University of Natural Resources and Life Sciences, Vienna

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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Name……..

Signature……...Date……..

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

µl	Microliter
µM	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 (FST = 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 60.2% 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 Actinopteryrgii (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 Loboi 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 (Agnèse 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 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; Rezania 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 (Njiruet 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).

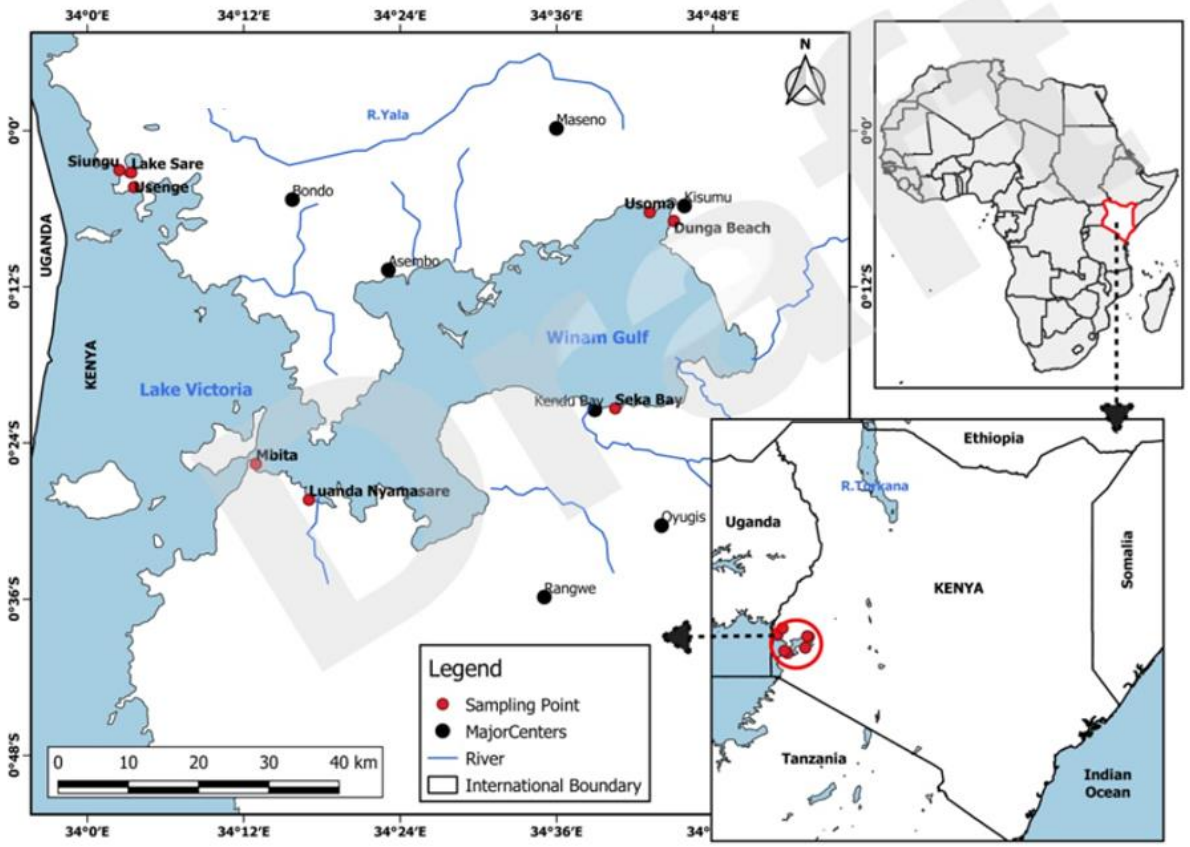


Figure 7: Map showing the sampling sites on Lake Victoria, Kenya

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

Species	Source of samples	Location		No. of samples
		Longitude (E)	Latitude (S)	
Oreochromis niloticus	Dunga	34°44'12''	0°08'41''	30
Oreochromis niloticus	Usenge	34° 3'34.722"	0° 4'20.526"	22
Oreochromis niloticus	Usoma	34° 43'8.382''	0°6'16.878''	32
Oreochromis niloticus	Siungu	34°2'27.882"	0° 3'2.652"	18
Oreochromis niloticus	Seka Bay	34°40'29.67"	0°21'20.724"	25
Oreochromis niloticus	Luanda Nyamasaria	34°16'59.64076"	0°28'23.81326"	23
Oreochromis niloticus	Mbita	34°12'55.24632"	0°25'36.76872"	25
Oreochromis leucostictus	Lake Sare	34°2'28.098"	0°3'7.836"	30
Oreochromis leucostictus	Siungu	34°2'27.882"	0° 3'2.652"	30
Oreochromis esculentus	Lake Sare	34°2'28.098"	0°3'7.836"	30
Tilapia zillii	Luanda Nyamasaria	34°16'59.64076"	0°28'23.81326"	14
Tilapia zillii	Mbita	34°44'12''	0°08'41''	30
Tilapia zillii	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^{o}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^{o}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^{o}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^{o}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(µl)	X96 plates Considering 15% pipetting errors (110 Reaction
1	Master Mix	2.5	275
2	Primer Mix	1.0	110
3	Distilled water	0.5	55
4	DNA Sample	1.0	110
	Total	**5.0**	**550**

For a 384 PCR plate per well, 2.5 µl of Master Mix was mixed with 1.0 µl of Primer Mix, 0.5 µl water and 1.0 µl 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 95^0C for 15 minutes, followed by denaturation for 30 cycles at 95^0C for 30 seconds, annealing at 55^0C for 1 minute and elongation for 1 minute at 72^0C and the last extension steps at 72^0C and incubation at 10^0C 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 µl HDGreen). 2.0µl of PCR product was mixed with 4.0µl 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 4.0µl 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, 2.86µl of Magnetic bead AMPure XPB was added in the PCR plates followed by 4.0µl 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 µl of 80% ethanol and then mixed for 45 seconds. The sample plate was transferred to the second wash plate that also contained 200 µl 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 µl 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 5.0µl of master mix into the Econospin plate followed by 2.0µl 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. 2.0µl of P7 was added into the plate followed by the addition of 1.0µl 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 Fst. 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 Fst 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 Fst value indicates differentiation between populations while low Fst 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 (Porras-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
Oreochromis niloticus	Dunga	30	25
Oreochromis niloticus	Usenge	22	22
Oreochromis niloticus	Usoma	32	20
Oreochromis niloticus	Siungu	18	7
Oreochromis niloticus	Seka Bay	25	16
Oreochromis niloticus	Luanda Nyamasaria	23	13
Oreochromis niloticus	Mbita	25	23
Oreochromis leucostictus	Lake Sare	30	28
Oreochromis leucostictus	Siungu	30	25
Oreochromis esculentus	Lake Sare	30	26
Tilapia zillii	Luanda Nyamasaria	14	14
Tilapia zillii	Mbita	30	23
Tilapia zillii	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 (He= 0.42±0.03) than observed heterozygosity (Ho=0.33±0.03) with *O. niloticus* populations having the highest observed heterozygosity (Ho=0.42±0.04) followed by *C. zillii* (Ho=0.35±0.06) while *O. leucostictus* had the least (Ho=0.28±0.05) (Table 3a). The average number of alleles (Na) was observed at 5.67±0.49 across all the populations with populations of *O. niloticus* having the highest (Na=11.52±1.25) followed by *C. zillii* populations (Na= 5.28±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±0.13) followed by *C. zillii* (I=0.89±0.11) while *O. leucostictus* had the least (I=0.57±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 (He=0.36±0.03) was higher than observed heterozygosity (Ho=0.30±0.03) (Table 3c). *C. zillii*

had a higher expected heterozygosity (He=0.44±0.05) followed by *O. esculentus* while *O. leucostictus* had the least expected heterozygosity (He=0.31±0.05), 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 (Na=5.28±0.54) while *O. esculentus* had the least (Na=2.83±0.42). Shannon information index was averagely 0.68±0.06 with *C. zillii* having the highest (I=0.89±0.11) while *O. leucostictus* had the least value (I=0.57±0.09) (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 (He=0.31±0.04) compared to the observed heterozygosity (Ho=0.29±0.04) (Table 3d). The expected heterozygosity was almost the same with *O. esculentus* populations having (0.32±0.05) and *O. leucostictus* with *(*He=0.31±0.05) (Table 3d).

Populations of *O. esculentus* had the highest number of alleles (Na=2.83±0.43) 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±0.10) while *O. leucostictus* had the least (I=0.56±0.07) (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).

