreochromis leucostictus</i>	Siungu	30	25
<i>Oreochromis esculentus</i>	Lake Sare	30	26
<i>Tilapia zillii</i>	Luanda Nyamasaria	14	14
<i>Tilapia zillii</i>	Mbita	30	23
<i>Tilapia zillii</i>	Siungu	13	12

3.2 Genetic differentiation and diversity of freshwater Tilapiines of Lake Victoria, Kenya

3.2.1 All tilapiines

Generally, all the tilapiines had a higher expected heterozygosity ($H_e = 0.42 \pm 0.03$) than observed heterozygosity ($H_o = 0.33 \pm 0.03$) with *O. niloticus* populations having the highest observed heterozygosity ($H_o = 0.42 \pm 0.04$) followed by *C. zillii* ($H_o = 0.35 \pm 0.06$) while *O. leucostictus* had the least ($H_o = 0.28 \pm 0.05$) (Table 3a). The average number of alleles (N_a) was observed at 5.67 ± 0.49 across all the populations with populations of *O. niloticus* having the highest ($N_a = 11.52 \pm 1.25$) followed by *C. zillii* populations ($N_a = 5.28 \pm 0.54$) while populations of *O. esculentus* had the least number of alleles (2.83 ± 0.42) (Table 3a).

The average Shannon Information Index (I) across the tilapiines was 0.87 ± 0.06 with the highest index across the populations observed in *O. niloticus* samples ($I = 1.45 \pm 0.13$) followed by *C. zillii* ($I = 0.89 \pm 0.11$) while *O. leucostictus* had the least ($I = 0.57 \pm 0.09$) (Table 3a).

The PCoA analysis for the tilapiines resulted in two clear groups. Cluster one comprised of *O. niloticus* populations while the second cluster comprised of *O. leucostictus*, *O. esculentus* and *C. zilli*. *O. esculentus*, *C. zilli* and *O. leucostictus* could not be differentiated (Figure 8a). The results are represented by 23.00% at the first axes, 8.94% at the second axes, and 6.45% at the third axes. The undifferentiated group shows some degree of admixture between these tilapiines. There are five samples of *C. zillii* that appear in the *O. niloticus* group and four samples of *O. niloticus* that appear in the cluster that composed *O. esculentus*, *C. zillii* and *O. leucostictus* (Figure 8a).

Considering *Oreochromis* species collected during the study (*O. niloticus*, *O. leucostictus* and *O. esculentus*), populations of *O. niloticus* formed an independent cluster while populations of *O. leucostictus* and *O. esculentus* formed another cluster (Figure 8b). Cluster containing *O. esculentus* and *O. leucostictus* could not be differentiated. At least five samples of *O. niloticus* were found in the cluster containing *O. leucostictus* and *O. esculentus* (Figure 8b). The results were represented by 29.77% at the first axes, 8.34% at the second axes, and 5.84% at the third axes.

Considering other tilapiines excluding *O. niloticus*, *C. zillii* showed higher values for the parameters tested compared to other fish species. The average expected heterozygosity ($H_e = 0.36 \pm 0.03$) was higher than observed heterozygosity ($H_o = 0.30 \pm 0.03$) (Table 3c). *C. zillii*

had a higher expected heterozygosity ($H_e=0.44\pm0.05$) followed by *O. esculentus* while *O. leucostictus* had the least expected heterozygosity ($H_e=0.31\pm0.05$), the similar trend was seen in the observed heterozygosity (Table 3c). The average number of alleles was 3.71 ± 0.29 with *C. zillii* having the highest ($N_a=5.28\pm0.54$) while *O. esculentus* had the least ($N_a=2.83\pm0.42$). Shannon information index was averagely 0.68 ± 0.06 with *C. zillii* having the highest ($I=0.89\pm0.11$) while *O. leucostictus* had the least value ($I=0.57\pm0.09$) (Table 3c).

The PCoA results showed no clear differentiation with most of the samples grouping together as explained by 15.53 %, 11.90% and 5.19% for axes one, two and three respectively (Figure 8c).

Generally, *Oreochromis* species collected from Lake Sare had the highest expected heterozygosity ($H_e=0.31\pm0.04$) compared to the observed heterozygosity ($H_o=0.29\pm0.04$) (Table 3d). The expected heterozygosity was almost the same with *O. esculentus* populations having (0.32 ± 0.05) and *O. leucostictus* with ($H_e=0.31\pm0.05$) (Table 3d).

Populations of *O. esculentus* had the highest number of alleles ($N_a=2.83\pm0.43$) while populations of *O. leucostictus* had the least value (2.62 ± 0.36) (Table 3d).

The average Shannon Information Index (I) across the *Oreochromis* populations was 0.56 ± 0.07 which lies within the recommended index ranges between 0 and 1 (NIST, n.d.). The highest index across the populations was observed in *O. esculentus* populations ($I=0.58\pm0.10$) while *O. leucostictus* had the least ($I=0.56\pm0.07$) (Table 3d).

The PCoA results formed two clusters; Cluster one comprised of *O. esculentus* while cluster two comprised of *O. leucostictus* with a few samples of *O. esculentus* indicating admixtures of *O. esculentus* and *O. leucostictus* (Figure 8d). Sample populations of *O. leucostictus* formed a sub-cluster (Figure 8d). These were explained by 19.98% for axis one followed by 10.79% for axis two while axis three had 6.80% genetic variation.

3.2.2 Genetic diversity of *Oreochromis niloticus* populations

Some samples of *Oreochromis niloticus* populations deviated from HWE (Table 4). Populations from Dunga showed a higher deviation with 23 out of 29 loci deviating from the HWE. Populations from Luanda, Seka Bay and Usenge showed a similar deviation of 17 loci out of 29 polymorphic loci tested. Least deviation was observed in the populations of Siungu with only three loci that deviated out of 29 tested (Table 4).

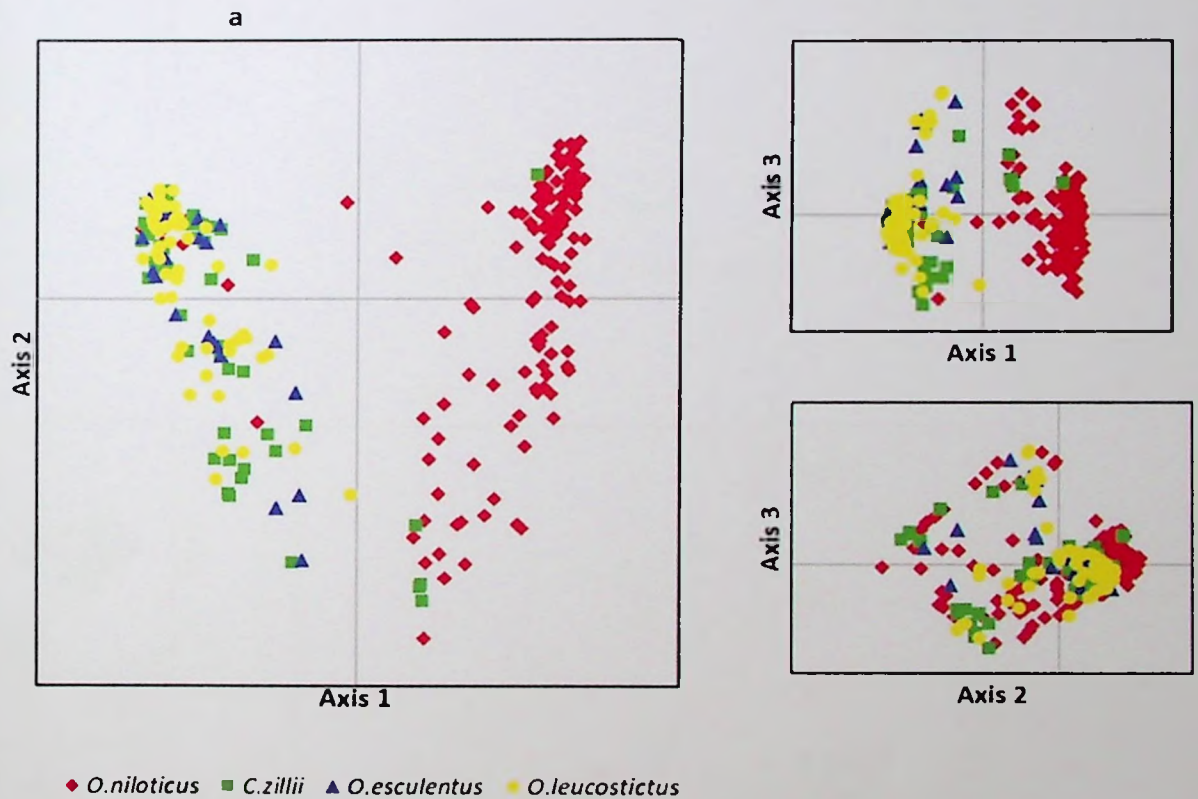
The heterozygosity, the pairwise comparison using Fst/NeiP distance, and the number of polymorphic loci were evaluated. Generally, *Oreochromis niloticus* populations had the highest expected heterozygosity ($H_e=0.54\pm0.02$) compared to the observed heterozygosity ($H_o=0.41\pm0.02$) (Table 3e). Populations from Dunga beach had the highest observed heterozygosity ($H_o=0.47\pm0.05$) while samples from other beaches had averagely 0.4 ± 0.05 as their observed heterozygosity (Table 3e). The highest expected heterozygosity was from Dunga beach samples (0.63 ± 0.04) and least was from Siungu ($H_e=0.43\pm0.05$). Samples from Dunga beach had the highest number of alleles ($N_a=7.24\pm0.69$) followed by Usenge beach ($N_a=6.66\pm0.72$) while Siungu had the least number of alleles ($N_a= 3.03\pm0.36$) (Table 3e). The average Shannon Information Index (I) across the *O. niloticus* populations was 1.12 ± 0.04 outside the recommended index ranges between 0 and 1 (NIST, n.d.) (Table 3e). The highest index across the populations was observed in the samples collected from Dunga beach ($I=1.39\pm0.11$) while the least was observed from Siungu beach ($I=0.79\pm0.11$) (Table 3e).

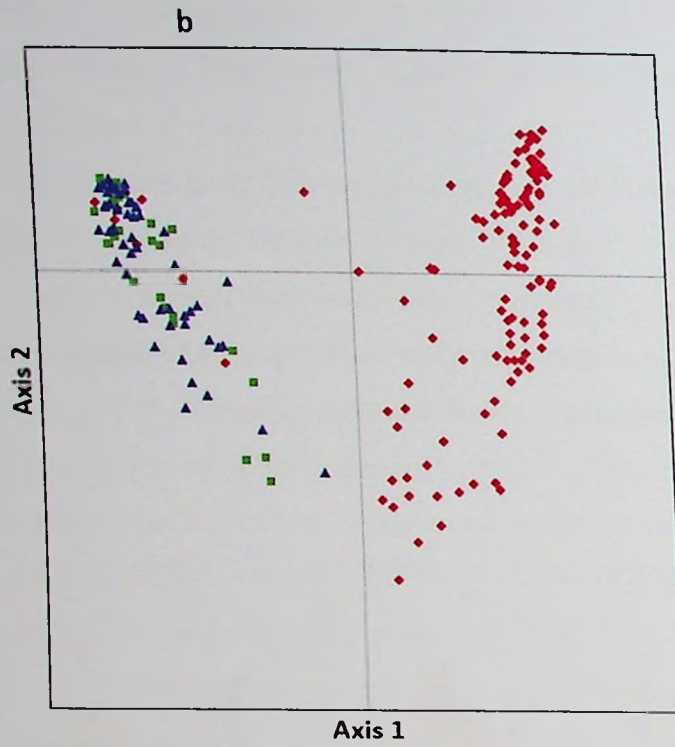
The principal coordinate analysis (PCoA) was conducted to differentiate the populations of *O. niloticus* from different landing beaches using the first three axes. The PCoA showed two clear groups: *O. niloticus* populations from Usenge, Mbita, Siungu, Luanda and Seka-Bay formed one cluster while populations from Dunga and Usoma formed another cluster (Figure 8e). In cluster one, populations from Usenge and Siungu were very close compared to populations from Mbita and Luanda. The percentage variation within the seven beaches was explained and represented by 12.38% at the first axes, 9.61% at the second axes, and 6.92% at the third axes (Figure 8e).

To investigate whether there was genetic differentiation between the two populations of *O. leucostictus* from Lake Victoria and Lake Sare, the PCoA results showed that *O. leucostictus* populations formed two cluster with Lake Sare populations forming one cluster while Victoria formed another cluster with a percentage variation represented by 15.69% for the first axis, 12.69% for the second axis while axis three had 6.75% (Figure 8f). The PCoA also shows some samples in intermediate positions between the groups; eight samples of *O. leucostictus* from Lake Sare and at least 10 samples from Lake Victoria. Seven samples from Lake Sare formed a subgroup far from the intermediates (Figure 8f).

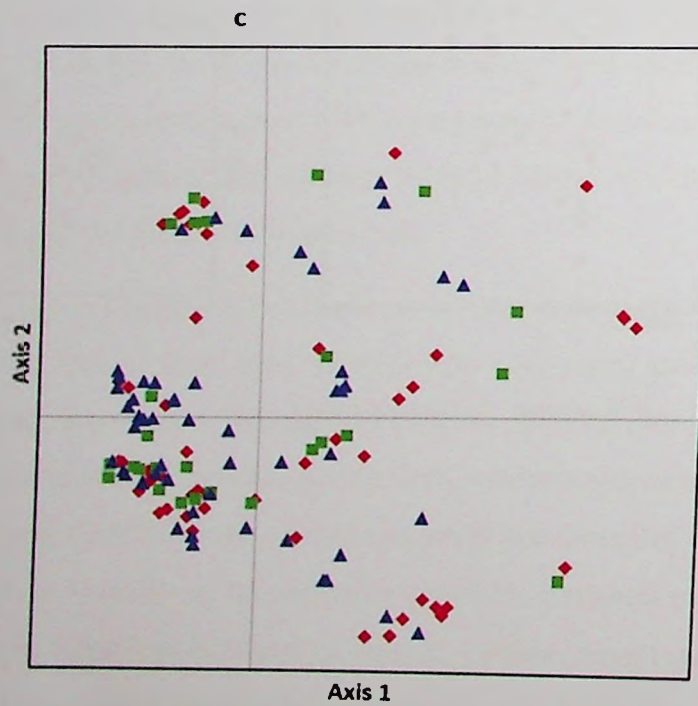
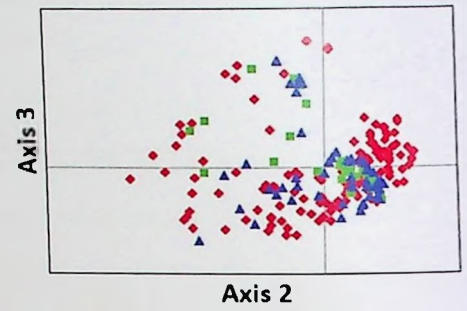
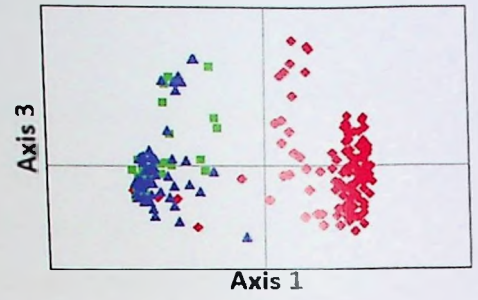
With *Oreochromis* species from Siungu, the PCoA results formed two clusters with one cluster containing *O. niloticus* while another cluster contained *O. leucostictus* populations (Figure 8g).

The comparison involving *O. esculentus*, *O. leucostictus* populations from Lake Sare with *O. leucostictus* populations from Lake Victoria showed no clear clustering of the populations. Few samples of *O. leucostictus* from Lake Sare formed a sub-cluster that comprised of five samples of *O. esculentus* from the same lake (Figure 8h). Generally, there was a high degree of the admixture of the *Oreochromis* populations.