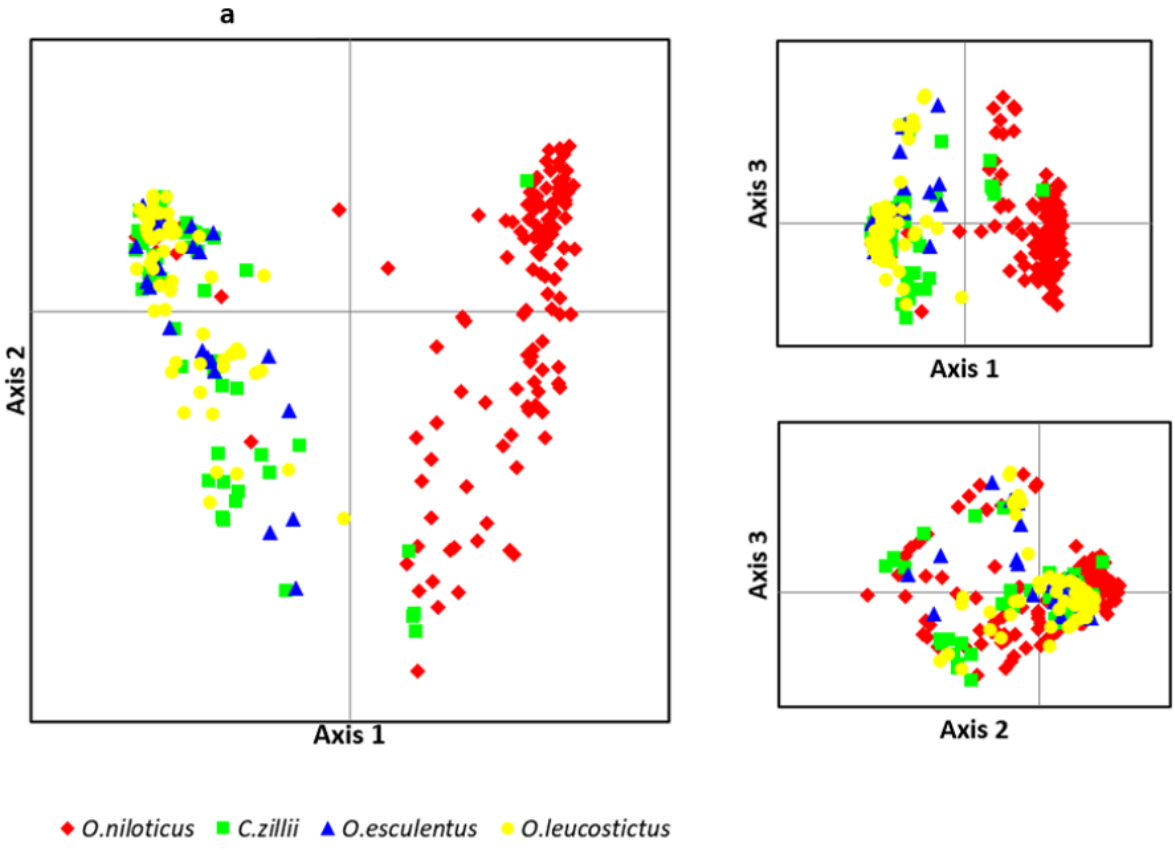
The heterozygosity, the pairwise comparison using Fst/NeiP distance, and the number of polymorphic loci were evaluated. Generally, *Oreochromis niloticus* populations had the highest expected heterozygosity (He=0.54±0.02) compared to the observed heterozygosity (Ho=0.41±0.02) (Table 3e). Populations from Dunga beach had the highest observed heterozygosity (Ho=0.47±0.05) 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 (He=0.43±0.05). Samples from Dunga beach had the highest number of alleles (Na=7.24±0.69) followed by Usenge beach (Na=6.66±0.72) while Siungu had the least number of alleles (Na= 3.03±0.36) (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±0.11) while the least was observed from Siungu beach (I=0.79±0.11) (Table 3e).

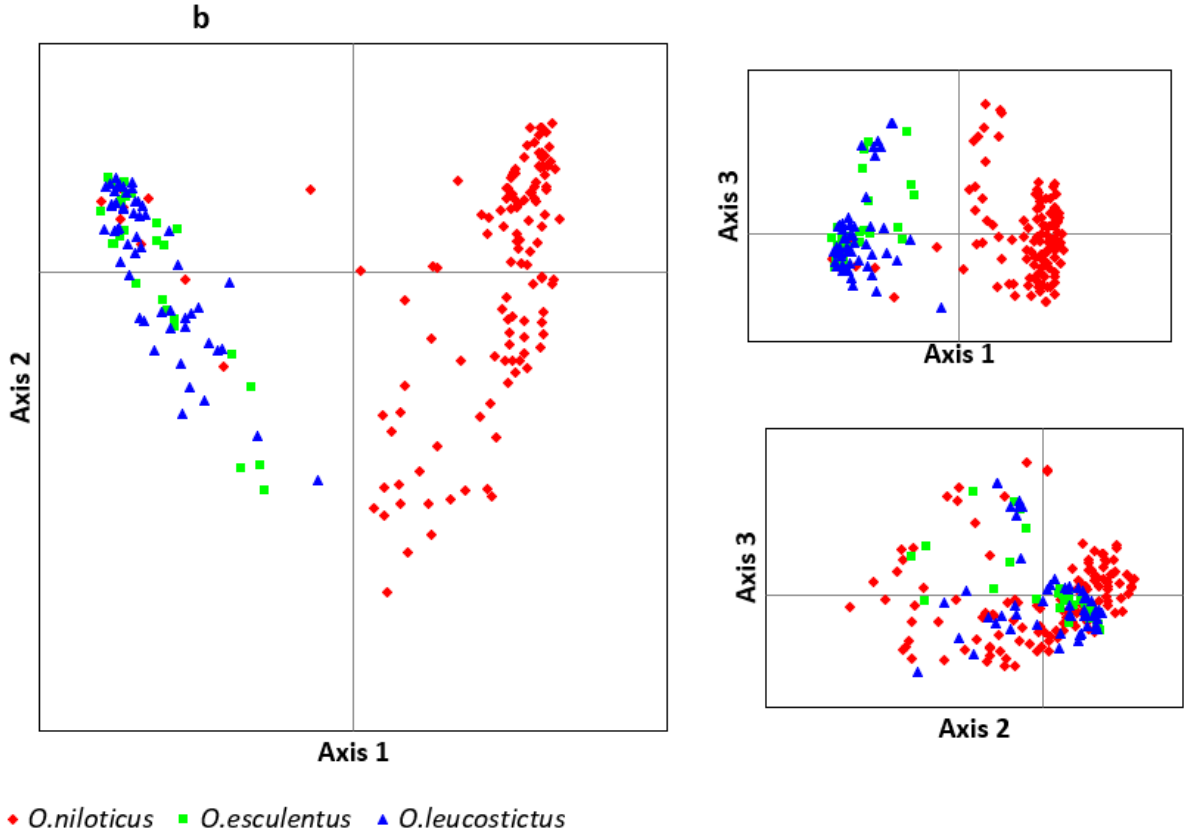
The principal coordinate analysis (PCoA) was conducted to differentiate the populations of *O. niloticus* from different landing beaches using the first three axes. The PCoA showed two clear groups: *O. niloticus* populations from Usenge, Mbita, Siungu, Luanda and Seka-Bay formed one cluster while populations 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).

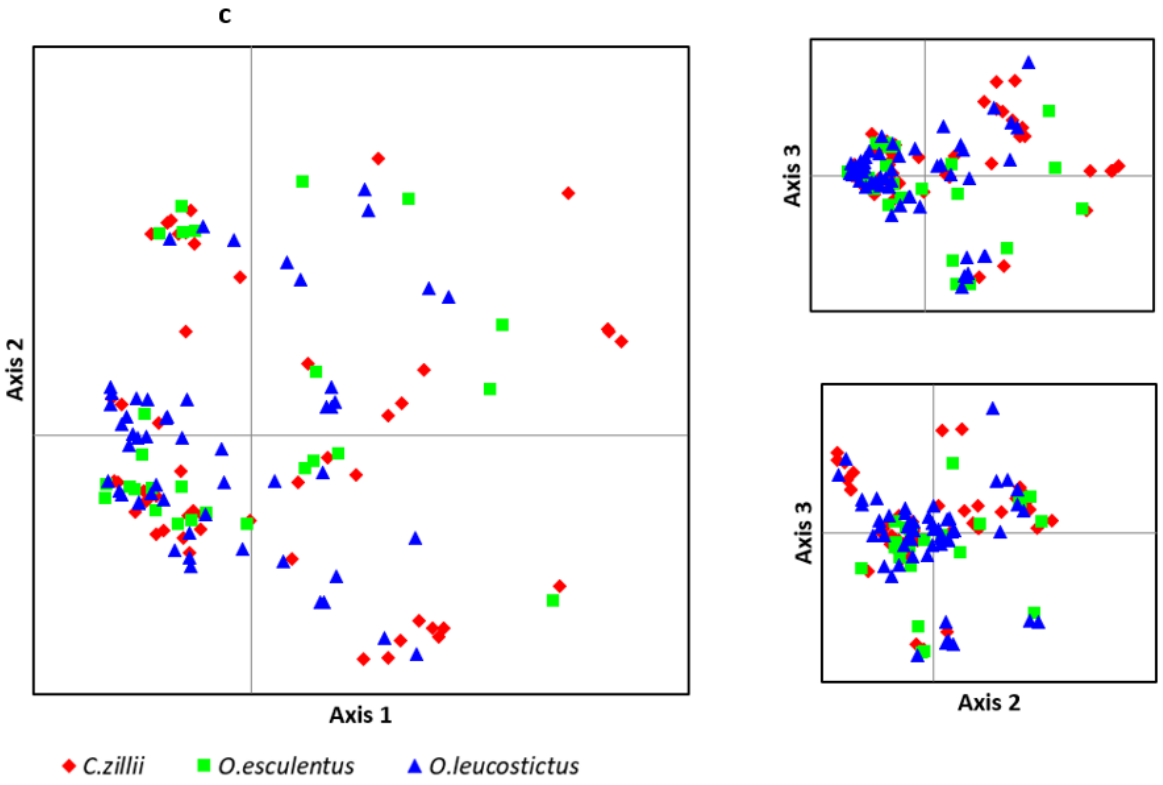
To investigate whether there was genetic differentiation between the two populations of *O. leucostictus* from Lake Victoria and Lake Sare, the PCoA results showed that *O. leucostictus* populations formed two cluster with Lake Sare populations forming one cluster while Victoria formed another cluster 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).

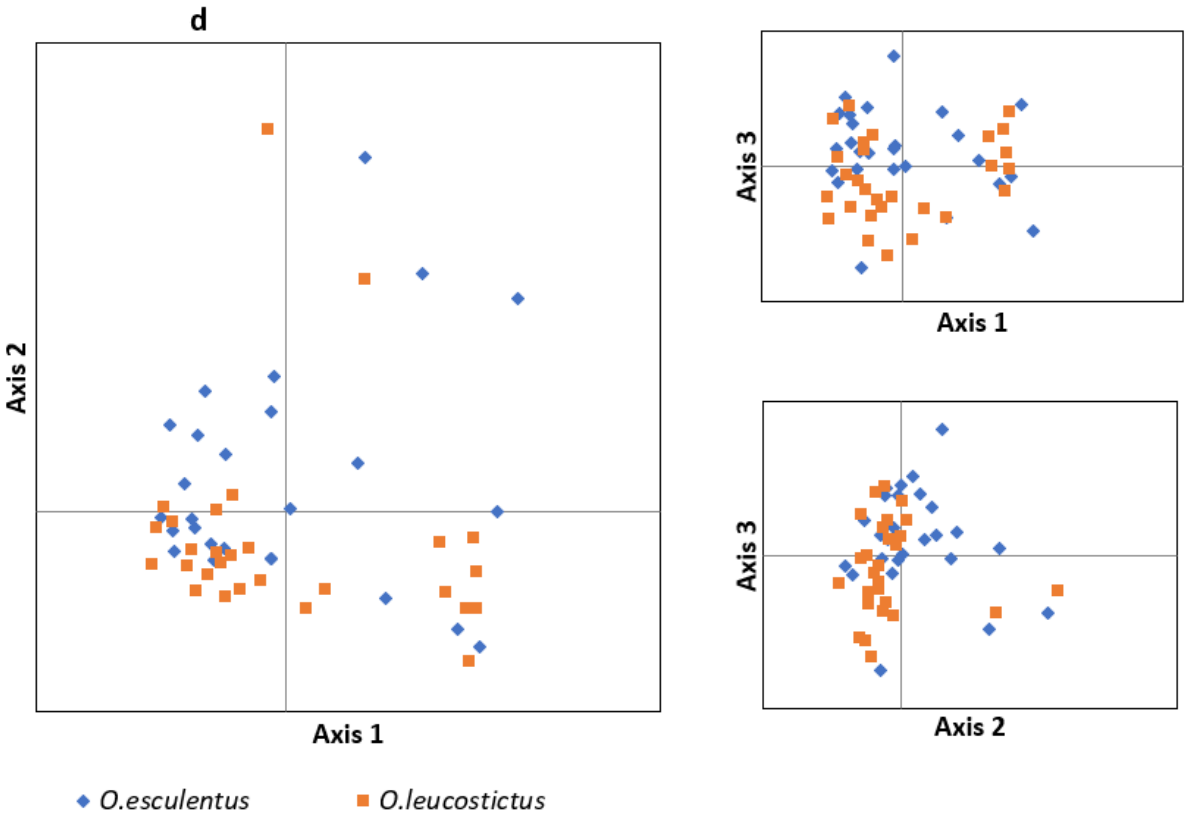
With *Oreochromis* species from Siungu, the PCoA results formed two clusters with one cluster containing *O. niloticus* while another cluster contained *O. leucostictus* populations (Figure 8g).

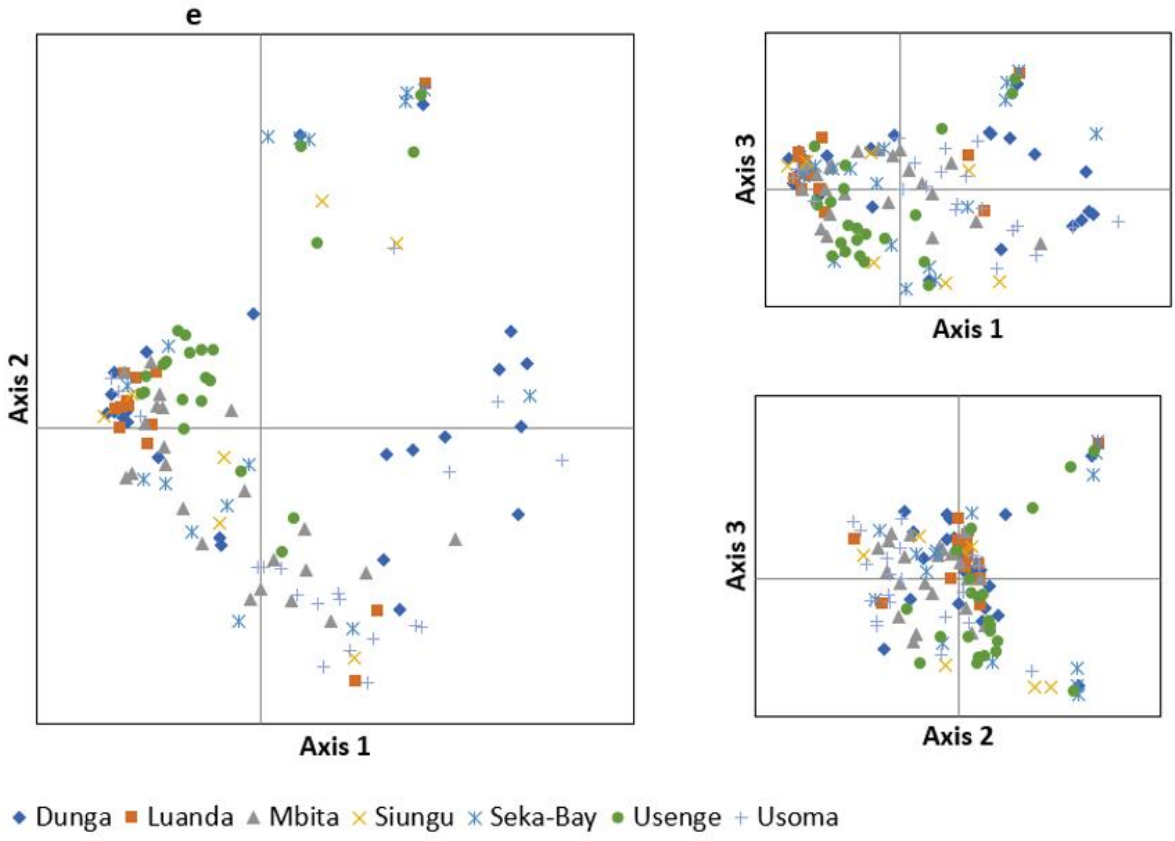
The comparison involving *O. esculentus, O. leucostictus* populations from Lake Sare with *O. leucostictus* populations from Lake Victoria showed no clear clustering of the populations. Few samples of *O. leucostictus* from Lake Sare formed a sub-cluster that comprised of five samples of *O. esculentus*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