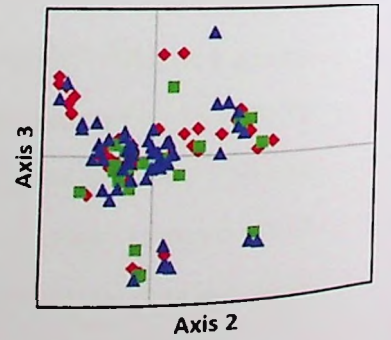
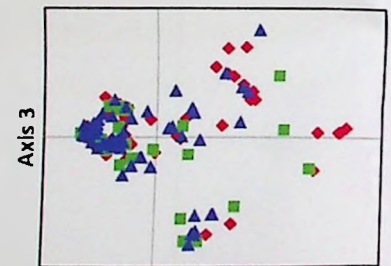


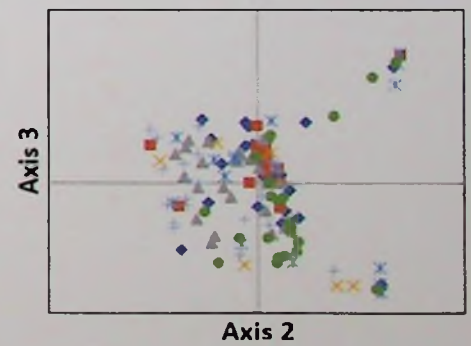
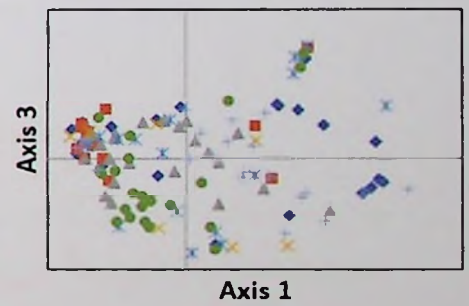
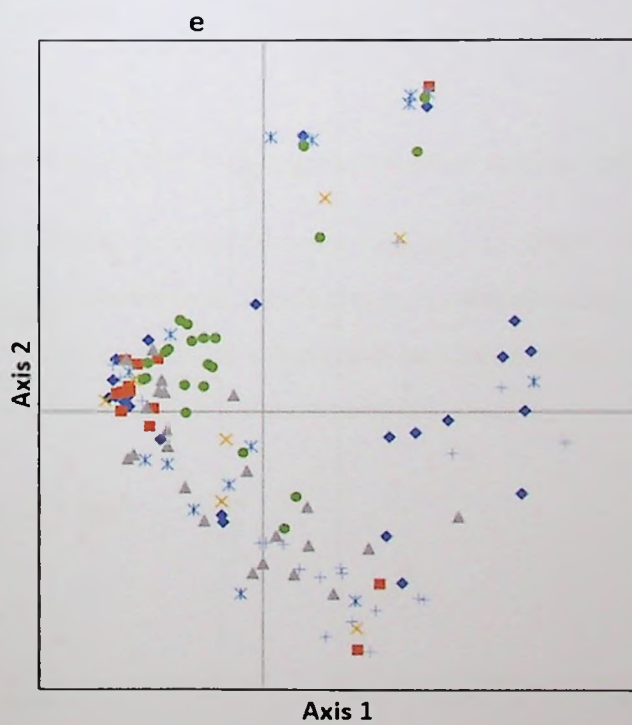
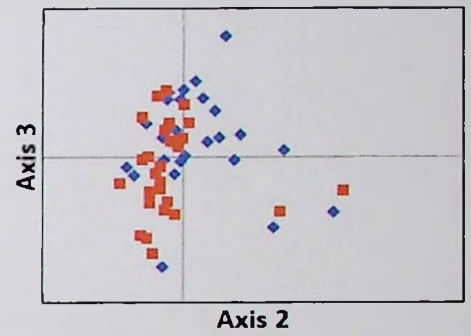
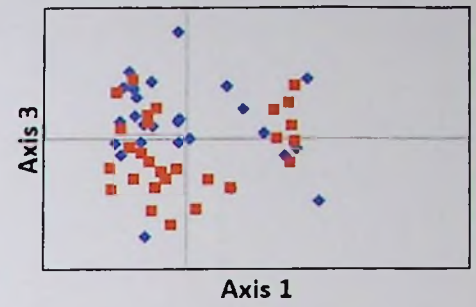
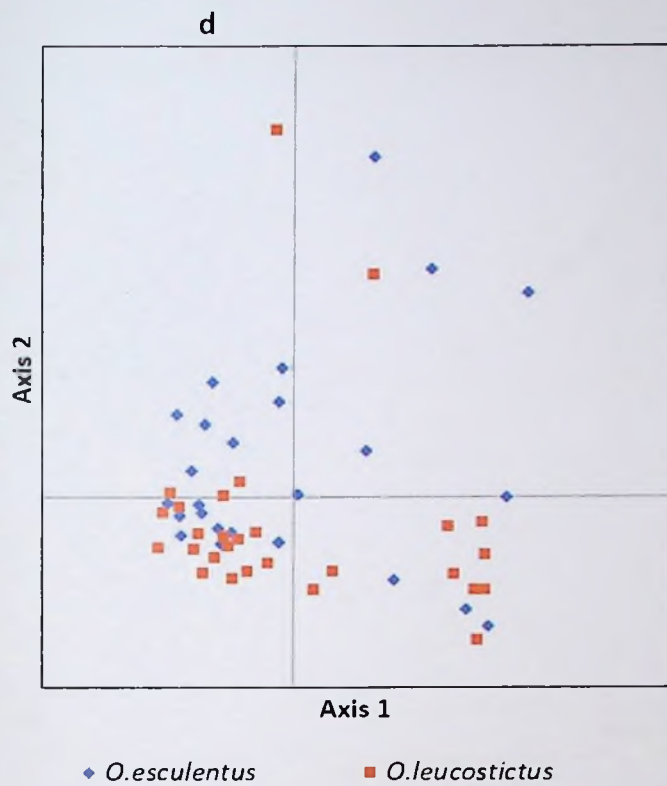


◆ *O. niloticus* ■ *O. esculentus* ▲ *O. leucostictus*

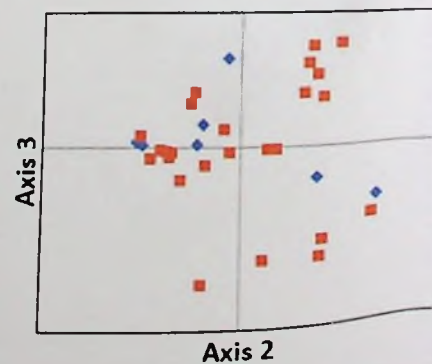
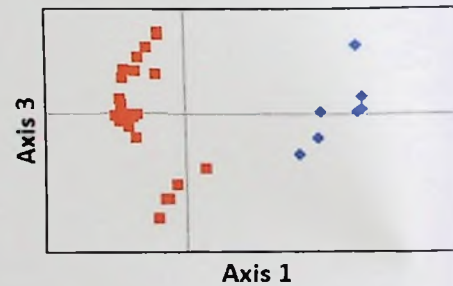
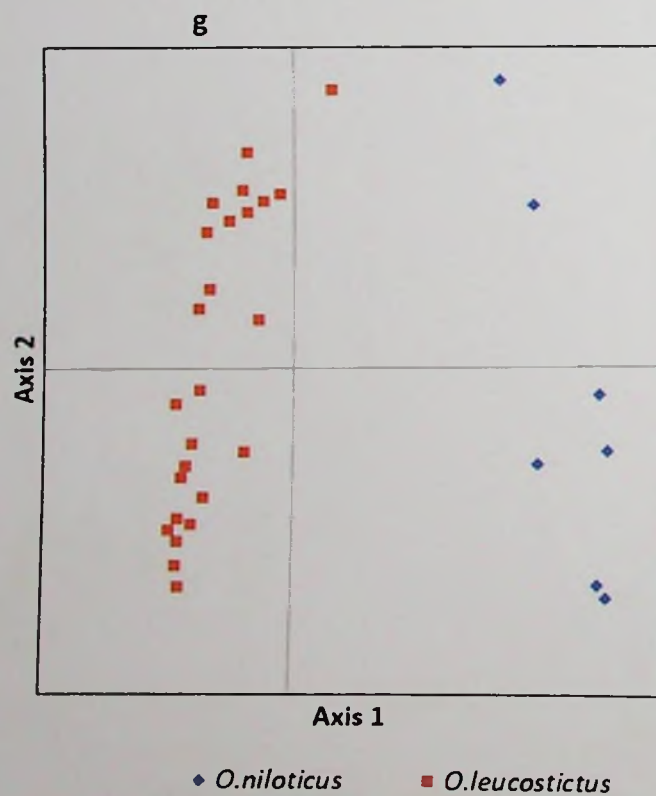
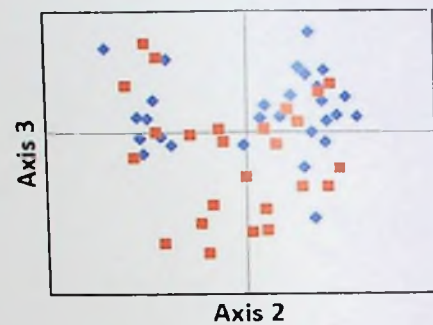
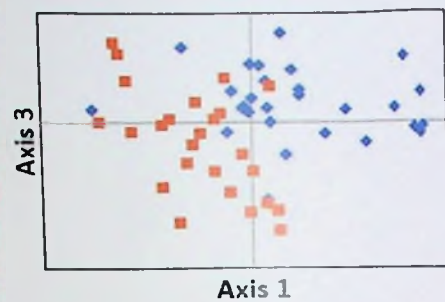
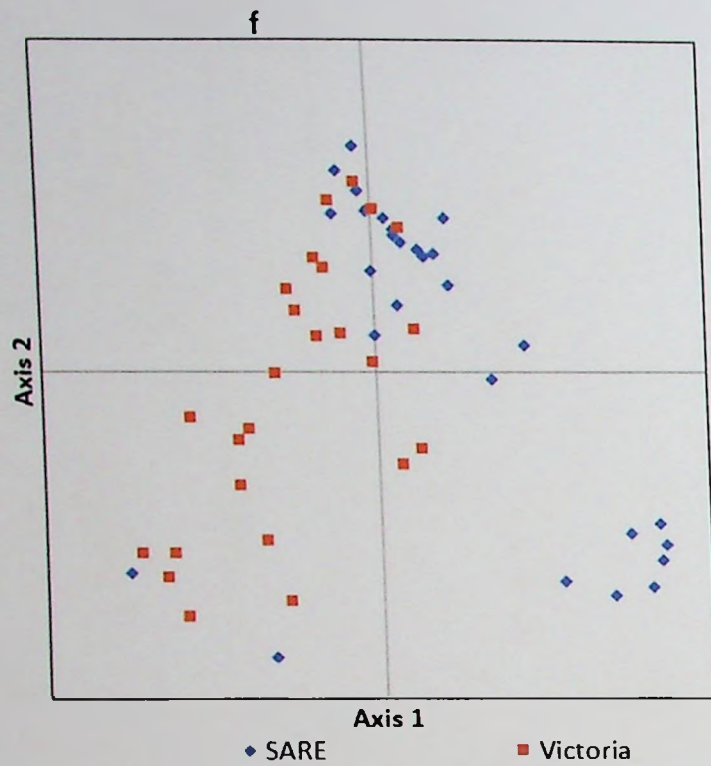


◆ *C. zillii* ■ *O. esculentus* ▲ *O. leucostictus*





◆ Dunga ■ Luanda ▲ Mbita × Siungu × Seka-Bay ● Usenge + Usoma



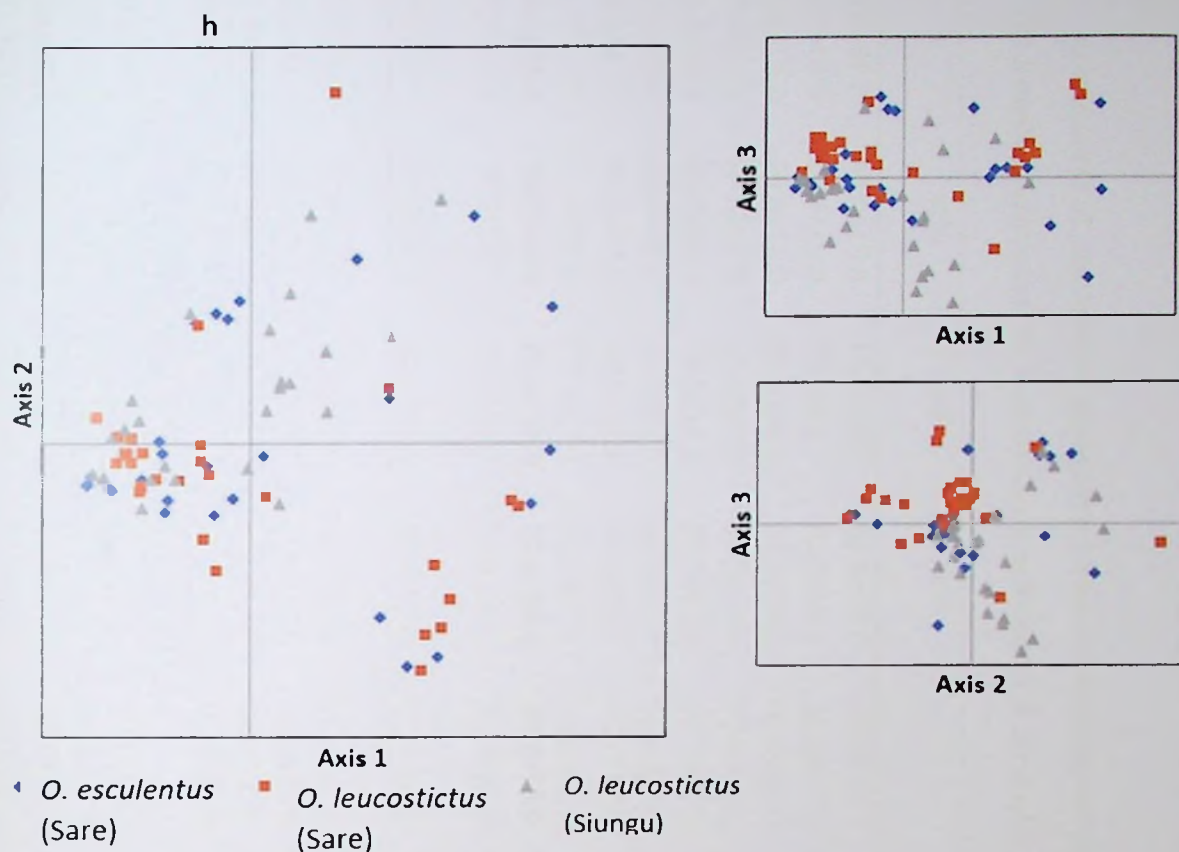


Figure 8: Scatter plots showing the tilapiine species collected during the study

a; All tilapiines, **b**; *Oreochromis* species, **c**; Other tilapiines excluding *O. niloticus*, **d**; *Oreochromis* species from Lake Sare, **e**; *O. niloticus* populations, **f**; *O. leucostictus* populations, **g**; *Oreochromis* species from Siungu beach, **h**; Comparison of Siungu and Sare samples. All the results are based on Principal component analysis.

Table 4: Heterozygosity, F-statistics, and Polymorphism by population for codominant data for tilapiines a; Tilapiines, b; *Oreochromis* species, c; Tilapiines excluding *O. niloticus*, d; *Oreochromis* species from Lake Sare, e; *O. niloticus* populations, f; *O. leuocostictus* (Mean $\pm$ SE).

Species	N	Na	Ne	I	Ho	He	uHe	F
a								
<i>O. niloticus</i>	88.79 $\pm$ 3.66	11.52 $\pm$ 1.25	3.99 $\pm$ 0.56	1.45 $\pm$ 0.13	0.42 $\pm$ 0.04	0.61 $\pm$ 0.04	0.61 $\pm$ 0.04	0.35 $\pm$ 0.04
<i>C. zillii</i>	33.14 $\pm$ 2.11	5.28 $\pm$ 0.54	2.24 $\pm$ 0.19	0.89 $\pm$ 0.10	0.35 $\pm$ 0.06	0.44 $\pm$ 0.05	0.47 $\pm$ 0.06	0.37 $\pm$ 0.09
<i>O. esculentus</i>	18.10 $\pm$ 1.29	2.83 $\pm$ 0.42	1.72 $\pm$ 0.17	0.58 $\pm$ 0.10	0.28 $\pm$ 0.06	0.32 $\pm$ 0.05	0.33 $\pm$ 0.06	0.07 $\pm$ 0.08
<i>O. leuocostictus</i>	37.83 $\pm$ 2.64	3.03 $\pm$ 0.46	1.69 $\pm$ 0.17	0.57 $\pm$ 0.09	0.28 $\pm$ 0.05	0.31 $\pm$ 0.05	0.32 $\pm$ 0.05	0.14 $\pm$ 0.06
Average	44.47 $\pm$ 2.79	5.66 $\pm$ 0.49	2.41 $\pm$ 0.18	0.87 $\pm$ 0.06	0.33 $\pm$ 0.03	0.42 $\pm$ 0.03	0.43 $\pm$ 0.03	0.26 $\pm$ 0.04
b								
<i>C. zillii</i>	33.14 $\pm$ 2.11	5.28 $\pm$ 0.54	2.24 $\pm$ 0.19	0.89 $\pm$ 0.11	0.35 $\pm$ 0.06	0.44 $\pm$ 0.05	0.47 $\pm$ 0.06	0.37 $\pm$ 0.09
<i>O. esculentus</i>	18.10 $\pm$ 1.29	2.83 $\pm$ 0.42	1.72 $\pm$ 0.17	0.58 $\pm$ 0.10	0.28 $\pm$ 0.06	0.32 $\pm$ 0.05	0.33 $\pm$ 0.06	0.07 $\pm$ 0.08
<i>O. leuocostictus</i>	37.83 $\pm$ 2.64	3.03 $\pm$ 0.46	1.69 $\pm$ 0.17	0.57 $\pm$ 0.09	0.28 $\pm$ 0.05	0.31 $\pm$ 0.05	0.32 $\pm$ 0.05	0.14 $\pm$ 0.06
Average	29.69 $\pm$ 1.49	3.71 $\pm$ 0.29	1.88 $\pm$ 0.11	0.68 $\pm$ 0.06	0.30 $\pm$ 0.03	0.36 $\pm$ 0.03	0.37 $\pm$ 0.03	0.22 $\pm$ 0.05

Table 5: Summary of Chi-Square Tests for Hardy-Weinberg Equilibrium for *O. niloticus* populations

Populations	Name Loci												
	TI1_TG	TI12_TAC	TI13_ATG	TI15_TGC	TI16_AAC	TI17_GAA	TI18_ATCT	TI22_CTAT	TI24_TTAC	TI26_ACAA	TI27_TTTG	TI31_CTAAT	TI32_AAAAT
Dunga	0.000	0.009	0.396	0.000	0.000	0.036	0.000	0.209	0.153	0.023	0.000	0.000	0.084
	***	**	ns	***	***	*	***	ns	ns	*	***	***	ns
Luanda	0.001	0.001	0.078	0.000	0.000	0.065	0.084	0.033	0.013	0.026	0.991	0.014	0.037
	***	**	ns	***	***	ns	ns	*	*	*	ns	*	*
Vlbita	0.000	0.001	0.764	0.915		0.365	0.678	0.073	0.680	0.352	0.913	0.001	0.059
	***	***	ns	ns		ns	ns	ns	ns	ns	ns	***	ns
Iungu	0.338	0.351	0.682			0.116	0.423	0.593	0.963	0.046		0.137	0.451
	ns	ns	ns			ns	ns	ns	ns	*		ns	ns
Seika Bay	0.003	0.251	0.000	0.000	0.001	0.008	0.134	0.001	0.001	0.405	0.994	0.459	0.091
	**	ns	***	***	**	**	ns	***	***	ns	ns	ns	ns
Senge	0.000	0.393	0.001	0.006	0.001	0.000	0.430	0.082	0.186	0.076	0.999	0.000	0.435
	***	ns	***	**	***	***	ns	ns	ns	ns	ns	***	ns
Yoma	0.949	0.700		0.000	0.000	0.000	0.172	0.000	0.928	0.775	0.894	0.000	0.438
	ns	ns		***	***	***	ns	***	ns	ns	ns	***	ns