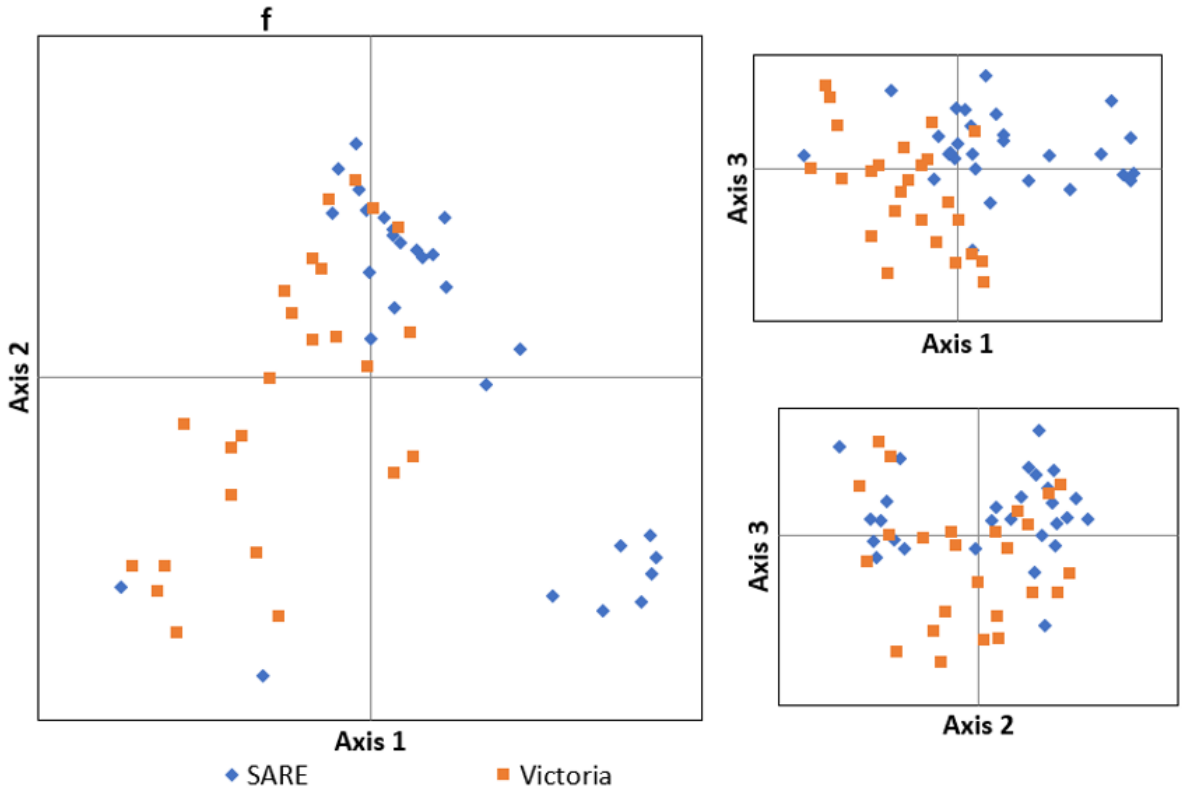


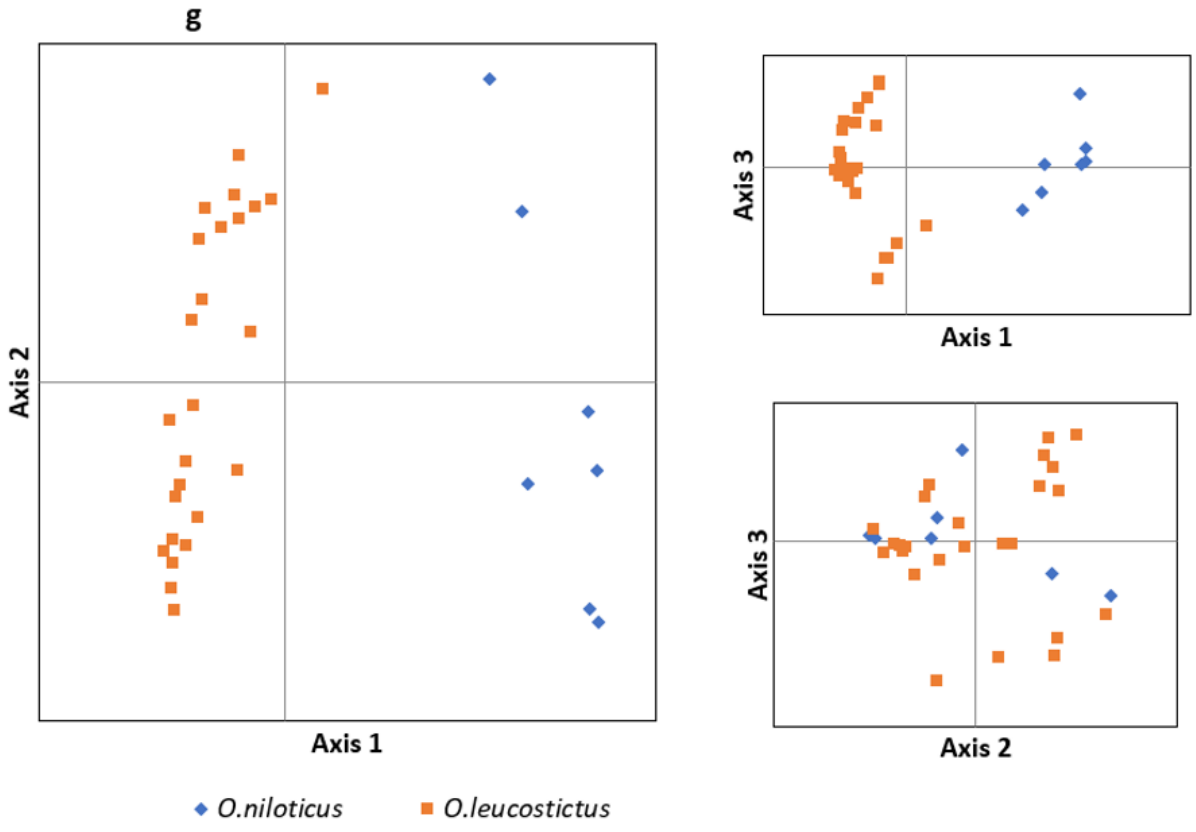

b
Axis 2
Axis 1
Axis 3
Axis 1
Axis 3
Axis 2
O.niloticus
O.esculentus
O.leucostictus


c
Axis 2
Axis 1
Axis 3
Axis 3
Axis 2
C.zillii
O.esculentus
O.leucostictus


d
Axis 2
Axis 1
Axis 3
Axis 1
Axis 3
Axis 2
O.esculentus
O.leucostictus


e
Axis 2
Axis 1
Axis 3
Axis 1
Axis 3
Axis 2
Dunga
Luanda
Mbita
Siungu
Seka-Bay
Usenge
Usoma


f
Axis 2
Axis 1
Axis 3
Axis 1
Axis 3
Axis 2
SARE
Victoria


g
Axis 2
Axis 1
Axis 3
Axis 1
Axis 3
Axis 2
O.niloticus
O.leucostictus

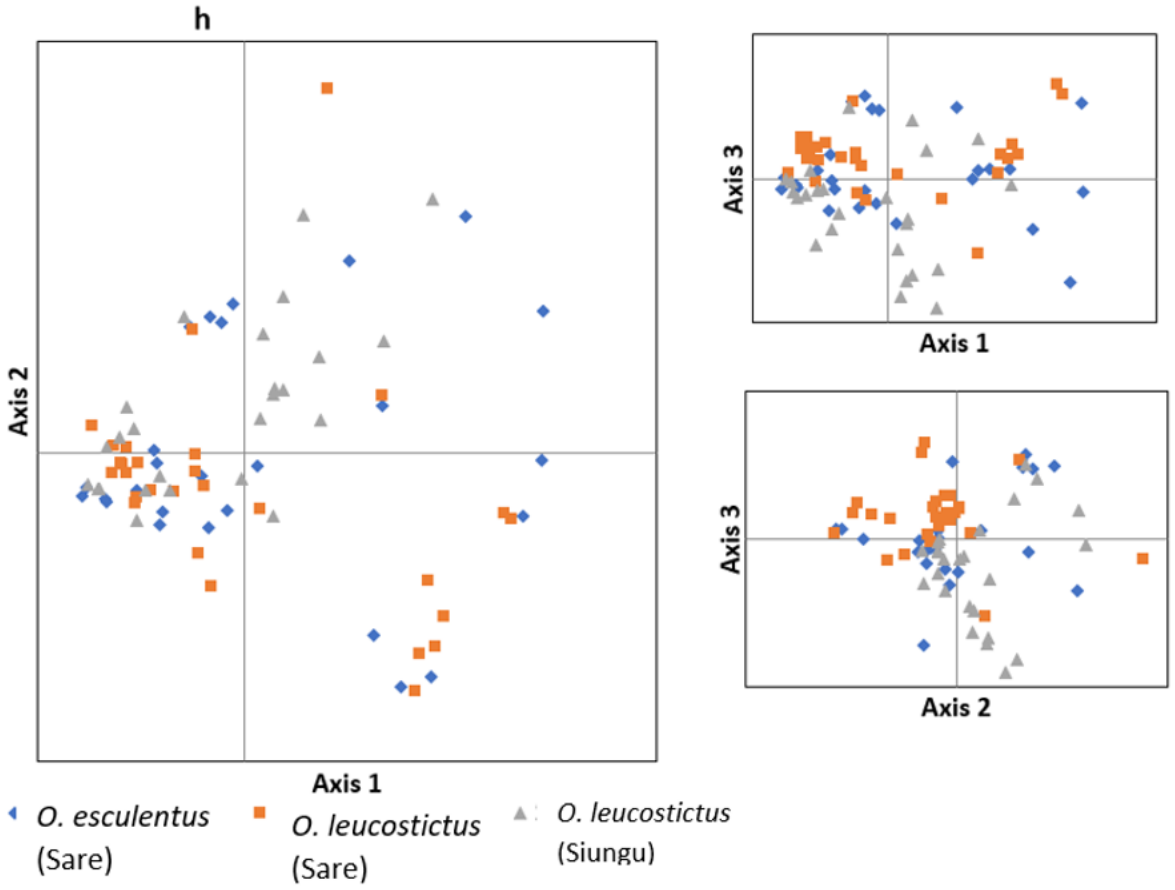


Figure 8: Scatter plots showing the tilapiine fish species collected during the study.

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. leucostictus* **(*Mean ± SE*).**

	Species	N	Na	Ne	I	Ho	He	uHe	F
a	***O. niloticus***	88.79±3.66	11.52±1.25	3.99±0.56	1.45±0.13	0.42±0.04	0.61±0.04	0.61±0.04	0.35±0.04
	C. zillii	33.14±2.11	5.28±0.54	2.24±0.19	0.89±0.10	0.35±0.06	0.44±0.05	0.47±0.06	0.37±0.09
	O. esculentus	18.10±1.29	2.83±0.42	1.72±0.17	0.58±0.10	0.28±0.06	0.32±0.05	0.33±0.06	0.07±0.08
	O. leucostictus	37.83±2.64	3.03±0.46	1.69±0.17	0.57±0.09	0.28±0.05	0.31±0.05	0.32±0.05	0.14±0.06
	Average	**44.47±2.79**	**5.66±0.49**	**2.41±0.18**	**0.87±0.06**	**0.33±0.03**	**0.42±0.03**	**0.43±0.03**	**0.26±0.04**
b	***C. zilli***	33.14±2.11	5.28±0.54	2.24±0.19	0.89±0.11	0.35±0.06	0.44±0.05	0.47±0.06	0.37±0.09
	O. esculentus	18.10±1.29	2.83±0.42	1.72±0.17	0.58±0.10	0.28±0.06	0.32±0.05	0.33±0.06	0.07±0.08
	O. leucostictus	37.83±2.64	3.034±0.46	1.69±0.17	0.57±0.09	0.28±0.05	0.31±0.05	0.32±0.05	0.14±0.06
	Average	**29.69±1.49**	**3.71±0.29**	**1.88±0.11**	**0.68±0.06**	**0.30±0.03**	**0.36±0.03**	**0.37±0.03**	**0.22±0.05**

c	***O. esculentus***	18.10±1.29	2.83±0.42	1.72±0.17	0.58±0.10	0.28±0.05	0.32±0.05	0.33±0.05	0.07±0.07
	O. leucostictus	19.66±1.56	2.62±0.37	1.65±0.16	0.54±0.09	0.29±0.06	0.31±0.05	0.32±0.05	0.07±0.07
	Average	**18.88±1.01**	**2.72±0.28**	**1.69±0.12**	**0.56±0.07**	**0.29±0.04**	**0.31±0.04**	**0.32±0.04**	**0.07±0.05**
d	**Dunga**	16.76±0.82	7.24±0.69	3.83±0.51	1.39±0.11	0.47±0.05	0.63±0.04	0.65±0.04	0.29±0.06
	Luanda	10.62±0.49	4.86±0.42	3.11±0.35	1.12±0.11	0.40±0.05	0.54±0.05	0.57±0.05	0.31±0.06
	Mbita	16.79±0.96	4.69±0.47	2.89±0.33	1.03±0.12	0.43±0.06	0.51±0.05	0.53±0.06	0.13±0.05
	Siungu	4.86±0.29	3.03±0.36	2.39±0.27	0.79±0.11	0.40±0.06	0.43±0.06	0.48±0.06	0.07±0.08
	Seka-Bay	10.48±0.54	4.93±0.43	3.28±0.28	1.23±0.09	0.35±0.05	0.62±0.03	0.66±0.04	0.47±0.07
	Usenge	17.62±0.99	6.66±0.72	3.73±0.52	1.29±0.13	0.40±0.05	0.58±0.05	0.60±0.05	0.33±0.05
	Usoma	11.66±0.88	4.48±0.55	2.93±0.39	1.01±0.12	0.38±0.05	0.49±0.05	0.53±0.05	0.23±0.09
	Average	**12.69±0.41**	**5.13±0.22**	**3.17±0.15**	**1.12±0.05**	**0.41±0.02**	**0.54±0.02**	**0.57±0.02**	**0.27±0.03**

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

	Name Loci												
Populations	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	*	*
Mbita	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
Siungu	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
Seka 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
Usenge	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
Usoma	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

Table 4 continued

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

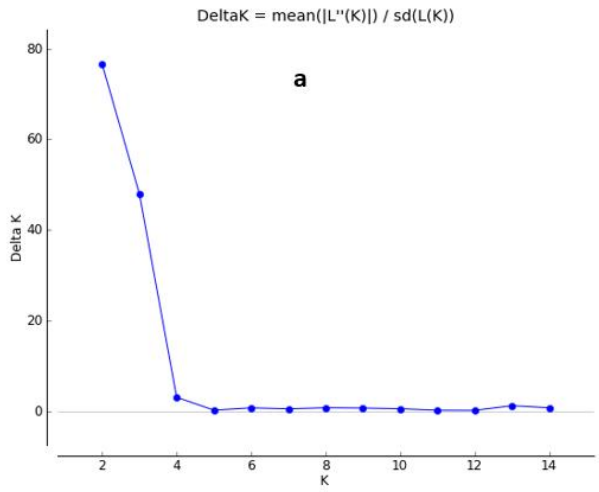
Key: ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

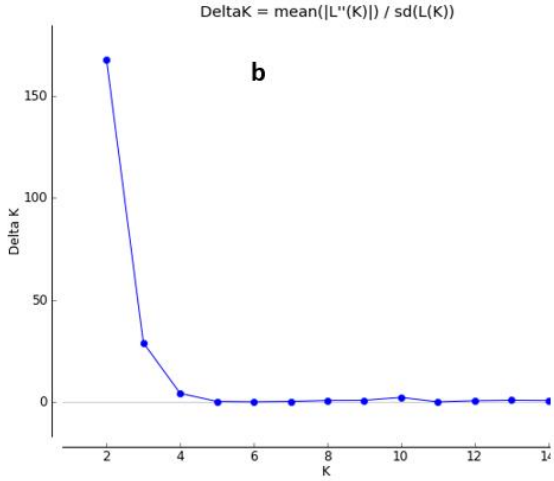
3.3 Sample cluster simulation using Structure harvester and STRUCTURE 2.3.4

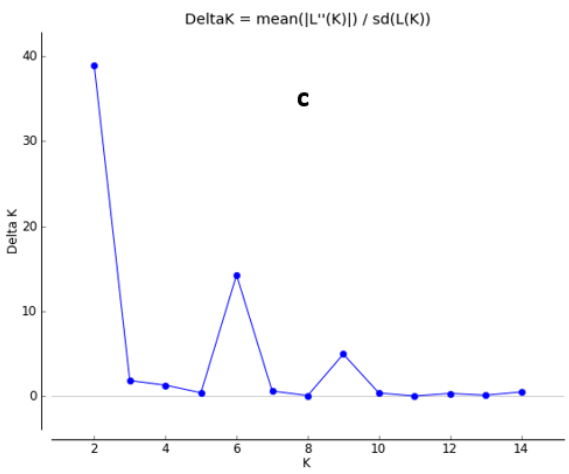
3.3.1 Structure harvester outputs

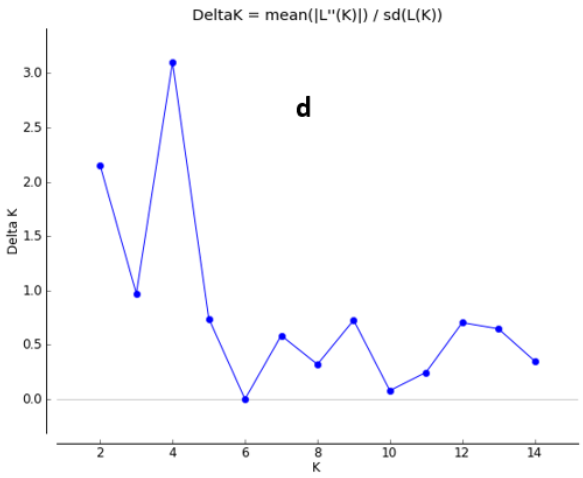
The data preparation resulted in a codominant matrix of allele frequencies. This matrix served as an input for STRUCTURE v. 2. 3. 4. Results of cluster assignment probabilities were analysed using the Structure harvester (http://taylor0.biology.ucla.edu/structureHarvester/), an online program that validates 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.

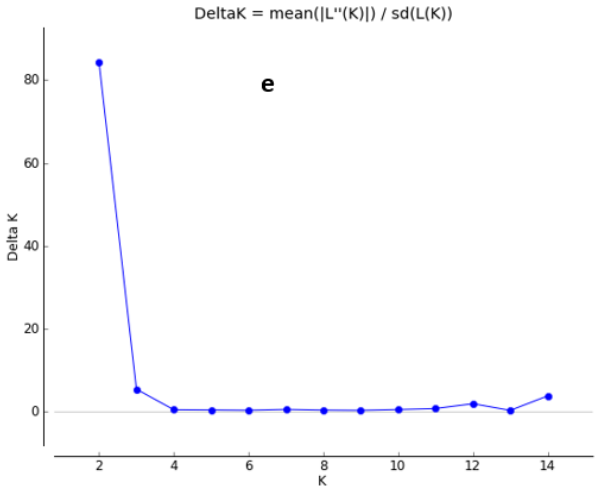
All the tilapiine samples, *Oreochromis* species, other tilapiines excluding *O. niloticus*, *O. niloticus*, *O. leucostictus,* Samples from Siungu and Species from Sare and Siungu collected during the study had a similar high K value (k=2) (Figures 9a, 9b, 9c, 9e, 9f, 9g and 9h). On the other hand,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

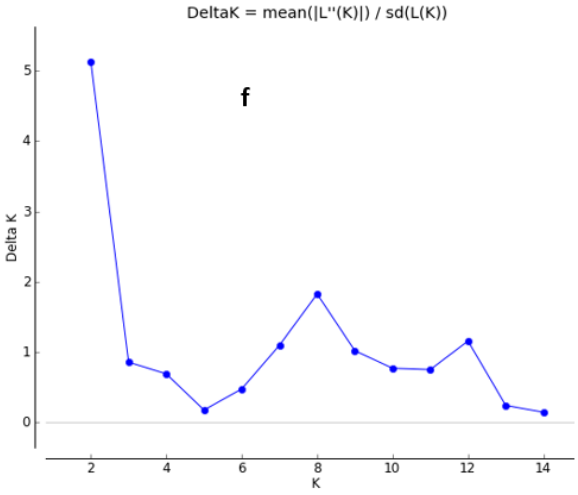





DeltaK = mean(|L''(K)|) / sd(L(K))
c
Delta K
K


DeltaK = mean(|L''(K)|) / sd(L(K))
d
Delta K
K


DeltaK = mean(|L''(K)|) / sd(L(K))
e
Delta K
K


DeltaK = mean(|L''(K)|) / sd(L(K))
f
Delta K
K

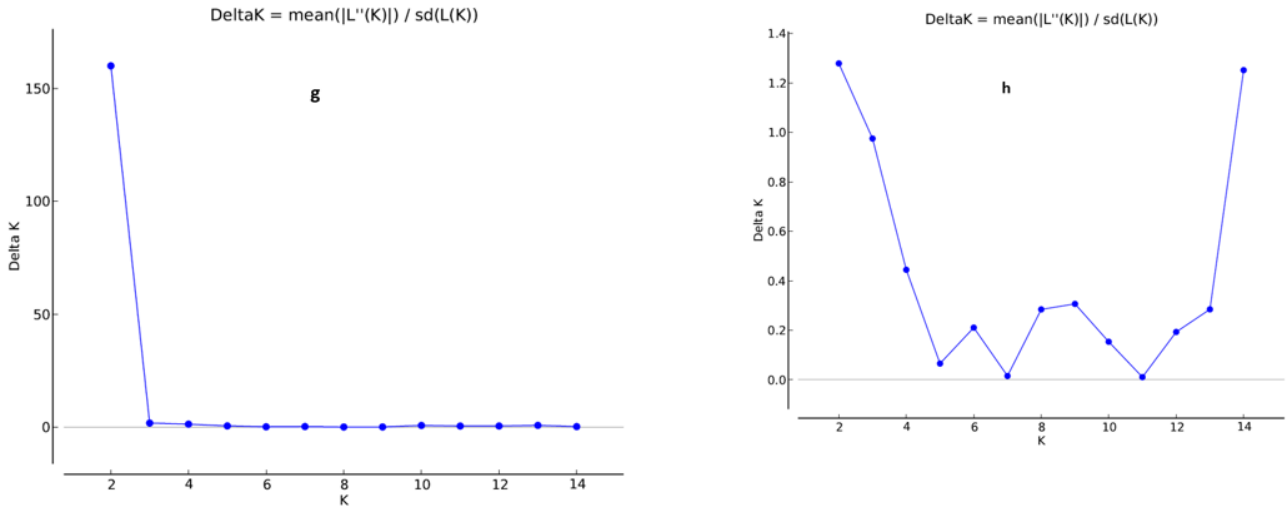


Figure 9: STRUCTURE HARVESTER plots showing K values for different tilapiines.