Populations	TI33_TTCAA	TI34_TCTCT	TI35_AAAAG	TI39_ATGG	TI4_GT	TI49_TGT	TI5_CA	TI51_TGT	TI52_TAT	TI55_TCTA	TI56_TGTT	TI57_TCCA	TI59_AGGA
Dunga	0.008 **	0.001 **	0.000 ***	0.000 ***	0.274 ns	0.000 ***	0.000 ***	0.002 **	0.008 **	0.001 ***	0.003 **	0.000 ***	0.291 ns
Luanda	0.727 ns	0.162 ns	0.059 ns	0.005 **	0.061 ns	0.002 **	0.369 ns	0.089 ns	0.054 ns	0.523 ns	0.019 *	0.001 ***	0.001 **
Mbita	0.570 ns	0.867 ns	0.302 ns	0.617 ns	0.102 ns	0.716 ns	0.759 ns	0.667 ns	0.054 ns	0.060 ns	0.038 *	0.901 ns	0.671 ns
Siungu	0.847 ns	0.366 ns	0.363 ns	0.540 ns	0.576 ns	0.576 ns	0.710 ns	0.414 ns	0.000 ns	0.137 ns	0.025 *	0.008 ns	0.120 ns
Seka Bay	0.975 ns	0.014 *	0.000 ***	0.067 ns	0.002 **	0.067 ns	0.118 ns	0.002 **	0.000 ***	0.256 ns	0.007 **	0.008 **	0.003 **
Usenge	0.058 ns	0.201 ns	0.000 ***	0.000 ***	0.019 *	0.066 ns	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.001 ***	0.147 ns	0.043 *
Usoma	0.173 ns	0.070 ns	0.022 *	0.001 **	0.157 ns	0.122 ns	0.649 ns	0.007 **	0.005 **	0.022 *	0.005 **	0.055 ns	0.055 ns

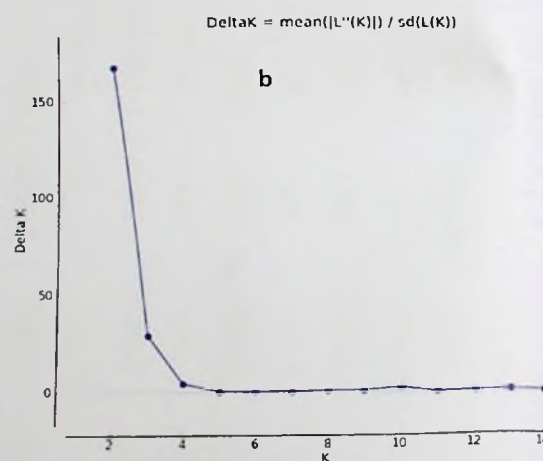
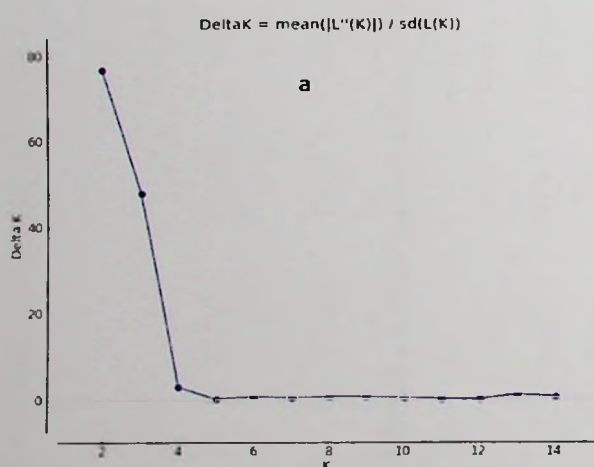
Key: ns=not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

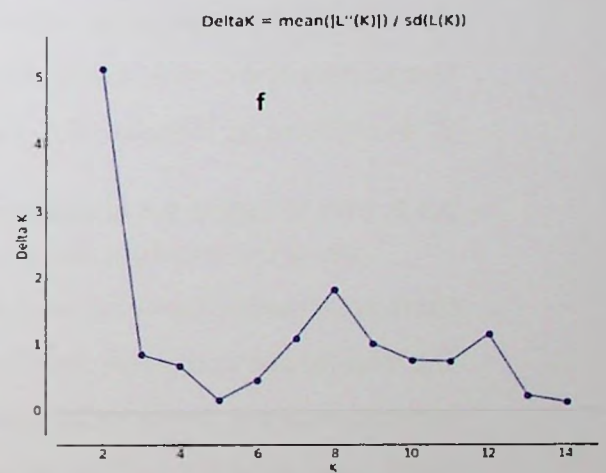
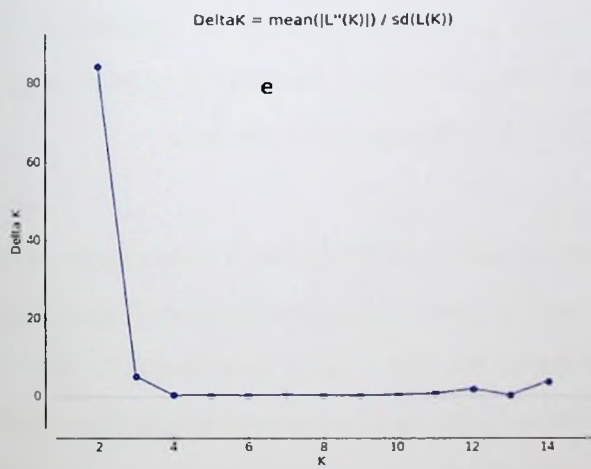
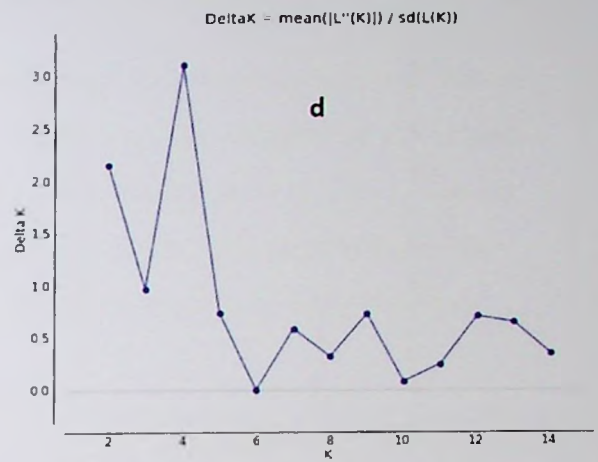
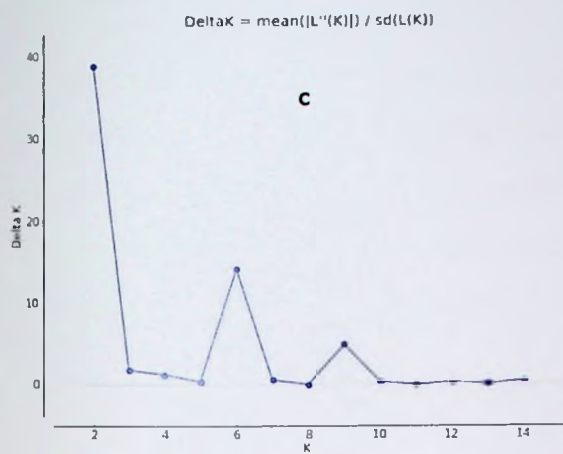
3.3 Sample cluster simulation using Structure harvester and STRUCTURE 2.3.4

3.3.1 Structure harvester outputs

The data preparation resulted in a codominant matrix of allele frequencies. This matrix served as an input for STRUCTURE v. 2.3.4. Results of cluster assignment probabilities were analysed using the Structure harvester (<http://taylor0.biology.ucla.edu/structureHarvester/>), an online program that validates multiple K values for maximum detection. The modal value of the distribution is the optimum K or the uppermost level of the structure. The K value stands for the number of genetic clusters that best fit the given datasets.

All the tilapiine samples, *Oreochromis* species, other tilapiines excluding *O. niloticus*, *O. niloticus*, *O. leuostictus*, Samples from Siungu and Species from Sare and Siungu collected during the study had a similar high K value ($k=2$) (Figures 9a, 9b, 9c, 9e, 9f, 9g and 9h). On the other hand, *Oreochromis* species from lake Sare had a higher $k=4$ which was high compared to other samples in the study (Figure 9d).





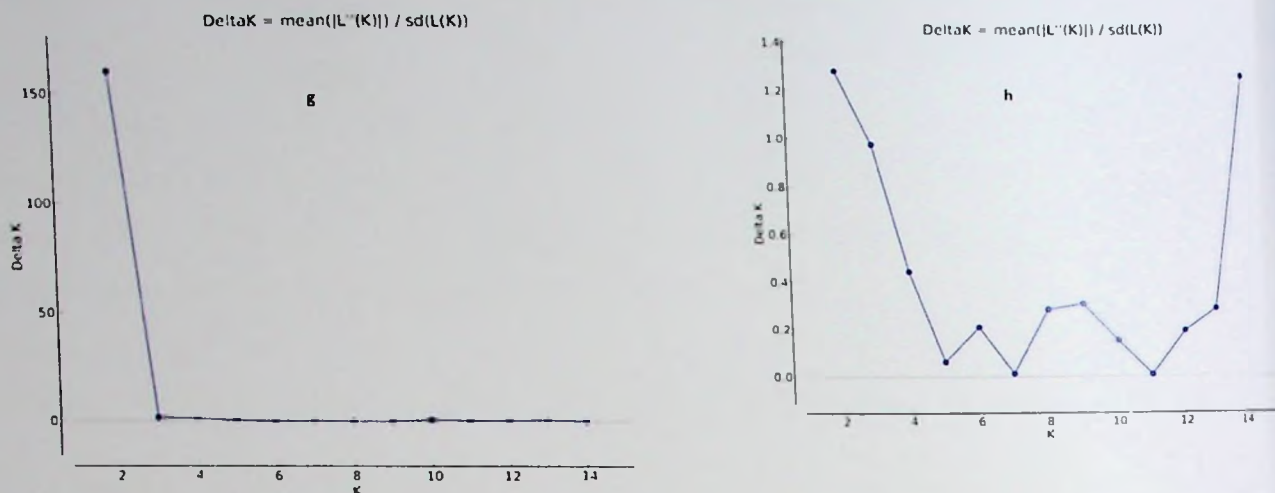


Figure 9: STRUCTURE HARVESTER plots showing K values for different tilapiines.

a; All tilapiine species, b; *Oreochromis* species, c; Other tilapiines (*O. esculentus*, *O. leucostictus* and *C. zillii*), d; *Oreochromis* species from Lake sare, e; *O. niloticus* populations; f; *O. leucostictus*, g; Samples from siungu, h; Samples from Siungu and Lake Sare

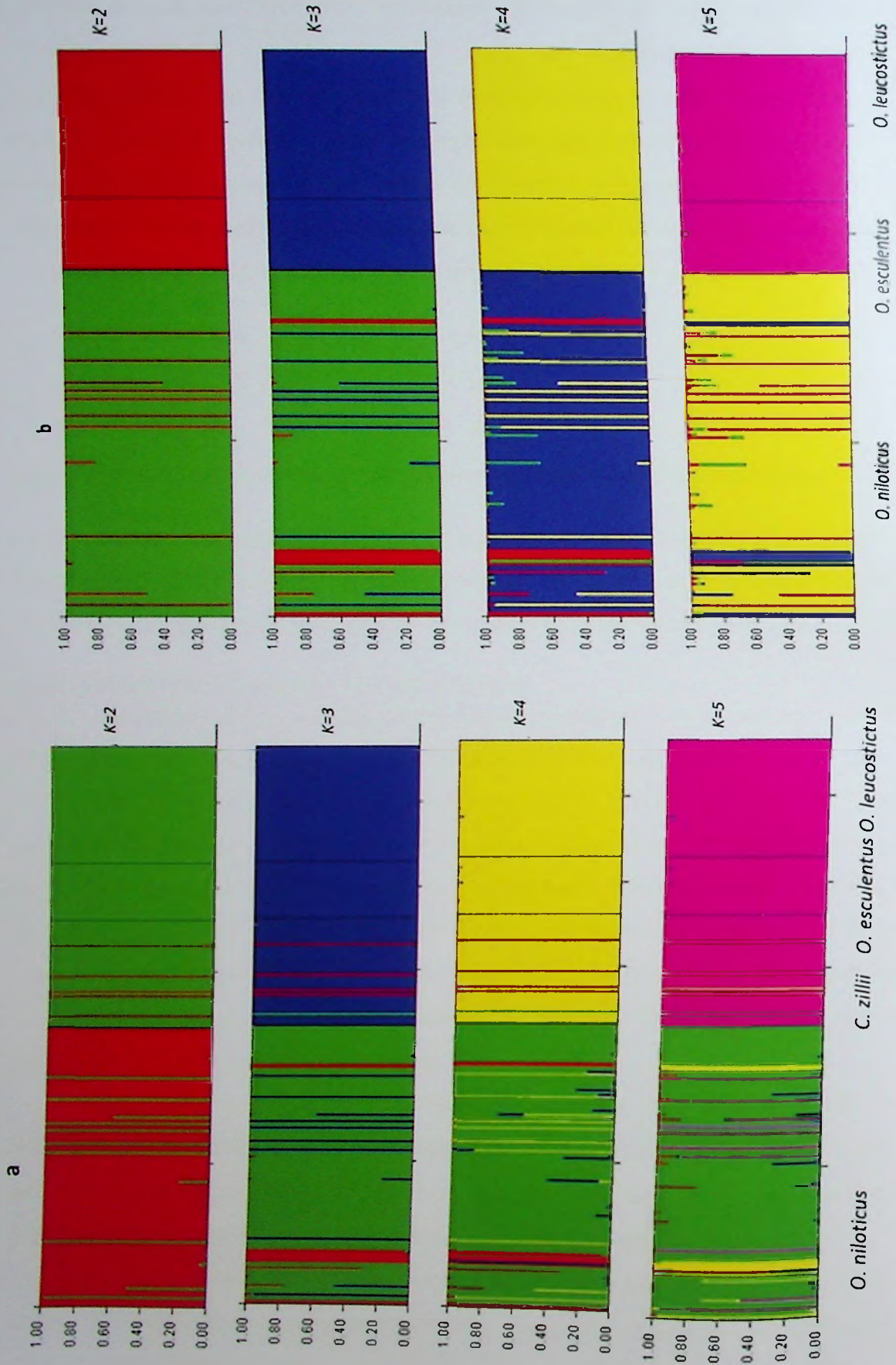
3.3.2. STRUCTURE 2.3.4 outputs for the tilapiines

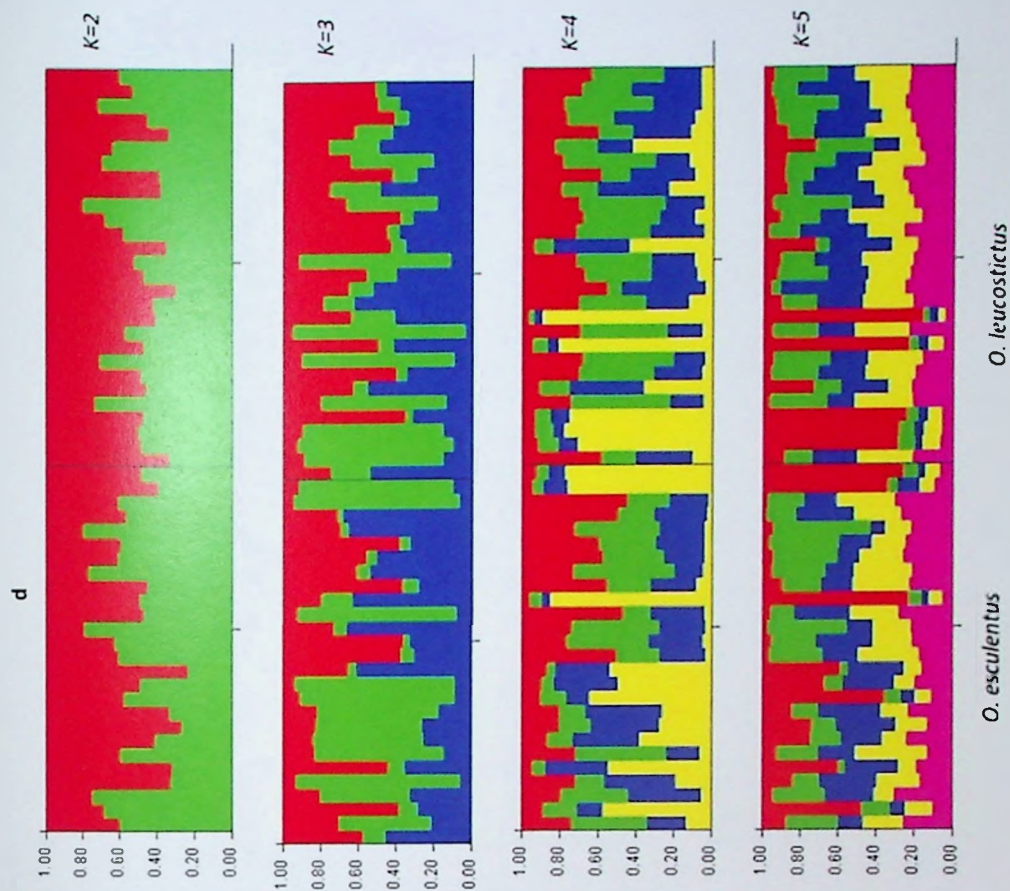
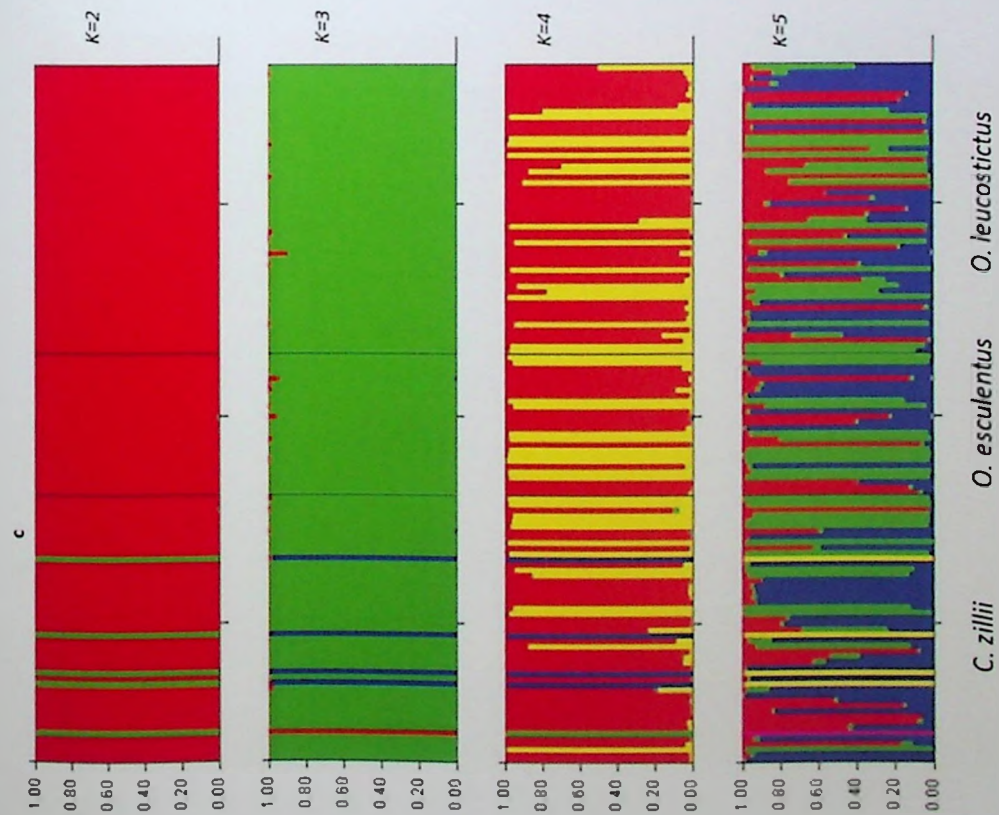
Using the structure analysis, all the tilapiines collected formed two main clusters with some individuals from *O. niloticus* populations having some degree of admixture (Figure 10a). The results are congruent with the PCoA in figure 8a. The cluster containing *C. zillii*, *O. esculentus* and *O. leucostictus* could not be differentiated. In pretty much all analyses, there are *O. niloticus* and *C. zillii*, individuals that are assigned to the other clusters (Figure 10a) and this is congruent with PCoA results in figure 8a. *Oreochromis* species collected during the study also formed two independent clusters with the cluster one containing *O. esculentus* and *O. leucostictus* while cluster two contained *O. niloticus* (Figure 10b), these results are congruent with the PCoA outputs in figure 8b.

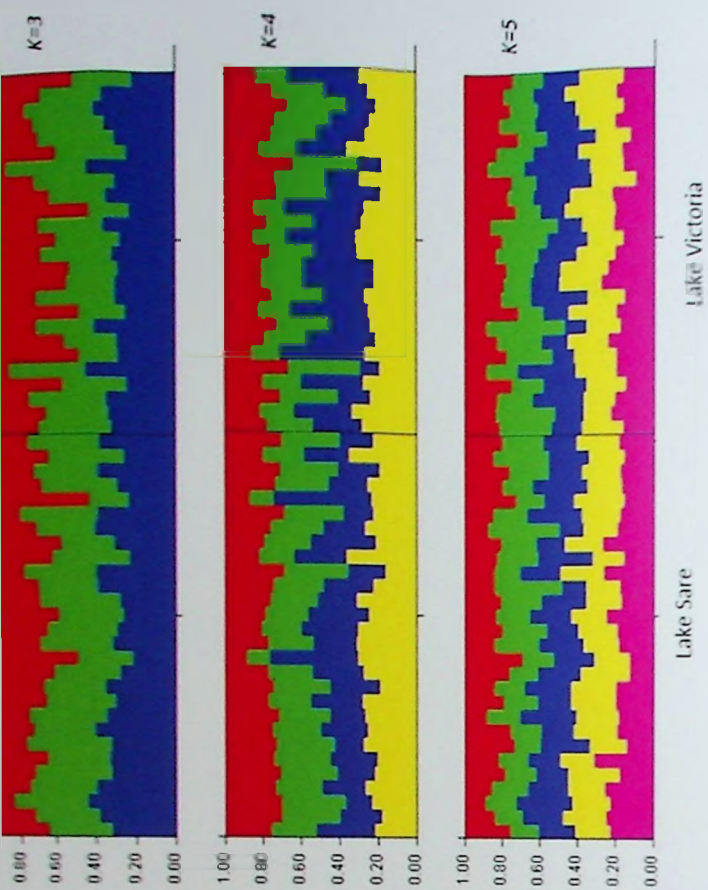
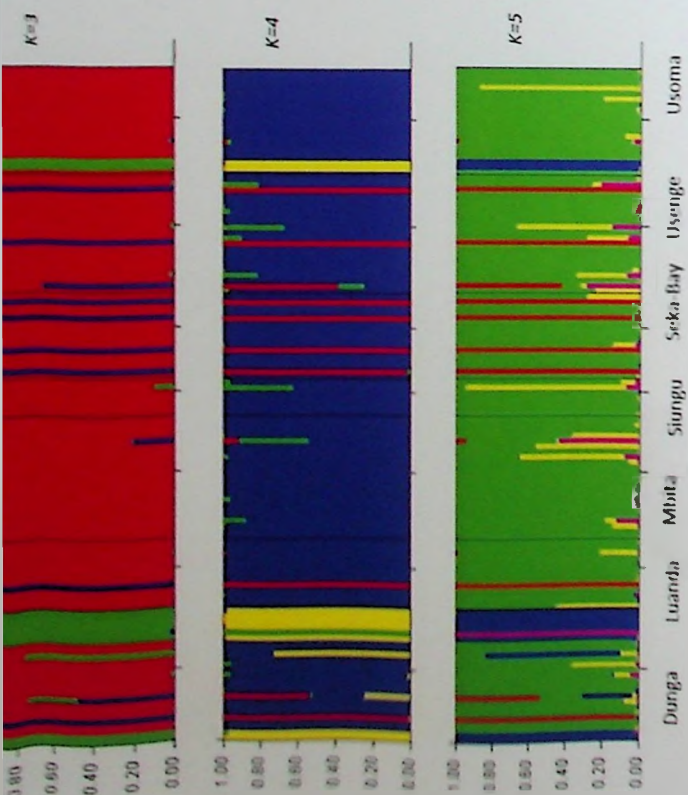
Considering the tilapiines excluding *O. niloticus*, the species could not be differentiated, and they showed some degree of admixtures at K=4 and 5 (Figure 10c). The same case was observed in *Oreochromis* species from Lake sare that could not be differentiated beyond K=2 and showed higher degree of admixtures (Figure 10d). The populations of *O. niloticus* from Dunga, Luanda, Seka-Bay and Usenge showed some degree of admixture while samples from Mbita, Siungu and Usoma beaches showed a pure

population (Figure 10e). Samples of *O. leucostictus* from both lakes did not show any clustering beyond $K=2$ (Figure 10f).

Oreochromis species from Siungu beach were differentiated with two clusters and this is congruent with results of PCoA in figure 8g. Degree of admixtures were observed at $k=3$ and above (Figure 10g). Comparing the *Oreochromis* species from Siungu and Sare, there was no differentiation at all (Figure 10h) which is also congruent with the PCoA results in figure 8h. Generally, all the structure outputs were congruent with the PCoA outputs using GenAlex.







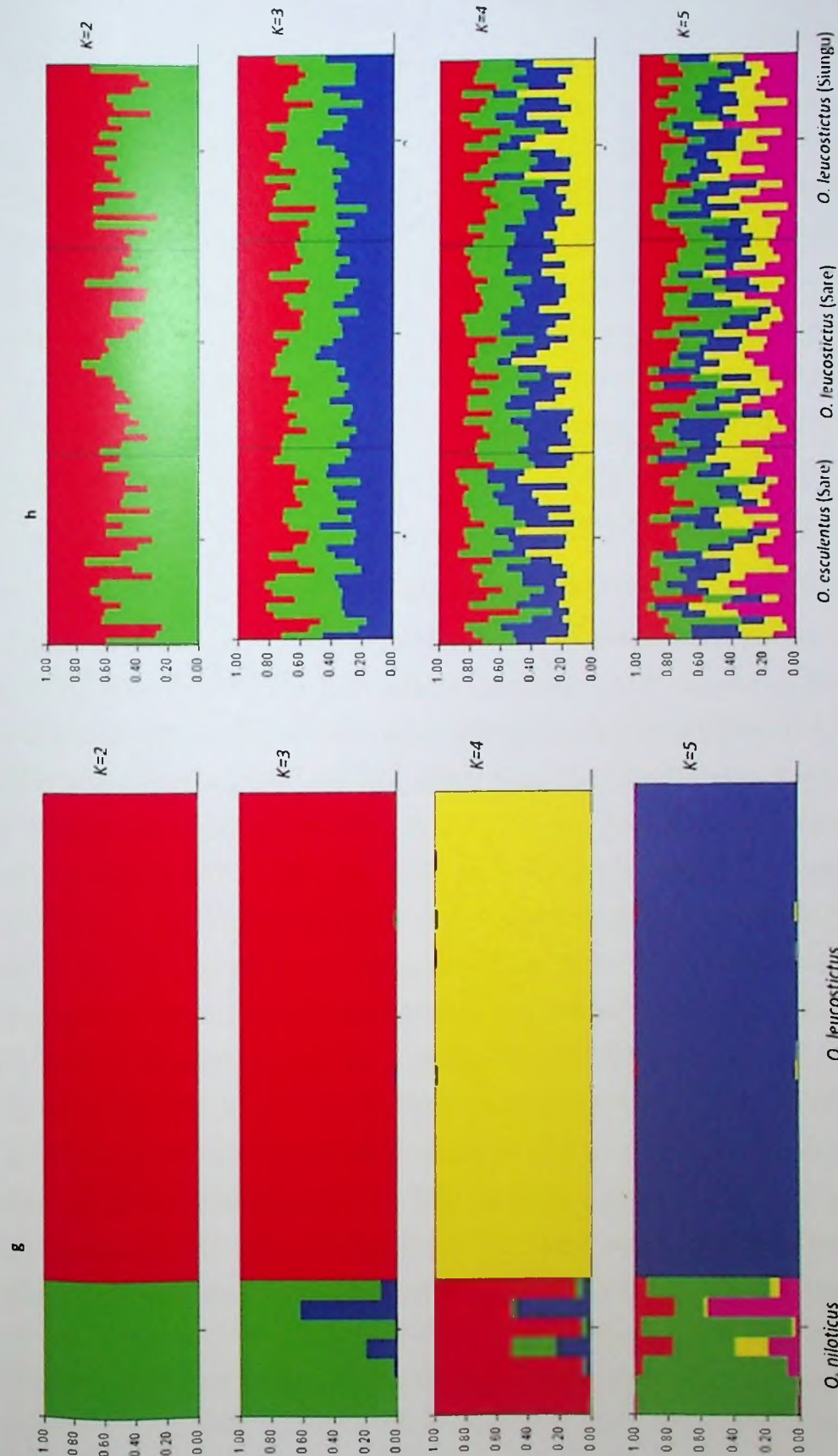


Figure 10: Bayesian structure analysis showing the clustering of different tilapia species. **a**; all tilapiines, **b**; *Oreochromis* species, **c**; Other tilapiines, **d**; Species from Sare, **e**; *O. niloticus*, **f**; *O. leucostictus*, **g**; Species from Siungu, **h**; Species from Siungu and Sare.

CHAPTER 4

4.0 DISCUSSION

Despite fisheries resources being useful, they are severely compromised by human induced activities that affect the sustainable utilization of such resources (Mboya et al., 2004). These anthropogenic activities include habitat destruction, overfishing and unregulated fish transfers (Eknath and Hulata, 2009). They have altered the natural genetic structure of different fish species through admixture and hybridization (Tibihika et al., 2020). Therefore, it is essential to understand the extent of genetic divergence of these fishes as this helps in the efficient management of wild fish populations and for aquaculture activities. This information can be achieved through differentiation of the tilapiines using highly informative genetic markers especially microsatellite genotyping.

4.1 Distribution of the tilapiines in the sampling sites

During sampling, few populations of *Oreochromis* species were collected especially *O. leucostictus* and *O. esculentus*. The low numbers could be attributed to factors like high fishing pressures, changes in water quality as well as interspecific breeding of *O. niloticus* with other *Oreochromis* species (Aloo, 2003). The higher fishing pressures and the introduction of the predatory Nile perch have drastically reduced the populations of the indigenous *Oreochromis* species especially *O. esculentus* and *O. variabilis* (Njiru et al., 2005). This explains why the study had few populations of *O. leucostictus* and *O. esculentus* (Hauser et al., 2002; Aloo, 2003). It is hypothesised that *O. niloticus* is native in places where Nile perch already existed so it may have adaptations to deal with its predation pressure that other species do not have. Notably, Interspecific breeding of the *O. niloticus* with other species results into hybrids that resemble more of *O. niloticus* than either of the other *Oreochromis* species, because of this they are hard to detect and therefore their numbers are underestimated (Champneys et al., 2020). Secondly, the higher pollution levels on Lake Victoria have deteriorated the water quality thus replacing the diatoms which is a major food for the native tilapiines with cyanobacteria that are less palatable, and this has hindered the survival of the indigenous fishes (Aloo, 2003; Njiru et al., 2012).

4.2 Genetic differentiation between the different tilapiines in the sampling sites

4.2.1 All tilapiines

Heterozygosity is used to compare the amount of genetic variation within different populations (Nagy et al., 2012; Gu et al., 2014; Chesnokov and Artemyeva, 2015). In the current study, the overall observed heterozygosity was lower than the expected heterozygosity for most tested tilapiines. Many studies have indicated that factors like presence of null alleles, sample size, inbreeding levels of the different tilapiines as well as Wahlund (1928) effect lowers the observed heterozygosity (Nei et al., 1975; D'amato et al., 2007; Kajungiro et al., 2019).

Studies by Kajungiro et al. 2019 observed lower heterozygosity in *O. niloticus* populations and attributed this to the small sample size which was used to infer the findings. In the current study, the obtained low heterozygosity of *O. leucostictus* and *O. esculentus* suggest low genetic variability for this species (López et al., 2007; Kajungiro et al., 2019) and this could be attributed to bottlenecks caused by fast reduction of population size due to predations and overfishing.

Contrary to our findings, studies by Angienda et al. (2011) obtained a higher observed heterozygosity in Lake Kanyaboli compared to our findings where the observed heterozygosity was lower in Lake Sare. The differences could be attributed to the types of microsatellite markers used, may be the markers used by Angienda et al. (2011) were more variable than the markers used in the current study. Secondly, Lake Sare being smaller than Kanyaboli limits the population size of the fish fauna thus low genetic diversity (Aloo, 2003). In the current study, the larger population sizes of *O. niloticus* populations generally translates to higher genetic diversity relative to other tilapiines (Hauser & Carvalho, 2008; Martinez et al., 2018). This indicates that the *O. niloticus* population is not affected by smaller amounts of genetic drift as populations are generally bigger thus having higher effective population size and consequently higher genetic diversity. (April et al., 2012; Martinez et al., 2018).

Various approaches using both multivariate analysis; Principal Coordinates Analysis (PCoA) and Bayesian clustering algorithms (STRUCTURE) were used in this study to evaluate genetic structure of the tilapiines. The results of PCoA showed two main clusters with *O. niloticus* populations forming an independent cluster while other species (*O. esculentus*, *O. leucostictus* and *C. zillii*) formed another cluster that could not be differentiated. The genetic

variation can be attributed to the differences in mutation, selection associated with the evolutionary history of populations as well as drift and migration linked with the effects of fragmentation of populations and their demographic background (Martinez et al., 2018). For example, *O. niloticus* is primarily a phytoplankton feeder and dominates areas of dense algal stocks (Jembe et al., 2006) while *O. esculentus* and *O. leucostictus* prefer habitats near papyrus fringes in littoral, shallow muddy bays, and lake inlets (Laurent et al., 2020). Probably such differences in habitat isolation contributes to the differences in PCoA results. Similarly, eight individuals of *O. niloticus* populations appeared in the cluster containing *O. esculentus* and *O. leucostictus* indicating that these individuals had some degree of admixture. The presence of *O. niloticus* populations in the other cluster could be due to the hybridization levels or potential misclassification of the species since admixed individuals resemble more *O. niloticus* and therefore they may have been misclassified (Angienda et al., 2011; Anane-Taabeah, 2019; Kariuku et al., 2021).

While hybridization events often occur among tilapiines following non-native species introductions into the natural environment, cases of hybridization between sympatric indigenous species are limited (Shechonge et al., 2018). In the study, the presence of the individuals of *O. niloticus* in a cluster containing *O. esculentus* and *O. leucostictus* indicates some degree of hybridization and this is supported by multiple studies (Mwanja and Kaufuma, 1995; Nyingi et al., 2009; Mwanja et al., 2010; Angienda et al., 2011; Firmat et al., 2013; Deines et al., 2014; Shechonge et al., 2018; Blackwell et al., 2020).