a; All tilapiine species, **b;** *Oreochromis* species, **c;** Other tilapiines (*O. esculentus, O. leucostictus* and *C. zilli*), **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

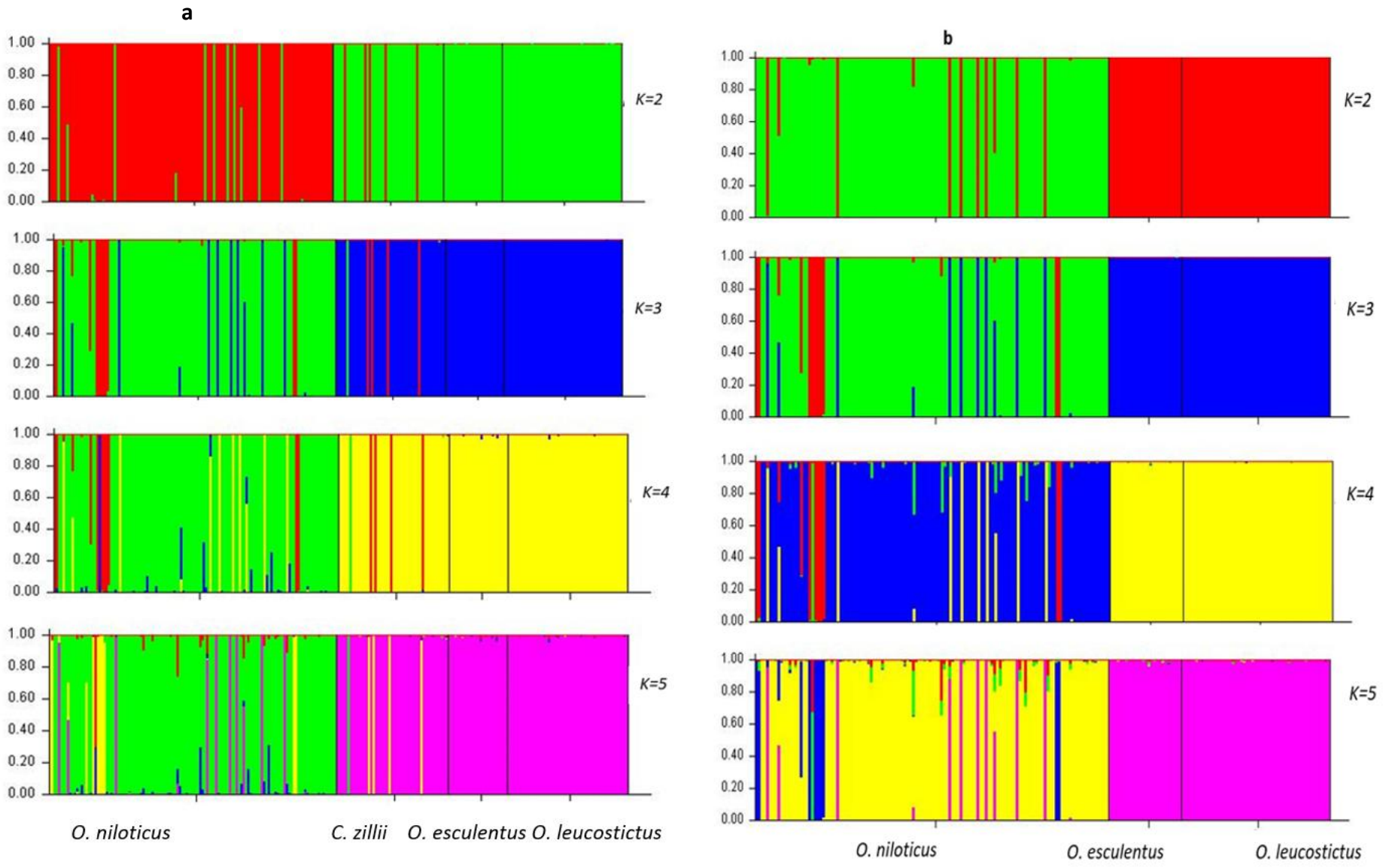
Using the structure analysis, all the tilapiines collected formed two main clusters with some individuals from *O. niloticus* populations having some degree of admixture (Figure 10a). The results are congruent with the PCoA in figure 8a. The cluster containing *C. zillii, O. esculentus* and *O. leucostictus* 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.

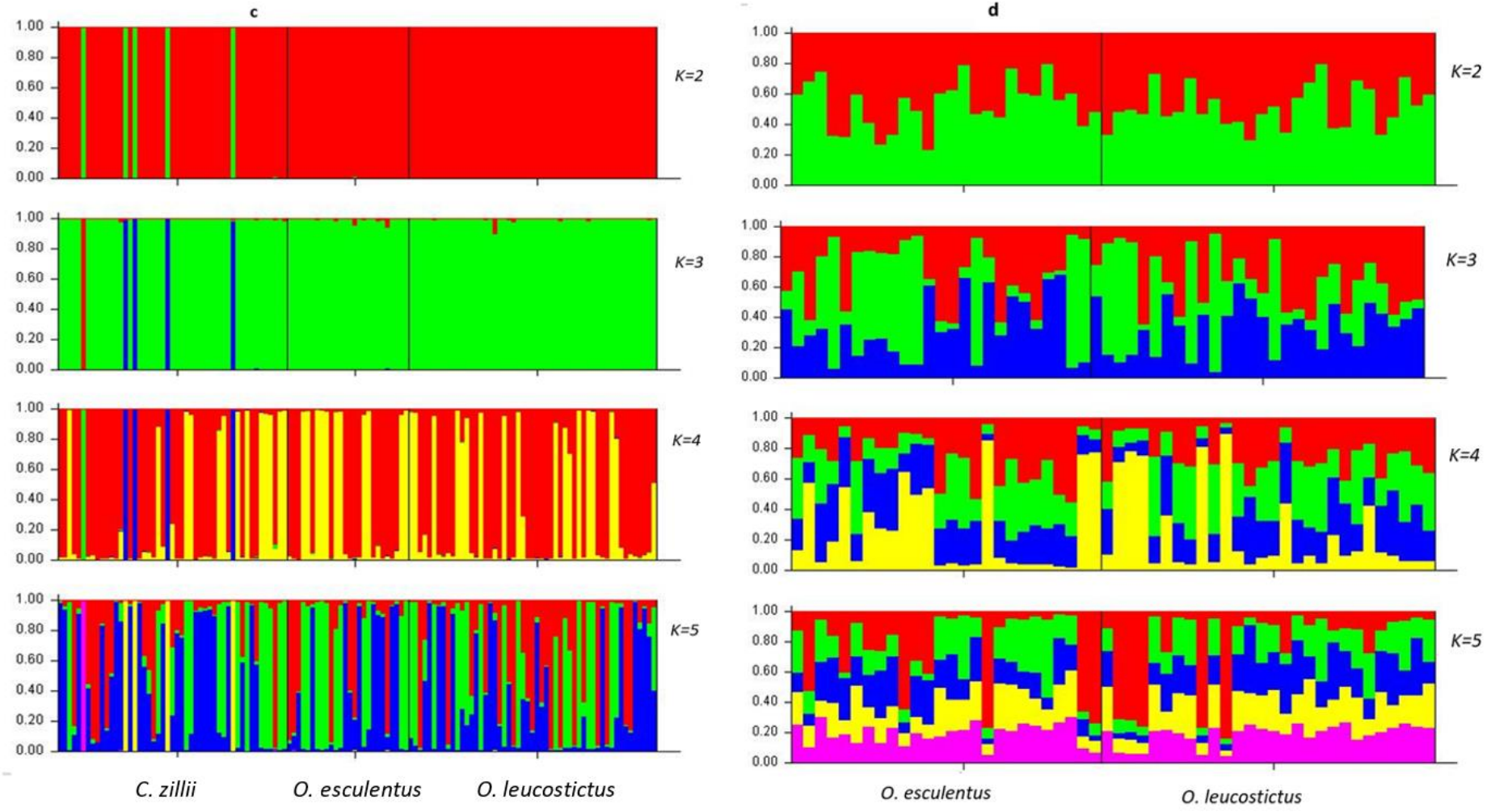
Considering the tilapiines excluding *O. niloticus*, the species could not be differentiated, and they showed some degree of admixtures at K=4 and 5 (Figure 10c). The same case was observed in Oreochromis species from Lake sare that could not be differentiated beyond K=2 and showed higher degree of admixtures (Figure 10d).

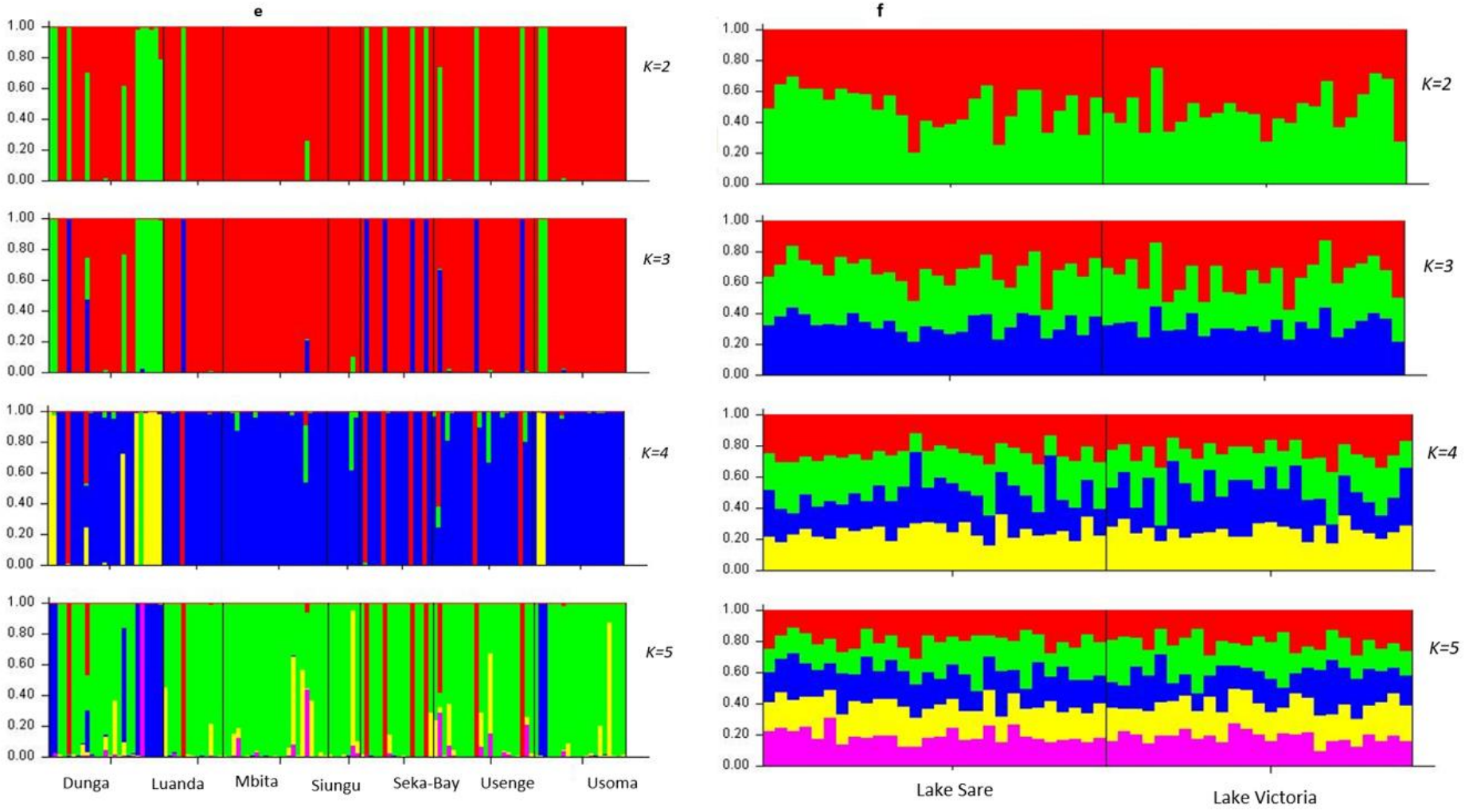
The populations of *O. niloticus* from Dunga, Luanda, Seka-Bay and Usenge showed some degree of admixture while samples from Mbita, Siungu and Usoma beaches showed a pure

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.


a
1.00
0.80
0.60
0.40
0.20
0.00
K=2
K=3
K=4
K=5
O. niloticus
C. zillii
O. esculentus
O. leucostictus
b
O. niloticus
O. esculentus
O. leucostictus


c
d
1.00
0.80
0.60
0.40
0.20
0.00
K=2
K=3
K=4
K=5
C. zillii
O. esculentus
O. leucostictus
O. esculentus
O. leucostictus


e
f
1.00
0.80
0.60
0.40
0.20
0.00
K=2
K=3
K=4
K=5
Dunga
Luanda
Mbita
Siungu
Seka-Bay
Usenge
Usoma
Lake Sare
Lake Victoria

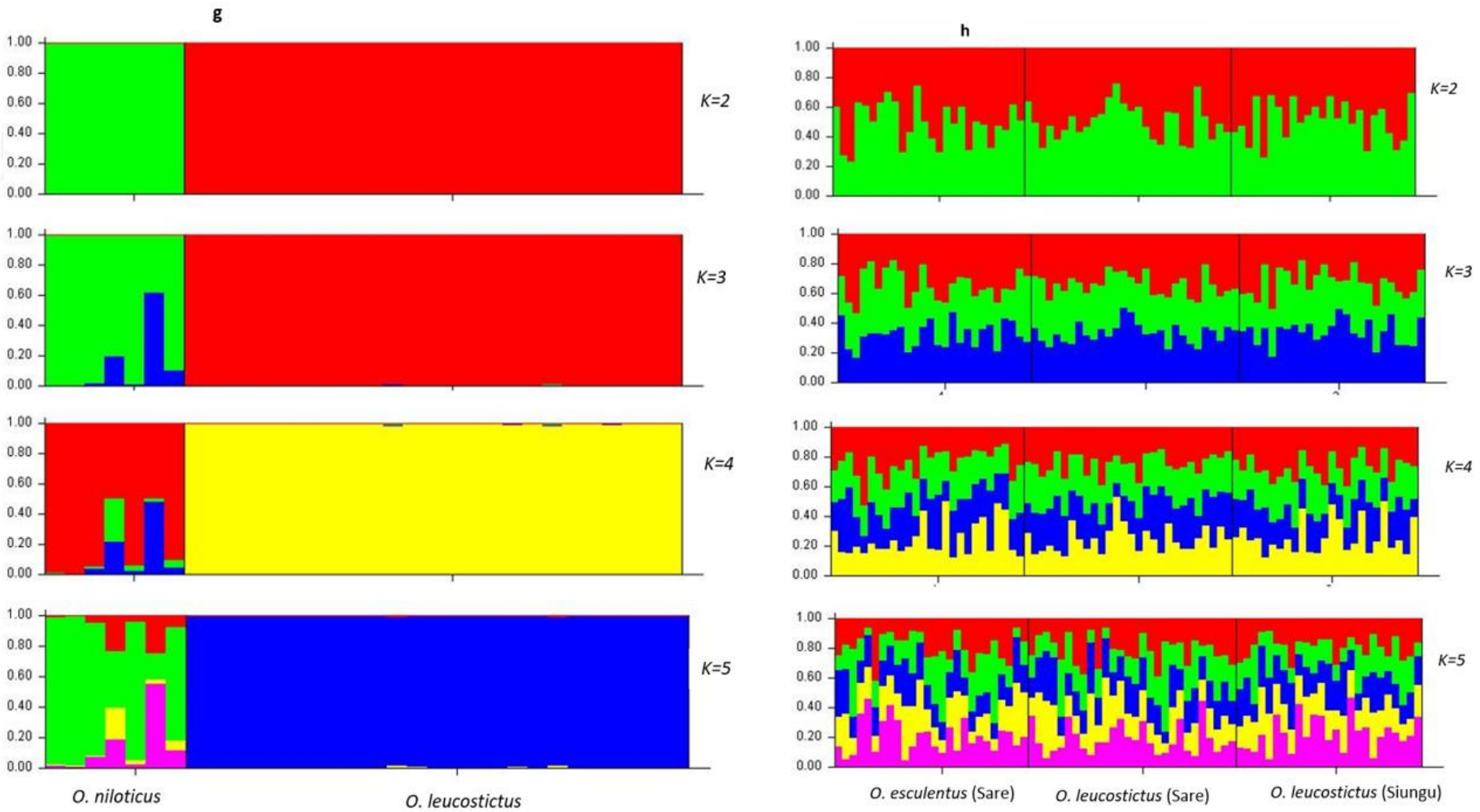


Figure 10: Bayesian structure analysis showing the clustering of different tilapiine species. ***a;*** *all tilapiines,* ***b;*** *Oreochromis species,* ***c;*** *Other tilapiines,* ***d;*** *Species from Sare,* ***e;*** *O. niloticus,* ***f;*** *O. leucostictus,* ***g;*** *Species from Siungu,* ***h;***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

Oreochrmois 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 (Ho = 0.53) of nine populations of *O. niloticus* in the Volta lake of Ghana was slightly higher than the expected heterozygosity (He = 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 Agnèse, 2007; Nyingi et al., 2007; Ndiwa et al., 2014). The current study showed low genetic differentiation between the populations from Mbita and Usoma (Fst=0.04), Luanda and Mbita (Fst=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 un-intentionally 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.