In Lake Victoria, studies by Mwanja and Kaufuma (1995) observed hybrids resulting from *O. niloticus* with *O. esculentus* in the satellite lakes concluding that no pure strains of *O. esculentus* existed. Nonetheless, findings by Angienda et al. (2011) discovered minimal nuclear gene transfer from *O. niloticus* to *O. esculentus* in Lake Kanyaboli and Namboyo, Kenya. This was attributed to the introduction of *O. niloticus* in Lake Kanyaboli with its high hybridization levels.

Study by Deines et al. (2014) found out that the native cichlids *Oreochromis macrochir* and *Oreochromis andersonii* hybridize in presence of the *O. niloticus*. The two native species do not naturally hybridise when in sympatry, suggesting that the presence of the non-native species facilitates hybridization events (Deines et al., 2014).

In Tanzania, studies by Blackwell et al. (2020) observed introgression levels between *Oreochromis niloticus* and *Oreochromis korogwe* Nambawala as well as hybrids between

Oreochromis urolepis and *Oreochromis korogwe* Mlingano. They attributed this to the introduced *Oreochromis niloticus* populations in Lake Nambawala that hybridized with the native species (Shechonge et al., 2019; Blackwell et al., 2020).

Similar cases of low hybridization levels between *Oreochromis niloticus* and *Oreochromis leucostictus* have been reported in Lake Naivasha Kenya (Ndiwa et al., 2014). Therefore, our results contribute further evidence to the hypothesis that there is a low level of introgression from *O. niloticus* into other *Oreochromis* species which likely threatens the conservation of this endangered species.

4.2.2 *Oreochromis* species from Lake Sare

Numerous studies on satellite lakes of Victoria basin have led to the discovery new fish species richness and genetic diversity which have not yet been sampled in the main Lake Victoria (Chapman et al., 2002; Mwanja, 2004; Abila et al., 2008). Sare; a satellite lake acts as a functional refugia for different fish fauna, it is connected to main Lake Victoria by extensive papyrus swamps that are anoxic to invasive predators like Nile perch and acts as well as entry of the *O. niloticus* species (Chapman et al., 1996; Abila et al., 2008). In the present study, the PCoA results showed clustering of the *Oreochromis* species into two groups that are close to each other with one group containing *O. esculentus*, another one containing *O. leucostictus* with a few samples of *O. esculentus*. The intermediate clustering between the two species could be associated with the hybridization which makes the species close to each other which makes them to occupy similar ecosystem (Angienda et al., 2011; Laurent et al., 2020; Kariuku et al., 2021). Studies show that *O. leucostictus* and *O. esculentus* prefer habitats near papyrus fringes in littoral, shallow muddy bays, and lake inlets (Laurent et al., 2020). Therefore, this enhances their ability to randomly mate thus admixtures among the species. Mwanja et al. (2010) observed that the populations of *O. esculentus* and *O. leucostictus* were close to each other than to *O. niloticus* and *O. variabilis*. This pattern of phylogenetically intermediates 'mixed' populations observed in the study provides strong evidence for the occurrence of hybridization and indicating the direction of introgression.

4.2.3 *Oreochromis niloticus* populations

Many loci showed significant deviations from the Hardy Weinberg Equilibrium in *O. niloticus* populations. The current findings are consistent with earlier findings in many strains of *Oreochromis* sp. (Bhassu et al., 2004; Li et al., 2008, Zhang et al., 2012). The deviation from

the HWE could be attributed to the presence of non-random mating, migration, hybridization, and selection or the existence of subpopulations (founder effect) (Frankham et al., 2002; Bhassu et al., 2004). Studies by Gu et al. (2014) state that selection has occurred in many *Oreochromis* species in China. Additionally, the wild *O. niloticus* populations have experienced higher fishing pressures thus many individuals are removed affecting the stability of the populations (Gu et al., 2014). Additionally, Bhassu et al. (2004) state that deviations from HWE in allele frequency is also related to sample size of the population. Carvalho and Hauser (1994) state that at least 50 individuals should be analysed to indicate the genotypic frequencies. In our study, due to sampling constraints, the maximum sample size we obtained was 30 for most of the populations. Thus, problems can arise when some of the measures of genetic distance used in the population studies are biased (they show systematic departures of the estimated mean from the true or expected value) at small sample sizes (Ruzzante, 1998). Thus, it is recommendable to increase the sample size for precise estimation of genetic distance and population structure (Ruzzante, 1998). Therefore, it is worth noting that these factors are affecting the *O. niloticus* populations in the sampled beaches of Lake Victoria.

Considering heterozygosity, the populations showed lower observed heterozygosity than expected heterozygosity. Many studies have stated that sample size, increased fishing pressure, mutation, migration, genetic drift, and selection affects heterozygosity of fish species (D'amato et al., 2007; Holsinger and Weir, 2009; Angienda et al., 2011; Bezault et al., 2011; Whitlock, 2011; Mireku et al., 2017). Secondly the presence of *O. niloticus* individuals with high degree of admixture or misassigned can also contribute to deviation from HWE. In the current study, few samples collected from Siungu, Luanda and Seka-Bay contributes to the low heterozygosity resulting into low genetic diversity. Therefore, the different subdivisions of *O. niloticus* populations from different beaches results to reduced genetic diversity due to their small size and genetic drift acting within each one of them (Mureki et al., 2017). Studies by Bezault et al. (2011) observed similar ranges of heterozygosity for the *O. niloticus* in the Kpando and Nyinuto portions of the Volta Lake. Gu et al. (2014) found that observed heterozygosity in six *Oreochromis* populations in the primary rivers of Guangdong province were lower than the expected heterozygosity. Studies by Hassanien and Gilbey (2005) also showed that the observed heterozygosity was lower than expected heterozygosity for all *O. niloticus* populations.

Contrary to our findings, Mireku et al. (2017) showed that observed heterozygosity ($H_o = 0.53$) of nine populations of *O. niloticus* in the Volta lake of Ghana was slightly higher than the expected heterozygosity ($H_e = 0.46$).

Results of PCoA indicate that *O. niloticus* populations from Usenge, Mbita, Siungu and Luanda clustered together while populations from Dunga and Usoma formed another cluster. The genetic structuring of the *O. niloticus* is attributed to the fact that the different beaches are geographically isolated from one another preventing movement of individuals between populations thus diminishing gene flow (Karn and Jasieniuk, 2017). Furthermore, there was strong genetic differentiation between Dunga and the four closely related populations of Usenge, Mbita, Siungu and Seka-Bay. The differences could be the result of geographical isolation which probably has act as a barrier to gene flow between those populations, leading to the suggested genetic structure that the STRUCTURE analysis revealed.

Since *O. niloticus* was introduced in 1940s, the results indicate that such durations could be sufficiently long enough for the introduced populations to have genetically diverged from one another through genetic drift. The strong founder effects during colonization into new habitats may also contribute to the substantial genetic differentiation among the populations.

Contrary, the admixture of the different populations in the beaches of Usenge and Siungu; Dunga and Usoma could be attributed to the random mating since the beaches are so close geographically by 15Km, uncontrolled movement of fish from one place to another as well as increased escapees from Aquaculture (Nyingi and Agnèsè, 2007; Nyingi et al., 2007; Ndiwa et al., 2014). The current study showed low genetic differentiation between the populations from Mbita and Usoma ($F_{st}=0.04$), Luanda and Mbita ($F_{st}=0.04$). The similarity among these populations is probably due to their origin from the same region of Lake Victoria as Luanda and Mbita are so close to each other geographically thus gene flow is expected to have happened among the admixed populations.

Secondly, it is likely that the unregulated translocations of fish in and outside of different beaches contributes to the suggested population admixture from Usenge, Mbita, Siungu and Luanda as they are closely clustered. All these confirm that there is human mediated geneflow between *O. niloticus* populations in the sampled beaches.

Notably, it is also likely that aquaculture activities might be contributing to the observed gene flow between the populations in these beaches. The increased aquaculture activities in the Rift Valley region have enhanced fish transfer from one drainage system to another and

allowing mixing between populations and or species (Ndiwa et al., 2014). The Economic stimulus program (ESP) introduced in 2009 by the Kenyan government has tremendously led to the establishment of many fishponds of which some are constructed near the wetlands, streams, rivers, and lakes (Munguti et al., 2014; Opiyo et al., 2018). During the heavy rains, the ponds get flooded leading to fish escape into Lake Victoria. Most of these ponds are not isolated from streams and wetlands, thus farmed fish can easily escape and *O. niloticus* hybridize with autochthonous (Angienda et al., 2011; Ndiwa et al., 2014). Therefore, all these factors confirm that there introgression levels exist within the *O. niloticus* populations.

4.2.4 *Oreochromis leucostictus*

In the present study, the distinct populations of *O. leucostictus* from Sare and Victoria could be attributed to the physical isolation created by the main road and swamp. The two lakes are separated by a natural wetland, the eco-physiological properties of these two lakes are different, which could also limit gene flow between the two populations by local adaptation or physiological barriers (Chapman et al., 2002; Crispo and Chapman, 2008). Although *O. leucostictus* from Victoria and Sare is generally differentiated based on the PCoA output, some samples occupied an intermediate position between the above-described 1st and 2nd clusters. Thus, it is clearly possible that multiple stockings/ fish transfers might have contributed to the gene-pool indicated by the intermediate populations. Secondly, since these lakes are so close to each other only separated by the main road and Yala swamp (Aloo, 2003). It is plausible that the fishermen can introduce the fish from one lake to another unintentionally and this results into inbreeding.

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Generally, expected heterozygosity was higher than observed heterozygosity throughout the samples. *Oreochromis niloticus* had higher heterozygosity, number of alleles, Shannon information index as well as number of expected alleles compared to other tilapiines. Increased fishing pressures and inbreeding levels of the different tilapiines lowers the genetic parameters.

The differentiation of the *O. niloticus* from other tilapiines implies the differences in the ecological requirements of the different species. On the other hand, the presence of *O. niloticus* populations in the other clusters indicates some degree of admixture. The results confirm the hypothesis that there is a low level of introgression from *O. niloticus* into other *Oreochromis* species like *O. esculentus*, and *O. leucostictus* which likely threatens the conservation of this endangered species.

With *O. niloticus* populations from different beaches, the results indicate that the genetic diversity and structure of *O. niloticus* populations from different beaches can be explained by their life history and geographical distribution. The close clustering of Usenge and Siungu, Mbita and Luanda populations and distinct separation of Dunga, suggests that these could be pure populations without admixture. The above should be taken into consideration in future wild populations conservation practices.

Factors like uncontrolled fish transfers and increased aquaculture activities resulting into fish escapes enhance the introgression and hybridization levels of the *Oreochromis* species as witnessed in the admixture of the different populations in Usenge and Siungu; Dunga and Usoma. Therefore, the findings add to a growing body of evidence that introduction of non-native fish species can lead to hybridization with indigenous species and threaten unique biodiversity.

5.2 Recommendations from the study that requires future research.

1. There is need to do a comparative study on the major lakes of East African countries to understand the extent of hybridization of the tilapiines in these natural environments. This would give a broader genetic diversity knowledge that could be used when designing conservation measures.
2. It is also prudent to do a general study on the hybridization levels of all tilapiines in the whole Lake Victoria to have a higher sample size as our study only focussed on Kenyan part.
3. Extensive research has been on the cichlid fishes in the greater lakes of East Africa (Victoria, Malawi, and Tanganyika). Among the cichlids, there is limited information on the hybridization of the different tilapiines. The study calls for extensive research on the tilapiines of greater lakes of East Africa.
4. Notwithstanding is the little knowledge available on the importance of the satellite lakes, yet they provide important refuges for the unique genetic diversity of fishes especially *O. esculentus* that are extinct in Lake Victoria. Therefore, there is need to do more research on the genetic diversity of the tilapiines in these satellite lakes.

5.3 Conservation recommendations

1. In situation where the endemic pure species co-exist with other introduced species, breeding actions of the native species should be enhanced.
2. Policies on the restrictions of fish translocations should be developed and implemented to avoid transfer of fish from one place to another without valid reason. This would help to prevent admixtures.
3. Zoning of aquacultural practices would also help to reduce on the spread of the invasive species in the wild. This strategy suggests that aquacultural and fisheries practices use only species that are native to each region.

6.0 REFERENCES

- Abila, R., Salzburger, W., Ndonga, M. F., Owiti, D. O., Barluenga, M., & Meyer, A. (2008). The role of the Yala swamp lakes in the conservation of Lake Victoria region haplochromine cichlids: Evidence from genetic and trophic ecology studies. *Lakes & Reservoirs: Research & Management*, 13(2), 95-104.
- Agnèse, J. F., Adépo-Gourène, B., Abban, E. K., & Fermon, Y. (1997). Genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae). *Heredity*, 79(1), 88-96.
- Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problems with hybrids: setting conservation guidelines. *Trends in ecology & evolution*, 16(11), 613-622.
- Aloo, P. A. (2002). Effects of climate and human activities on the ecosystem of Lake Baringo, Kenya. In *the East African great lakes: Limnology, palaeolimnology and biodiversity* (pp. 335-347). Springer, Dordrecht.
- Aloo, P. A. (2003). Biological diversity of the Yala Swamp lakes, with special emphasis on fish species composition, in relation to changes in the Lake Victoria Basin (Kenya): threats and conservation measures. *Biodiversity & Conservation*, 12(5), 905-920.
- Anane-Taabeah, G. (2019). *Characterization of the molecular genetic variation in wild and farmed Nile tilapia Oreochromis niloticus in Ghana for conservation and aquaculture development* (Doctoral dissertation, Virginia Tech).
- 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. *Conservation genetics*, 12(1), 243-255.
- Antoniou, A., Kasapidis, P., Kotoulas, G., Mylonas, C. C., & Magoulas, A. (2017). Genetic diversity of Atlantic Bluefin tuna in the Mediterranean Sea: insights from genome-wide SNPs and microsatellites. *Journal of Biological Research-Thessaloniki*, 24(1), 1-14.
- April, J., Hanner, R. H., Dion-Côté, A. M., & Bernatchez, L. (2013). Glacial cycles as an allopatric speciation pump in north-eastern American freshwater fishes. *Molecular Ecology*, 22(2), 409-422.
- Arcos-Burgos, M., & Muenke, M. (2002). Genetics of population isolates. *Clinical genetics*, 61(4), 233-247.

- Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A. and Johnson, E.A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PloS one*, 3(10), p.e3376.
- Balirwa, J. S. (1992). The evolution of the fishery of *Oreochromis niloticus* (Pisces: Cichlidae) in Lake Victoria. *Hydrobiologia*, 232(1), 85-89.
- 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, J. E., Abila, R., Grobler, J. P., Dangasuk, O. G., Njahira, M. N., & Kaunda-Arara, B. (2014). Genetic diversity and gene flow in *Clarias gariepinus* from Lakes Victoria and Kanyaboli, Kenya. *African Journal of Aquatic Science*, 39(3), 287-293.
- Basiita, R. K., Zenger, K. R., Mwanja, M. T., & Jerry, D. R. (2015). Development of genome-wide microsatellite genetic resources in a commercially important African freshwater fish species-the Nile perch, *Lates niloticus*. *Animal genetics*, 46(3), 340.
- Basir, Z., & Peyghan, R. (2018). Study of the effect of poultry by-product replacement instead of fish meal on skin cell profile of Nile tilapia (*Oreochromis niloticus*). *Journal of the Persian Gulf*, 9(31), 53-60.
- Bezault, E., Balaesque, P., Toguyeni, A., Fermon, Y., Araki, H., Baroiller, J. F., & Rognon, X. (2011). Spatial and temporal variation in population genetic structure of wild Nile tilapia (*Oreochromis niloticus*) across Africa. *BMC genetics*, 12(1), 1-16.
- Bhassu, S., Yusoff, K., Panandam, J. M., Embong, W. K., Oyyan, S., & Tan, S. G. (2004). The genetic structure of *Oreochromis* spp. (Tilapia) populations in Malaysia as revealed by microsatellite DNA analysis. *Biochemical Genetics*, 42(7), 217-229.
- Blackwell, T., Ford, A. G., Ciezarek, A. G., Bradbeer, S. J., Gracida Juarez, C. A., Smith, A. M., Ngatunga, B.P., Shechonge, A., Tamatamah, R., Etherington, G. & Haerty, W. (2020). Newly discovered cichlid fish biodiversity threatened by hybridization with non-native species. *Molecular Ecology*.
- Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114-2120.
- Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I. & Corner, R. (2010). Aquaculture: global status and trends.

Philosophical Transactions of the Royal Society B: *Biological Sciences*, 365(1554), 2897-2912.

- Bradbeer, S. J., Harrington, J., Watson, H., Warraich, A., Shechonge, A., Smith, A., Tamatamah, R., Ngatunga, B.P., Turner, G.F. & Genner, M. J. (2019). Limited hybridization between introduced and Critically Endangered indigenous tilapia fishes in northern Tanzania. *Hydrobiologia*, 832(1), 257-268.
- Britton, J. R., Boar, R. R., Grey, J., Foster, J., Lugonzo, J., & Harper, D. M. (2007). From introduction to fishery dominance: the initial impacts of the invasive carp *Cyprinus carpio* in Lake Naivasha, Kenya, 1999 to 2006. *Journal of Fish biology*, 71, 239-257.
- Brown, J.K., Taggart, J.B., Bekaert, M., Wehner, S., Palaiokostas, C., Setiawan, A.N., Symonds, J.E. and Penman, D.J. (2016). Mapping the sex determination locus in the hāpuku (*Polyprion oxygeneios*) using ddRAD sequencing. *BMC genomics*, 17(1), pp.1-12.
- Bush, A., Hermoso, V., Linke, S., Nipperess, D., Turak, E., & Hughes, L. (2014). Freshwater conservation planning under climate change: demonstrating proactive approaches for Australian Odonata. *Journal of Applied Ecology*, 51(5), 1273-1281.
- Cai, J., Quagrainie, K., & Hishamunda, N. (2017). Social and Economic Performance of Tilapia Farming in Africa. *FAO Fisheries and Aquaculture Circular*, (C1130).
- Camargo, J. A., & Alonso, Á. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environment international*, 32(6), 831-849.
- Campbell, M.T., Bandillo, N., Al Shiblawi, F.R.A., Sharma, S., Liu, K., Du, Q., Schmitz, A.J., Zhang, C., Véry, A.A., Lorenz, A.J. & Walia, H. (2017). Allelic variants of OsHKT1; 1 underlie the divergence between indica and japonica subspecies of rice (*Oryza sativa*) for root sodium content. *PLoS genetics*, 13(6), p.e1006823.
- Candy, J. R., Campbell, N. R., Grinnell, M. H., Beacham, T. D., Larson, W. A., & Narum, S. R. (2015). Population differentiation determined from putative neutral and divergent adaptive genetic markers in Eulachon (*Thaleichthys pacificus*), an anadromous Pacific smelt. *Molecular Ecology Resources*, 15(6), 1421-1434.
- Canonico, G. C., Arthington, A., McCrary, J. K., & Thieme, M. L. (2005). The effects of introduced tilapias on native biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 15(5), 463-483.

- Carvalho, G. R., & Hauser, L. (1994). Genetic impacts of fish introductions: an African perspective. *Species Changes in African Lakes*. Chapman & Hall, London.
- Champneys, T., Genner, M. J., & Ioannou, C. C. (2020). Invasive Nile tilapia dominates a threatened indigenous tilapia in competition over shelter. *Hydrobiologia*, 1-16.
- Chapman, L. J., Chapman, C. A., & Chandler, M. (1996). Wetland ecotones as refugia for endangered fishes. *Biological Conservation*, 78(3), 263-270.
- Chapman, L. J., Chapman, C. A., Nordlie, F. G., & Rosenberger, A. E. (2002). Physiological refugia: swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 133(3), 421-437.
- Chemoiwa, E. J., Abila, R., Macdonald, A., Lamb, J., Njenga, E., & Barasa, J. E. (2013). Genetic diversity and population structure of the endangered ripon barbel, *Barbus altianalis* (Boulenger, 1900) in Lake Victoria catchment, Kenya based on mitochondrial DNA sequences. *Journal of Applied Ichthyology*, 29(6), 1225-1233.
- Crispo, E., & Chapman, L. J. (2008). Population genetic structure across dissolved oxygen regimes in an African cichlid fish. *Molecular Ecology*, 17(9), 2134-2148.
- D'Amato, M. E., Esterhuysen, M. M., Van Der Waal, B. C., Brink, D., & Volckaert, F. A. (2007). Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. *Conservation Genetics*, 8, 475-488.
- Deines, A. M., Bbole, I., Katongo, C., Feder, J. L., & Lodge, D. M. (2014). Hybridisation between native *Oreochromis* species and introduced Nile tilapia *O. niloticus* in the Kafue River, Zambia. *African Journal of Aquatic Science*, 39(1), 23-34.
- Dilyt , J., Sabatino, S., Godinho, R., & Brito, J. C. (2020). Diversification and gene flow of tilapia species driven by ecological changes in lowland and mountain areas of southern Mauritania. *Evolutionary Ecology*, 34(1), 133-146.
- Eccles, D.H., 1992. FAO species identification sheets for fishery purposes. Field guide to the freshwater fishes of Tanzania. Prepared and published with the support of the United Nations Development Programme (project URT/87/016). FAO, Rome. 145 p.
- Ekmath, A. E., & Hulata, G. (2009). Use and exchange of genetic resources of Nile tilapia (*Oreochromis niloticus*). *Reviews in Aquaculture*, 1(3-4), 197-213.

- Fatsi, P. S. K., Hashem, S., Kodama, A., Appiah, E. K., Saito, H., & Kawai, K. (2020). Population genetics and taxonomic signatures of wild Tilapia in Japan based on mitochondrial DNA control region analysis. *Hydrobiologia*, 847(6), 1491-1504.
- Firmat, C., Alibert, P., Losseau, M., Baroiller, J. F., & Schlieuwen, U. K. (2013). Successive invasion-mediated interspecific hybridizations and population structure in the endangered cichlid *Oreochromis mossambicus*. *PLoS One*, 8(5), e63880.
- Food and Agriculture Organization. (2020). Sustainability in action. *State of World Fisheries and Aquaculture*. Rome, 200.
- Froese, R., Tsikliras, A. C., & Stergiou, K. I. (2011). Editorial note on weight-length relations of fishes. *Acta Ichthyologica et Piscatoria*, 41(4), 261-263.
- Gherardi, F., Britton, J. R., Mavuti, K. M., Pacini, N., Grey, J., Tricarico, E., & Harper, D. M. (2011). A review of biodiversity in Lake Naivasha, Kenya: Developing conservation actions to protect East African lakes from the negative impacts of alien species. *Biological Conservation*, 144(11), 2585-2596.
- Goudswaard, P. C., Witte, F., & Katunzi, E. F. B. (2002). The tilapiine fish stock of Lake Victoria before and after the Nile perch upsurge. *Journal of Fish Biology*, 60(4), 838-856.
- Gu, D. E., Mu, X. D., Song, H. M., Luo, D., Xu, M., Luo, J. R., & Hu, Y. C. (2014). Genetic diversity of invasive *Oreochromis* sp.(tilapia) populations in Guangdong province of China using microsatellite markers. *Biochemical Systematics and Ecology*, 55, 198-204.
- Hamilton, M. G., Lind, C. E., Barman, B. K., Velasco, R. R., Danting, M., Jodecel, C., & Benzie, J. A. (2020). Distinguishing Between Nile Tilapia Strains Using a Low-Density Single-Nucleotide Polymorphism Panel. *Frontiers in genetics*, 11, 1434.
- Harrison, R. G., & Larson, E. L. (2014). Hybridization, introgression and the nature of species boundaries. *Journal of Heredity*, 105(S1), 795-809.
- Hassanien, H. A., & Gilbey, J. (2005). Genetic diversity and differentiation of Nile tilapia (*Oreochromis niloticus*) revealed by DNA microsatellites. *Aquaculture Research*, 36(14), 1450-1457.
- Hassanien, H. A., Elnady, M., Obeida, A., & Itriby, H. (2004). Genetic diversity of Nile tilapia populations revealed by randomly amplified polymorphic DNA (RAPD). *Aquaculture Research*, 35(6), 587-593.
- Hauser, L., Adcock, G. J., Smith, P. J., Ramírez, J. H. B., & Carvalho, G. R. (2002). Loss of microsatellite diversity and low effective population size in an overexploited

- population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences*, 99(18), 11742-11747.
- Hickley, P., Bailey, R., Harper, D. M., Kundu, R., Muchiri, M., North, R., & Taylor, A. (2002). The status and future of the Lake Naivasha fishery, Kenya. *Hydrobiologia*, 488(1-3), 181-190.
- Hohenlohe, P. A., Amish, S. J., Catchen, J. M., Allendorf, F. W., & Luikart, G. (2011). Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and west slope cutthroat trout. *Molecular ecology resources*, 11, 117-122.
- Hong, B., Swaney, D.P., Mörtz, C.M., Smedberg, E., Hägg, H.E., Humborg, C., Howarth, R.W., & Bouraoui, F. (2012). Evaluating regional variation of net anthropogenic nitrogen and phosphorus inputs (NANI/NAPI), major drivers, nutrient retention pattern and management implications in the multinational areas of Baltic Sea basin. *Ecological Modelling*, 227, pp.117-135.
- Horreo, J. L., Martinez, J. L., Ayllon, F., Pola, I. G., Monteoliva, J. A., Heland, M., & GARCIA-VAZQUEZ, E. V. A. (2011). Impact of habitat fragmentation on the genetics of populations in dendritic landscapes. *Freshwater Biology*, 56(12), 2567-2579.
- Hosoya, S., Kikuchi, K., Nagashima, H., Onodera, J., Sugimoto, K., Satoh, K., Matsuzaki, K., Yasugi, M., Nagano, A.J., Kumagayi, A., & Ueda, K. (2018). Assessment of genetic diversity in Coho salmon (*Oncorhynchus kisutch*) populations with no family records using ddRAD-seq. *BMC research notes*, 11(1), pp.1-5.
- Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular ecology resources*, 9(5), 1322-1332.
- IUCN (International Union for Conservation of Nature). (2014). IUCN Red List of threatened species. Available at: <http://www.iucnredlist.org/details/15457/0> (Accessed 2nd December 2020)
- Jembe, B. T., Boera, P. N., & Okeyo Owuor, J. B. (2006). Distribution and association of tilapiine unit stocks in the Lake Victoria catchment (Kenya).
- Kajungiro, R. A., Palaikostas, C., Pinto, F. A. L., Mmochi, A. J., Mtolera, M., Houston, R. D., & De Koning, D. J. (2019). Population structure and genetic diversity of Nile tilapia (*O. niloticus*) strains cultured in Tanzania. *Frontiers in genetics*, 10, 1269.

- 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 tilapiine species relevant for aquaculture. *Aquaculture*, 736501.
- Kateregga, E., & Sterner, T. (2009). Lake Victoria fish stocks and the effects of water hyacinth. *The Journal of Environment & Development*, 18(1), 62-78.
- Kayombo, S., & Jorgensen, S. E. (2006). Lake Victoria. *Experience and Lessons Learned Brief Dar es Salaam University, Dar es Salaam, Tanzania, kayombo@wrep.udsm.ac.tz & Royal Danish University of Pharmaceutical Sciences, Copenhagen, Denmark.*
- Keyombe, J. L. A., Kevin, O., Waithaka, E., Nicholas, O., Oscar, D., & Kyule, D. N. (2020). Some aspects of fish growth in Redbelly Tilapia (*Coptodon zilli*) and Largemouth Bass (*Micropterus salmoides*) in Lake Naivasha, Kenya. *Fisheries and Aquaculture Journal*, 11(3), 1d-1d.
- Keyombe, J. L., Andrew, Y. W., & Dalmas, O. O. (2017). Comparative assessment of diet and condition factor of *Cyprinus carpio* and *Oreochromis leucostictus* in Lake Naivasha, Kenya. *International Journal of Aquatic Biology*, 5(3), 228-335.
- Kigano, S. (2016). *Genetic diversity, population structure and morphological characterization of the silver cyprinid Rastrineobola argentea (Pellegrin) in Port Victoria, Mbita and Nyanza Gulf of Lake Victoria (Kenya)* (Doctoral dissertation, Jomo Kenyatta University of Agriculture and Technology).
- Kocher, T. D., Lee, W. J., Sobolewska, H., Penman, D., & McAndrew, B. (1998). A genetic linkage map of a cichlid fish, the tilapia (*Oreochromis niloticus*). *Genetics*, 148(3), 1225-1232.
- Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular ecology resources*, 15(5), 1179-1191.
- Laugen, A.T., Engelhard, G.H., Whitlock, R., Arlinghaus, R., Dankel, D.J., Dunlop, E.S., Eikeset, A.M., Enberg, K., Jørgensen, C., Matsumura, S. & Nusslé, S. (2014). Evolutionary impact assessment: accounting for evolutionary consequences of fishing in an ecosystem approach to fisheries management. *Fish and Fisheries*, 15(1), pp.65-96.
- Laurent, C., Yongo, E., Waithaka, E., & Mutethya, E. (2020). Analysis of some biological aspects of the Blue-spotted tilapia, *Oreochromis leucostictus* (Trewavas, 1933) in Lake Naivasha, Kenya.

- Li, H. L., Gu, X. H., Li, B. J., Chen, C. H., Lin, H. R., & Xia, J. H. (2017). Genome-wide QTL analysis identified significant associations between hypoxia tolerance and mutations in the GPR132 and ABCG4 genes in Nile tilapia. *Marine biotechnology*, 19(5), 441-453.
- Martinez, A. S., Willoughby, J. R., & Christie, M. R. (2018). Genetic diversity in fishes is influenced by habitat type and life-history variation. *Ecology and evolution*, 8(23), 12022-12031.
- Martins, C., Oliveira, C., Wasko, A. P., & Wright, J. M. (2004). Physical mapping of the Nile tilapia (*Oreochromis niloticus*) genome by fluorescent in situ hybridization of repetitive DNAs to metaphase chromosomes—a review. *Aquaculture*, 231(1-4), 37-49.
- Martins, C., Wasko, A. P., Oliveira, C., & Wright, J. M. (2000). Nucleotide sequence of 5S rDNA and localization of the ribosomal RNA genes to metaphase chromosomes of the tilapiine cichlid fish, *Oreochromis niloticus*. *Hereditas*, 133(1), 39-46.
- Matsuishi, T., Muhoozi, L., Mkumbo, O., Budeba, Y., Njiru, M., Asila, A., Othina, A. and Cowx, I.G., 2006. Are the exploitation pressures on the Nile perch fisheries resources of Lake Victoria a cause for concern?. *Fisheries Management and Ecology*, 13(1), pp.53-71.
- Mboya, D. O., Manyala, J. O., & Ngugi, C. C. (2004). Fish introductions and their impact on the biodiversity and the fisheries of Lake Victoria.
- Mengistu, S., Komen, J., Benzie, J. A., Khaw, H. L., & Mulder, H. A. (2018). Genotype by environment interaction for growth and survival of Nile tilapia in aerated and non-aerated ponds. In *Session abstracts ISGA 2018*.
- Meyer, B. S., Matschiner, M., & Salzburger, W. (2015). A tribal level phylogeny of Lake Tanganyika cichlid fishes based on a genomic multi-marker approach. *Molecular phylogenetics and evolution*, 83, 56-71.
- Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., Islam, K., & Latif, M. A. (2013). A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International journal of molecular sciences*, 14(11), 22499-22528.
- Mireku, K. K., Kassam, D., Changadeya, W., Attipoe, F. Y. K., & Adinortey, C. A. (2017). Assessment of genetic variations of Nile Tilapia (*Oreochromis niloticus* L.) in the Volta Lake of Ghana using microsatellite markers. *African Journal of Biotechnology*, 16(7), 312-321.

- Moen, T., Agresti, J. J., Cnaani, A., Moses, H., Famula, T. R., Hulata, G., ... & May, B. (2004). A genome scan of a four-way tilapia cross supports the existence of a quantitative trait locus for cold tolerance on linkage group 23. *Aquaculture Research*, 35(9), 893-904.
- Munguti, J. M., Kim, J. D., & Ogello, E. O. (2014). An overview of Kenyan aquaculture: Status, challenges and opportunities for future development. *Fisheries and Aquatic sciences*, 17(1), 1-11.
- 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. *Aquatic Ecosystem Health & Management*, 13(4), 442-450.
- Mwanja, W., Booton, G. C., Kaufman, L., Chandler, M., Fuerst, P., Donaldson, E., & MacKinlay, D. (1996). Population and stock characterization of Lake Victoria. Tilapiine fishes based on RAPD markers. In *Aquaculture Biotechnology Symposium Proceedings of the International Congress on the Biology of Fishes (ed, by EM Donaldson & DD Mackinlay)* (pp. 115-124).
- Nagl, S., Tichy, H., Mayer, W. E., Samonte, I. E., McAndrew, B. J., & Klein, J. (2001). Classification and phylogenetic relationships of African tilapiine fishes inferred from mitochondrial DNA sequences. *Molecular phylogenetics and evolution*, 20(3), 361-374.
- Nagy, S., Poczai, P., Cernák, I., Gorji, A. M., Hegedűs, G., & Taller, J. (2012). PICcalc: an online program to calculate polymorphic information content for molecular genetic studies. *Biochemical genetics*, 50(9-10), 670-672.
- Ndiwa, T. C., Nyingi, D. W., & Agnese, J. F. (2014). An important natural genetic resource of *Oreochromis niloticus* (Linnaeus, 1758) threatened by aquaculture activities in Lobo drainage, Kenya. *PloS One*, 9(9), e106972.
- Nei, M., Maruyama, T., & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution*, 1-10.
- Njiru, M., Mkumbo, O. C., & van der Knaap, M. (2010). Some possible factors leading to decline in fish species in Lake Victoria. *Aquatic Ecosystem Health & Management*, 13(1), 3-10.
- Njiru, M., Nyamweya, C., Gichuki, J., Mugidde, R., Mkumbo, O., & Witte, F. (2012). Increase in anoxia in Lake Victoria and its effects on the fishery. *Anoxia*, 99-128.

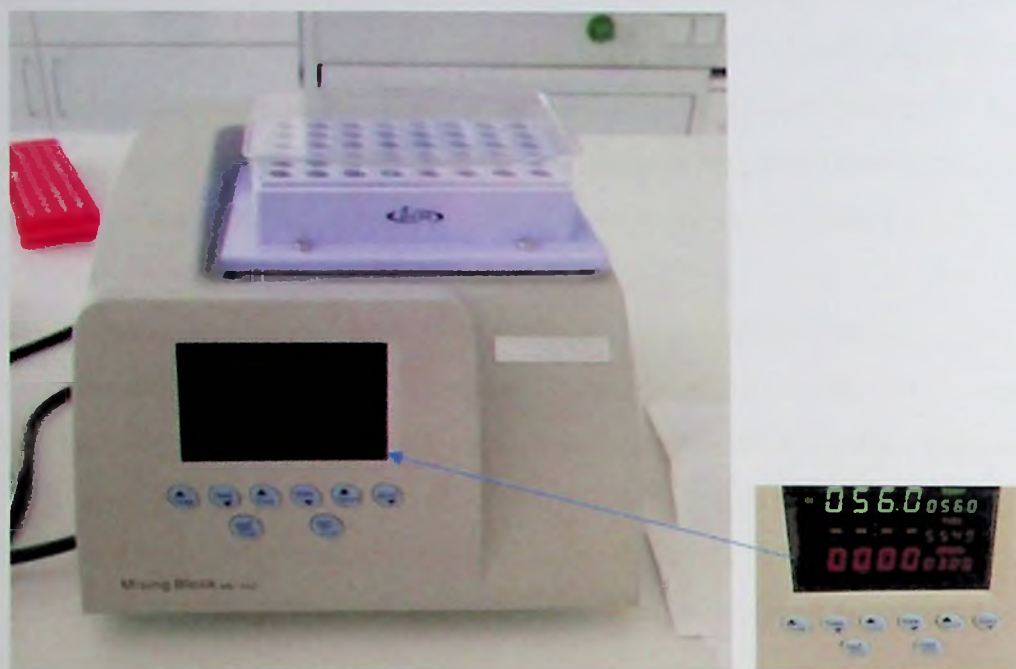
- Njiru, M., Waithaka, E., Muchiri, M., Van Knaap, M., & Cowx, I. G. (2005). Exotic introductions to the fishery of Lake Victoria: What are the management options?. *Lakes & Reservoirs: Research & Management*, 10(3), 147-155.
- Ogutu-Ohwayo, R. (1990). The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*. *Environmental biology of fishes*, 27(2), 81-96.
- Ogutu-Ohwayo, R., & Hecky, R. E. (1991). Fish introductions in Africa and some of their implications. *Canadian Journal of Fisheries and Aquatic Sciences*, 48(51), 8-12.
- 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. *African Journal of Aquatic Science*, 38(sup1), 1-14.
- Ojuok, J. E., Njiru, M., Ntiba, M. J., & Mavuti, K. M. (2007). The effect of overfishing on the life-history strategies of Nile tilapia, *Oreochromis niloticus* (L.) in the Nyanza Gulf of Lake Victoria, Kenya. *Aquatic Ecosystem Health & Management*, 10(4), 443-448.
- Okaronon, J. O., Katunzi, E. F. B., & Asila, A. A. (1998). Fish species identification guide for Lake Victoria: enumerator's field guide.
- Okwiri, B., Donde, O. O., & Kibet, C. J. (2019). Status and impacts of non-native freshwater fish on fisheries biodiversity and biogeography in Kenya: A management perspective. *Lakes & Reservoirs: Research & Management*, 24(4), 332-343.
- Opiyo, M. A., Marijani, E., Muendo, P., Odede, R., Leschen, W., & Charo-Karisa, H. (2018). A review of aquaculture production and health management practices of farmed fish in Kenya. *International Journal of Veterinary Science and Medicine*, 6(2), 141-148.
- Peakall, R. O. D., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular ecology notes*, 6(1), 288-295.
- Peter, B. M., & Slatkin, M. (2015). The effective founder effect in a spatially expanding population. *Evolution*, 69(3), 721-734.
- Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PloS one*, 7(5), e37135.

- Porras-Hurtado, L., Ruiz, Y., Santos, C., Phillips, C., Carracedo, Á., & Lareu, M. (2013). An overview of STRUCTURE: applications, parameter settings, and supporting software. *Frontiers in genetics*, 4, 98.
- Pullin, R. S., & Lowe-McConnell, R. H. (Eds.). (1982). *The Biology and Culture of Tilapias: Proceedings of the International Conference on the Biology and Culture of Tilapias, 2-5 September 1980 at the Study and Conference Center of the Rockefeller Foundation, Bellagio, Italy* (Vol. 7). WorldFish.
- Rezania, S., Ponraj, M., Din, M. F. M., Songip, A. R., Sairan, F. M., & Chelliapan, S. (2015). The diverse applications of water hyacinth with focus on sustainable energy and production for new era: An overview. *Renewable and Sustainable Energy Reviews*, 41, 943-954.
- Reznick, D. N., & Ghalambor, C. K. (2001). The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Microevolution rate, pattern, process*, 183-198.
- Rognon, X., & Guyomard, R. (2003). Large extent of mitochondrial DNA transfer from *Oreochromis aureus* to *O. niloticus* in West Africa. *Molecular Ecology*, 12(2), 435-445.
- Serrote, C. M. L., Reiniger, L. R. S., Silva, K. B., dos Santos Rabaiolli, S. M., & Stefanel, C. M. (2020). Determining the Polymorphism Information Content of a molecular marker. *Gene*, 726, 144175.
- Salzburger, W., & Meyer, A. (2004). The species flocks of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics. *Naturwissenschaften*, 91(6), 277-290.
- Shechonge, A., Ngatunga, B. P., Bradbeer, S. J., Day, J. J., Freer, J. J., Ford, A. G. P., & Genner, M. J. (2019). Widespread colonization of Tanzanian catchments by introduced *Oreochromis* tilapia fishes: The legacy from decades of deliberate introduction. *Hydrobiologia*, 832, 235-253.
- Shechonge, A., Ngatunga, B. P., Tamatamah, R., Bradbeer, S. J., Harrington, J., Ford, A. G. P., Turner, G. F., & Genner, M. J. (2018). Losing cichlid fish biodiversity: Genetic and morphological homogenization of tilapia following colonization by introduced species. *Conservation Genetics*, 19, 1199-1209.
- Smith, P. J., Francis, R. I. C. C., & McVeagh, M. (1991). Loss of genetic diversity due to fishing pressure. *Fisheries research*, 10(3-4), 309-316.

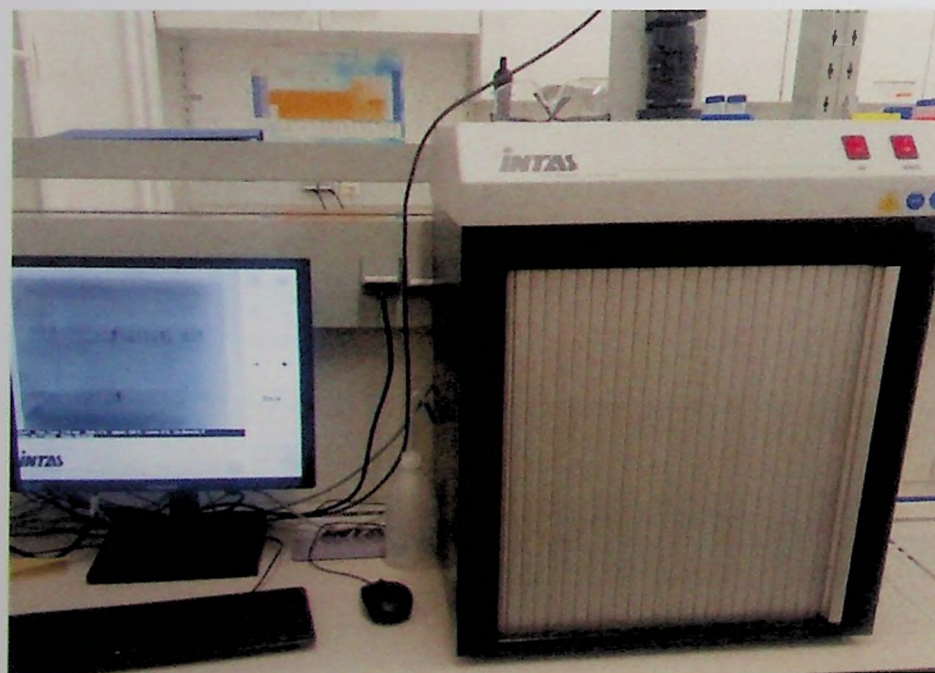
- Sodsuk, P., & McAndrew, B. J. (1991). Molecular systematics of three tilapine genera *Tilapia*, *Sarotherodon* and *Oreochromis* using allozyme data. *Journal of Fish Biology*, 39, 301-308.
- Soliman, T., Aly, W., Fahim, R. M., Berumen, M. L., Jenke-Kodama, H., & Bernardi, G. (2017). Comparative population genetic structure of redbelly tilapia (*Coptodon zillii* (Gervais, 1848)) from three different aquatic habitats in Egypt. *Ecology and evolution*, 7(24), 11092-11099.
- Taslima, K., Taggart, J. B., Wehner, S., McAndrew, B. J., & Penman, D. J. (2017). Suitability of DNA sampled from Nile tilapia skin mucus swabs as a template for ddRAD-based studies. *Conservation genetics resources*, 9(1), 39-42.
- 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 evolutionary biology*, 20(1), 16.
- 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*. *Conservation Genetics*, 20(2), 357-372.
- Tidwell, J. H., & Allan, G. L. (2001). Fish as food: aquaculture's contribution. *EMBO reports*, 2(11), 958-963.
- Todesco, M., Pascual, M. A., Owens, G. L., Ostevik, K. L., Moyers, B. T., Hübner, S. & Rieseberg, L. H. (2016). Hybridization and extinction. *Evolutionary applications*, 9(7), 892-908.
- Trewavas, E. (1982). Genetic groupings of *Tilapiines* used in aquaculture. *Aquaculture*, 27(1), 79-81.
- UN FAO. (2016). The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all.
- Villamagna, A. M., & Murphy, B. R. (2010). Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): a review. *Freshwater biology*, 55(2), 282-298.
- vonHoldt, B. M., Brzeski, K. E., Wilcove, D. S., & Rutledge, L. Y. (2018). Redefining the role of admixture and genomics in species conservation. *Conservation Letters*, 11(2), e12371.
- Williams, A. E., Duthie, H. C., & Hecky, R. E. (2005). Water hyacinth in Lake Victoria: Why did it vanish so quickly and will it return?. *Aquatic Botany*, 81(4), 300-314.

- Wilson, J.R., Ajuonu, O., Center, T.D., Hill, M.P., Julien, M.H., Katagira, F.F., Neuenschwander, P., Njoka, S.W., Ogwang, J., Reeder, R.H. & Van, T. (2007). The decline of water hyacinth on Lake Victoria was due to biological control by *Neochetina* spp. *Aquatic Botany*, 87(1),90-93.
- Witte, F., Wanink, J. H., Kishe-Machumu, M., Mkumbo, O. C., Goudswaard, P. C., & Seehausen, O. (2007). Differential decline and recovery of haplochromine trophic groups in the Mwanza Gulf of Lake Victoria. *Aquatic Ecosystem Health & Management*, 10(4), 416-433.
- Wolf, J. B., Bayer, T., Haubold, B., Schilhabel, M., Rosenstiel, P., & Tautz, D. (2010). Nucleotide divergence vs. gene expression differentiation: comparative transcriptome sequencing in natural isolates from the carrion crow and its hybrid zone with the hooded crow. *Molecular ecology*, 19, 162-175.
- Wu, L., & Yang, J. (2012). Identifications of captive and wild tilapia species existing in Hawaii by mitochondrial DNA control region sequence. *PLoS One*, 7(12), e51731.
- Zengeya, T., Ivey, P., Woodford, D.J., Weyl, O., Novoa, A., Shackleton, R., Richardson, D. & Van Wilgen, B. (2017). Managing conflict-generating invasive species in South Africa: Challenges and trade-offs. *Bothalia-African Biodiversity & Conservation*, 47(2),1-11.

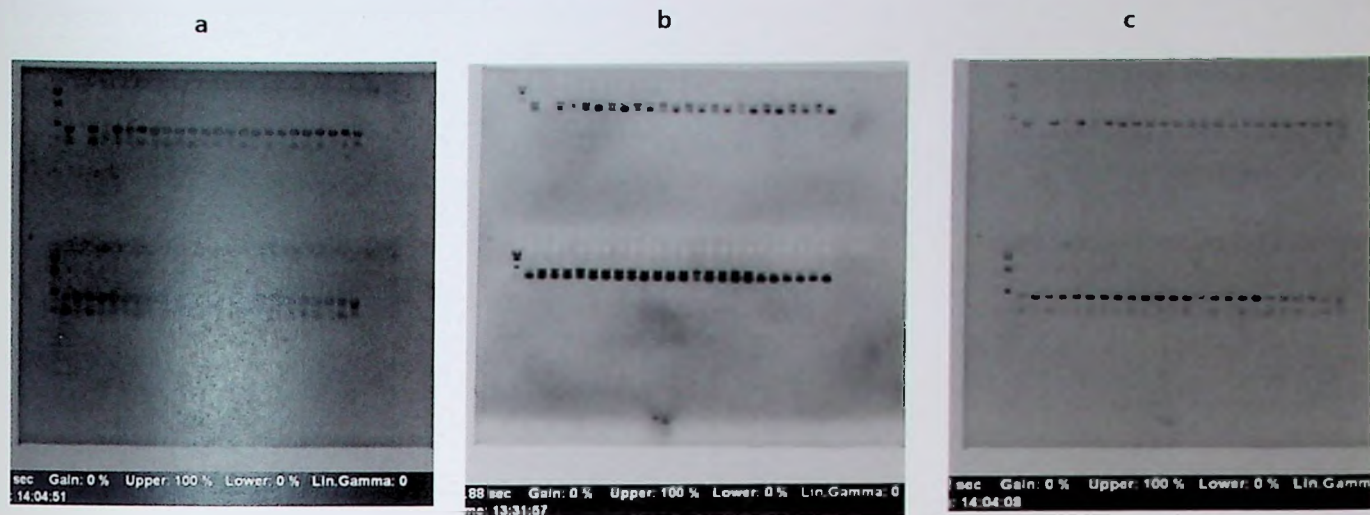
7.0 APPENDIX



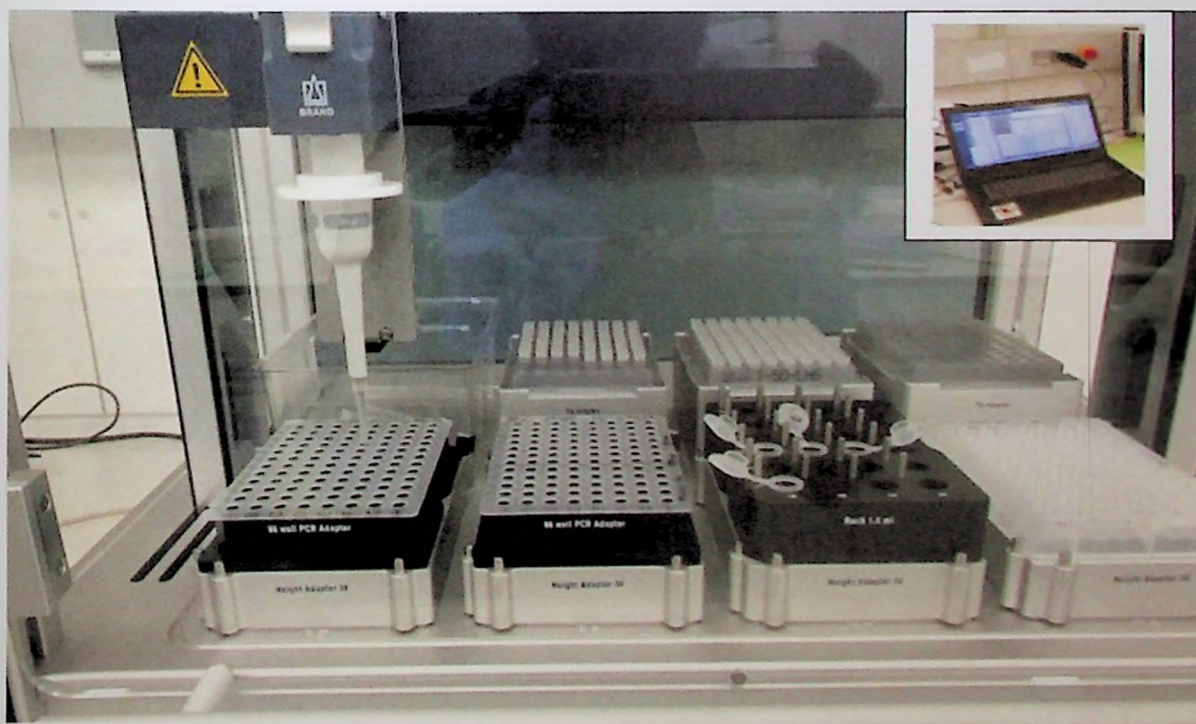
Appendix 1: Mixing block (PEQLAB) with calibrations



Appendix 2: Intas GEL IX IMAGER, Germany



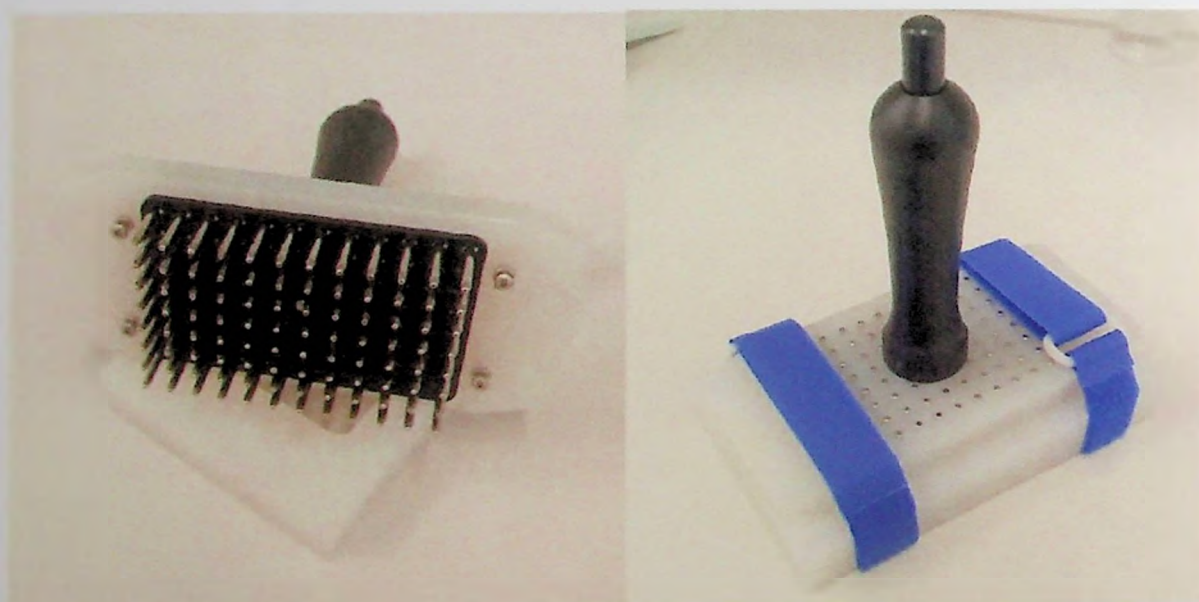
Appendix 3:DNA images visualized under INTAS Imaging Machine; a: Dark run, b: Normal run, C: Light run



Appendix 4:Liquid Handling Station robot with a data pool version 2.1.14



Appendix 5:T100 Thermocycler (Bio-Rad Laboratories, USA)



Appendix 6:Inverted magnetic bead extraction device VP 407-AM-N (V & P Scientific, Inc.)

Appendix 7:Evanno table showing the tilapiines from different beaches with best K-value highlighted in yellow

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	Delta K
1	13	-15993.038462	4.312296	—	—	—
2	13	-9990.130769	65.381208	6002.907692	5008.207692	76.600110
3	13	-8995.430769	20.347088	994.700000	976.489231	47.991597
4	15	-8977.220000	25.759803	18.210769	78.598462	3.051206
5	13	-9037.607692	160.085261	-60.387692	33.220000	0.207514
6	13	-9131.215385	125.747318	-93.607692	89.492308	0.711684
7	13	-9314.315385	377.125536	-183.100000	185.984615	0.493164
8	13	-9311.430769	230.883670	2.884615	169.607692	0.734602
9	13	-9478.153846	396.847445	-166.723077	265.176923	0.668209
10	13	-9379.700000	323.605202	98.453846	167.069231	0.516275
11	13	-9448.315385	332.844100	-68.615385	66.192308	0.198869
12	13	-9450.738462	296.925483	-2.423077	48.930769	0.164791
13	13	-9404.230769	351.025394	46.507692	417.376923	1.189022
14	13	-9775.100000	708.512322	-370.869231	517.707692	0.730697
15	13	-9628.261538	675.809917	146.838462	—	—

Appendix 8:Evanno table showing *Oreochromis* species from Lake Sare samples, best K-value highlighted in yellow

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	Delta K
1	13	-1363.792308	1.898008	—	—	—
2	14	-1396.907143	32.242076	-33.114835	69.354945	2.151069
3	13	-1499.376923	146.491508	-102.469780	142.254396	0.971076
4	13	-1459.592308	55.760776	39.784615	172.861538	3.100056
5	13	-1592.669231	184.361793	-133.076923	136.746154	0.741727
6	13	-1589.000000	169.784849	3.669231	0.138462	0.000816
7	13	-1585.469231	61.680148	3.530769	36.130769	0.585776
8	13	-1618.069231	129.724685	-32.600000	41.576923	0.320501
9	13	-1609.092308	55.851730	8.976923	40.607692	0.727062
10	13	-1640.723077	196.404040	-31.630769	15.538462	0.079115
11	13	-1656.815385	323.771272	-16.092308	80.107692	0.247421
12	13	-1592.800000	303.820056	64.015385	214.076923	0.704617
13	13	-1742.861538	349.098115	-150.061538	225.984615	0.647338
14	13	-1666.938462	182.057379	75.923077	63.876923	0.350861
15	13	-1654.892308	218.283735	12.046154	—	—

Appendix 9: Evanno table for *Oreochromis niloticus* populations from different beaches best k-value highlighted in yellow.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	Delta K
1	13	-7511.769231	2.741771	—	—	—
2	13	-5979.307692	15.731839	1532.461538	1326.646154	84.328738
3	13	-5773.492308	152.291215	205.815385	810.453846	5.321737
4	13	-6378.130769	2329.615473	-604.638462	855.538462	0.367244
5	13	-7838.307692	3979.088344	-1460.176923	1247.415385	0.313493
6	13	-8051.069231	5280.333776	-212.761538	1261.504103	0.238906
7	15	-7002.326667	2996.415211	1048.742564	1323.085128	0.441556
8	13	-7276.669231	1821.350721	-274.342564	508.211282	0.279030
9	13	-8059.223077	2340.708732	-782.553846	509.700000	0.217755
10	13	-9351.476923	2150.667433	-1292.253846	888.607692	0.413178
11	13	-11532.338462	4481.289234	-2180.861538	2935.646154	0.655090
12	13	-10777.553846	3313.648450	754.784615	5986.830769	1.806719
13	13	-16009.600000	4670.452195	-5232.046154	998.692308	0.213832
14	13	-20242.953846	6709.264431	-4233.353846	24872.215385	3.707145
15	13	-49348.523077	73538.706143	-29105.569231	—	—

Appendix 10: Table showing summary of Chi-Square Tests for Hardy-Weinberg Equilibrium for all tilapiines.

No. Loci	29
No. Samples	254
No. Pops.	4

Pop	Locus	DF	ChiSq	Prob	Signif
<i>O. niloticus</i>	TI1_TG	55	326.736	0.000	***
<i>O. niloticus</i>	TI12_TAC	15	103.748	0.000	***
<i>O. niloticus</i>	TI13_ATG	10	73.882	0.000	***
<i>O. niloticus</i>	TI15_TGC	21	397.597	0.000	***
<i>O. niloticus</i>	TI16_AAC	28	496.699	0.000	***
<i>O. niloticus</i>	TI17_GAA	105	733.500	0.000	***
<i>O. niloticus</i>	TI18_ATCT	171	479.817	0.000	***
<i>O. niloticus</i>	TI22_CTAT	351	1052.182	0.000	***

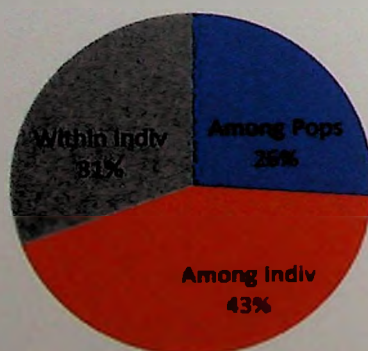
<i>O. niloticus</i>	TI24_TTAC	91	163.327	0.000	***
<i>O. niloticus</i>	TI26_ACAA	28	89.583	0.000	***
<i>O. niloticus</i>	TI27_TTTG	15	140.896	0.000	***
<i>O. niloticus</i>	TI31_CTAAT	105	575.183	0.000	***
<i>O. niloticus</i>	TI32_AAAAT	406	623.252	0.000	***
<i>O. niloticus</i>	TI33_TTCAA	36	35.816	0.477	ns
<i>O. niloticus</i>	TI34_TCTCT	210	701.824	0.000	***
<i>O. niloticus</i>	TI35_AAAAG	276	905.692	0.000	***
<i>O. niloticus</i>	TI39_ATGG	55	469.499	0.000	***
<i>O. niloticus</i>	TI4_GT	36	139.875	0.000	***
<i>O. niloticus</i>	TI49_TGT	55	284.763	0.000	***
<i>O. niloticus</i>	TI5_CA	36	148.948	0.000	***
<i>O. niloticus</i>	TI51_TGT	45	246.963	0.000	***
<i>O. niloticus</i>	TI52_TAT	28	178.983	0.000	***
<i>O. niloticus</i>	TI55_TCTA	105	329.948	0.000	***
<i>O. niloticus</i>	TI56_TGTT	10	110.190	0.000	***
<i>O. niloticus</i>	TI57_TCCA	3	54.094	0.000	***
<i>O. niloticus</i>	TI59_AGGA	15	79.196	0.000	***
<i>O. niloticus</i>	TI6_GA	36	193.290	0.000	***
<i>O. niloticus</i>	TI61_TGGA	28	286.004	0.000	***
<i>O. niloticus</i>	TI8_AC	15	220.972	0.000	***
<i>T. zilli</i>	TI1_TG	21	106.782	0.000	***
<i>T. zilli</i>	TI12_TAC	15	45.226	0.000	***
<i>T. zilli</i>	TI13_ATG	1	35.000	0.000	***
<i>T. zilli</i>	TI15_TGC	10	136.800	0.000	***
<i>T. zilli</i>	TI16_AAC	15	100.872	0.000	***
<i>T. zilli</i>	TI17_GAA	28	121.360	0.000	***
<i>T. zilli</i>	TI18_ATCT	21	69.799	0.000	***
<i>T. zilli</i>	TI22_CTAT	45	212.446	0.000	***
<i>T. zilli</i>	TI24_TTAC	1	41.000	0.000	***
<i>T. zilli</i>	TI26_ACAA	10	66.425	0.000	***

<i>T. zilli</i>	TI27_TTTG	3	36.416	0.000	***
<i>T. zilli</i>	TI31_CTAAT	28	184.113	0.000	***
<i>T. zilli</i>	TI32_AAAAT	28	76.682	0.000	***
<i>T. zilli</i>	TI33_TTCAA	1	1.000	0.317	ns
<i>T. zilli</i>	TI34_TCTCT	55	140.414	0.000	***
<i>T. zilli</i>	TI35_AAAAG	21	152.716	0.000	***
<i>T. zilli</i>	TI39_ATGG	45	368.121	0.000	***
<i>T. zilli</i>	TI4_GT	3	35.013	0.000	***
<i>T. zilli</i>	TI49_TGT	36	109.876	0.000	***
<i>T. zilli</i>	TI5_CA	3	82.000	0.000	***
<i>T. zilli</i>	TI51_TGT	3	46.250	0.000	***
<i>T. zilli</i>	TI52_TAT	1	32.000	0.000	***
<i>T. zilli</i>	TI55_TCTA	3	6.800	0.079	ns
<i>T. zilli</i>	TI56_TGTT	1	42.000	0.000	***
<i>T. zilli</i>	TI57_TCCA	1	42.000	0.000	***
<i>T. zilli</i>	TI59_AGGA	Monomorphic			
<i>T. zilli</i>	TI6_GA	15	37.013	0.001	**
<i>T. zilli</i>	TI61_TGGA	10	27.000	0.003	**
<i>T. zilli</i>	TI8_AC	21	91.205	0.000	***
<i>O. esculentus</i>	TI1_TG	6	4.759	0.575	ns
<i>O. esculentus</i>	TI12_TAC	6	5.577	0.472	ns
<i>O. esculentus</i>	TI13_ATG	Monomorphic			
<i>O. esculentus</i>	TI15_TGC	Monomorphic			
<i>O. esculentus</i>	TI16_AAC	1	6.644	0.010	**
<i>O. esculentus</i>	TI17_GAA	3	21.320	0.000	***
<i>O. esculentus</i>	TI18_ATCT	15	33.749	0.004	**
<i>O. esculentus</i>	TI22_CTAT	36	57.420	0.013	*
<i>O. esculentus</i>	TI24 TTAC	Monomorphic			
<i>O. esculentus</i>	TI26_ACAA	3	1.009	0.799	ns
<i>O. esculentus</i>	TI27_TTTG	1	0.198	0.656	ns
<i>O. esculentus</i>	TI31_CTAAT	10	31.155	0.001	***

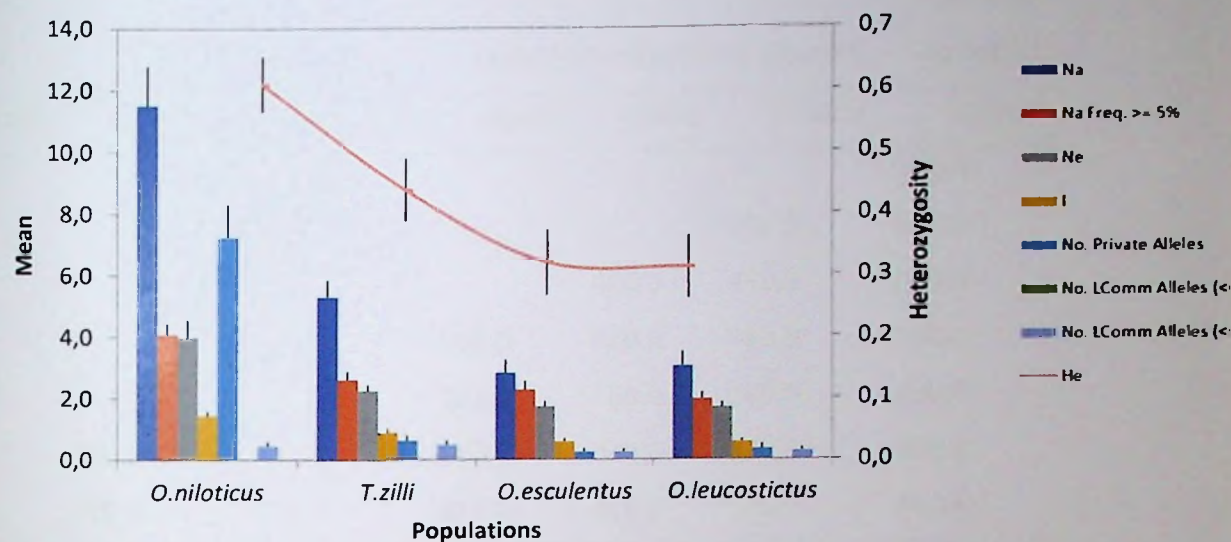
<i>O. esculentus</i>	TI32_AAAAT	6	6.768	0.343	ns
<i>O. esculentus</i>	TI33_TTCAA	Monomorphic			
<i>O. esculentus</i>	TI34_TCTCT	10	15.890	0.103	ns
<i>O. esculentus</i>	TI35_AAAAG	1	0.618	0.432	ns
<i>O. esculentus</i>	TI39_ATGG	1	0.102	0.750	ns
<i>O. esculentus</i>	TI4_GT	1	2.865	0.091	ns
<i>O. esculentus</i>	TI49_TGT	28	42.806	0.036	*
<i>O. esculentus</i>	TI5_CA	Monomorphic			
<i>O. esculentus</i>	TI51_TGT	Monomorphic			
<i>O. esculentus</i>	TI52_TAT	Monomorphic			
<i>O. esculentus</i>	TI55_TCTA	Monomorphic			
<i>O. esculentus</i>	TI56_TGTT	1	0.011	0.917	ns
<i>O. esculentus</i>	TI57_TCCA	Monomorphic			
<i>O. esculentus</i>	TI59_AGGA	Monomorphic			
<i>O. esculentus</i>	TI6_GA	10	6.543	0.768	ns
<i>O. esculentus</i>	TI61_TGGA	1	5.000	0.025	*
<i>O. esculentus</i>	TI8_AC	6	6.667	0.353	ns
<i>O. leucostictus</i>	TI1_TG	6	2.225	0.898	ns
<i>O. leucostictus</i>	TI12_TAC	15	11.722	0.700	ns
<i>O. leucostictus</i>	TI13_ATG	Monomorphic			
<i>O. leucostictus</i>	TI15_TGC	Monomorphic			
<i>O. leucostictus</i>	TI16_AAC	1	7.734	0.005	**
<i>O. leucostictus</i>	TI17_GAA	28	79.430	0.000	***
<i>O. leucostictus</i>	TI18_ATCT	6	10.703	0.098	ns
<i>O. leucostictus</i>	TI22_CTAT	28	34.073	0.198	ns
<i>O. leucostictus</i>	TI24_TTAC	Monomorphic			
<i>O. leucostictus</i>	TI26_ACAA	3	0.165	0.983	ns
<i>O. leucostictus</i>	TI27_TTTG	1	0.120	0.729	ns
<i>O. leucostictus</i>	TI31_CTAAT	6	43.705	0.000	***
<i>O. leucostictus</i>	TI32_AAAAT	28	89.972	0.000	***
<i>O. leucostictus</i>	TI33_TTCAA	Monomorphic			

<i>O. leucostictus</i>	TI34_TCTCT	10	14.341	0.158	ns
<i>O. leucostictus</i>	TI35_AAAAG	1	0.078	0.780	ns
<i>O. leucostictus</i>	TI39_ATGG	1	0.002	0.963	ns
<i>O. leucostictus</i>	TI4_GT	1	0.973	0.324	ns
<i>O. leucostictus</i>	TI49_TGT	21	43.155	0.003	**
<i>O. leucostictus</i>	TI5_CA	Monomorphic			
<i>O. leucostictus</i>	TI51_TGT	Monomorphic			
<i>O. leucostictus</i>	TI52_TAT	Monomorphic			
<i>O. leucostictus</i>	TI55_TCTA	Monomorphic			
<i>O. leucostictus</i>	TI56_TGTT	Monomorphic			
<i>O. leucostictus</i>	TI57_TCCA	Monomorphic			
<i>O. leucostictus</i>	TI59_AGGA	Monomorphic			
<i>O. leucostictus</i>	TI6_GA	10	18.293	0.050	ns
<i>O. leucostictus</i>	TI61_TGGA	3	4.900	0.179	ns
<i>O. leucostictus</i>	TI8_AC	6	23.633	0.001	***

Key: ns=not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$



Appendix 11: Graph showing results of Analysis of Molecular Variance for all tilapiines.



Appendix 12: Graph showing allelic patterns for codominant data of all the tilapiines.

Appendix 3: Table showing summary AMOVA Table of *O. niloticus* populations

Source	df	SS	MS	Est. Var.	%
Among Populations	6	200.037	33.340	0.535	5%
Among Individuals	119	1715.685	14.418	5.108	52%
Within Individuals	126	529.500	4.202	4.202	43%
Total	251	2445.222		9.845	100%

F-Statistics	Value	P (rand $\geq$ data)
Fst	0.054	0.001
Fis	0.549	0.001
Fit	0.573	0.001

Appendix 14: Table showing Fst Values for *Oreochromis niloticus* populations on Lake Victoria.

Nei

Genetic

Distance Pairwise Population Fst Values

Dunga	Luanda	Mbita	Siungu	Seka-Bay	Usenge	Usoma	
0.000							Dunga
0.053	0.000						Luanda
0.050	0.039	0.000					Mbita
0.094	0.087	0.066	0.000				Siungu
0.063	0.060	0.063	0.112	0.000			Seka-Bay
0.056	0.054	0.056	0.089	0.060	0.000		Usenge
0.044	0.051	0.036	0.078	0.077	0.064	0.000	Usoma