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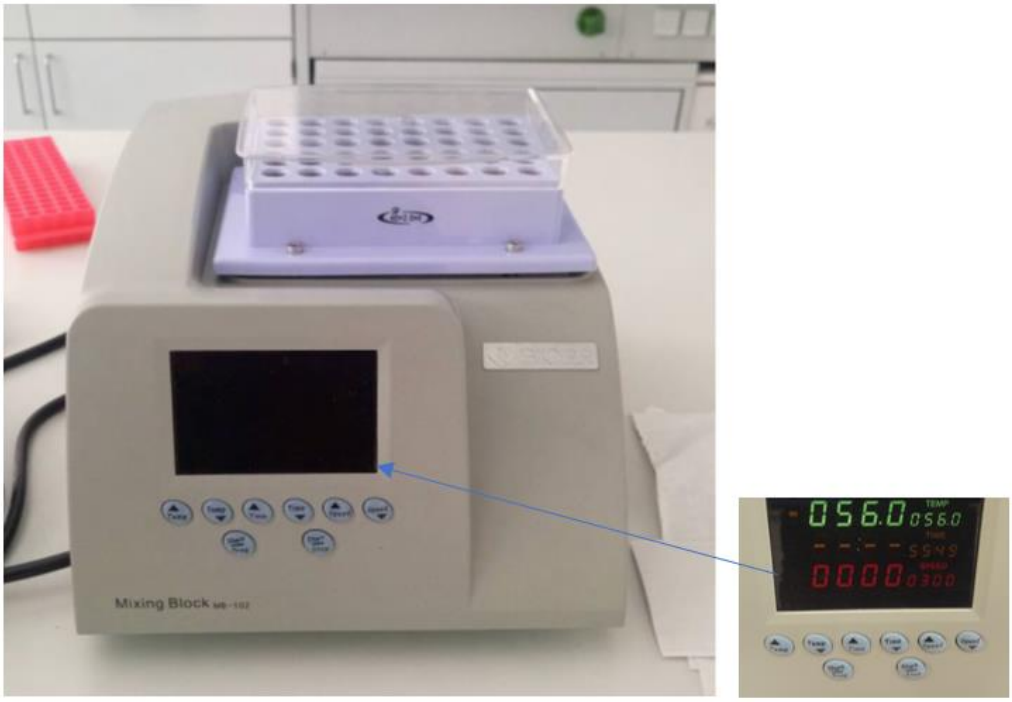
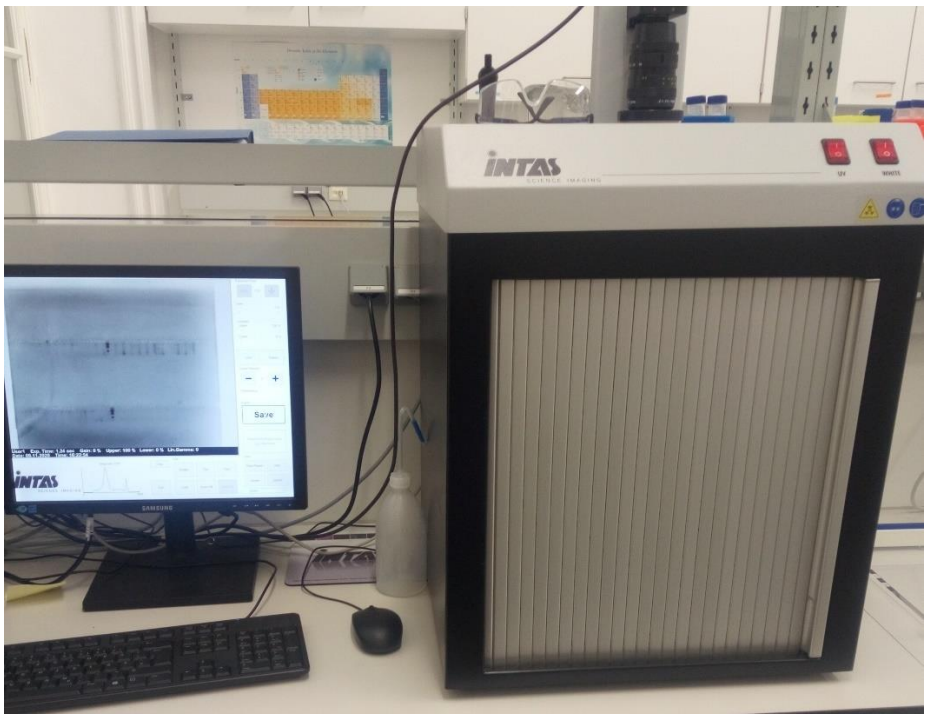
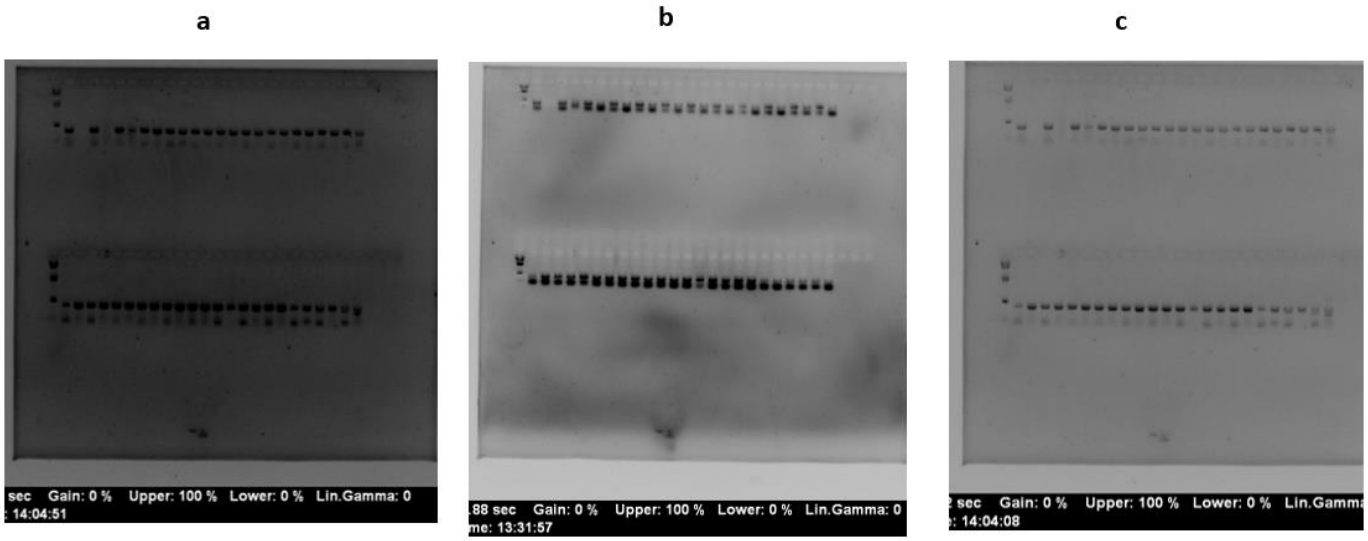
7.0 APPENDIX



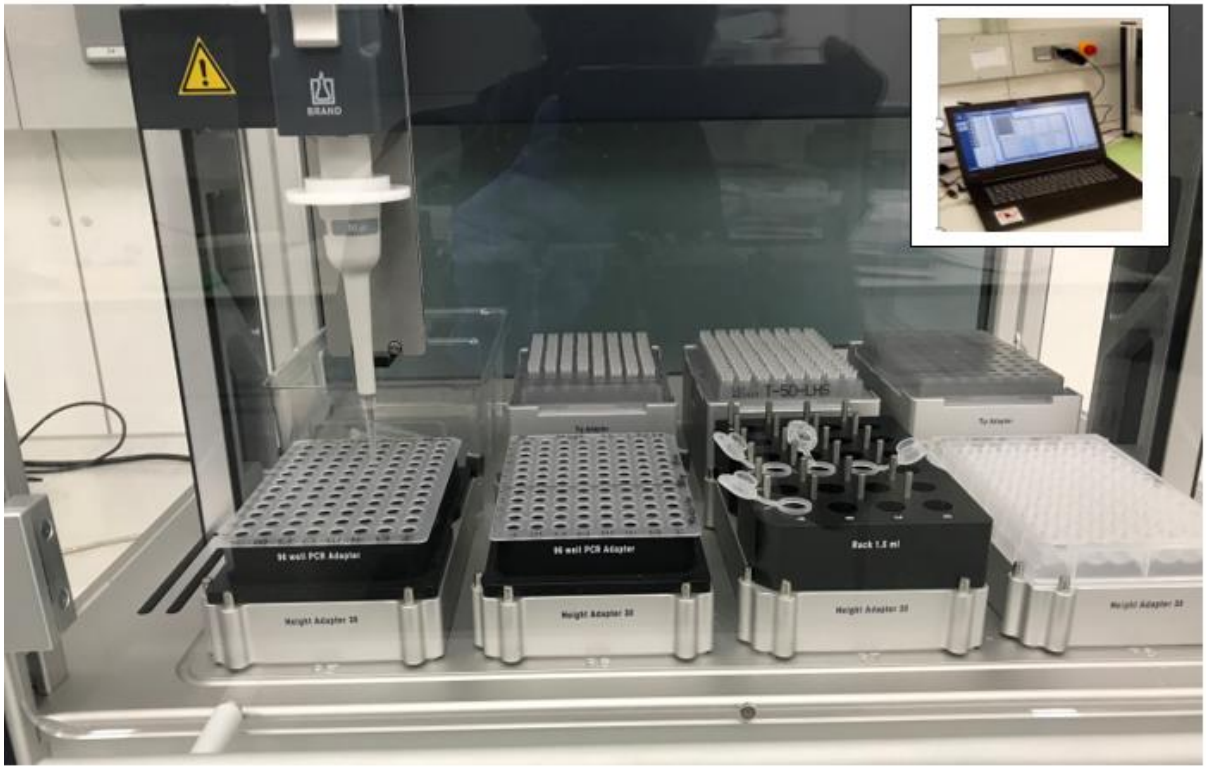
Appendix 1: Mixing block (PEQLAB) with calibrations



Appendix 2: Intas GEL IX IMAGER, Germany

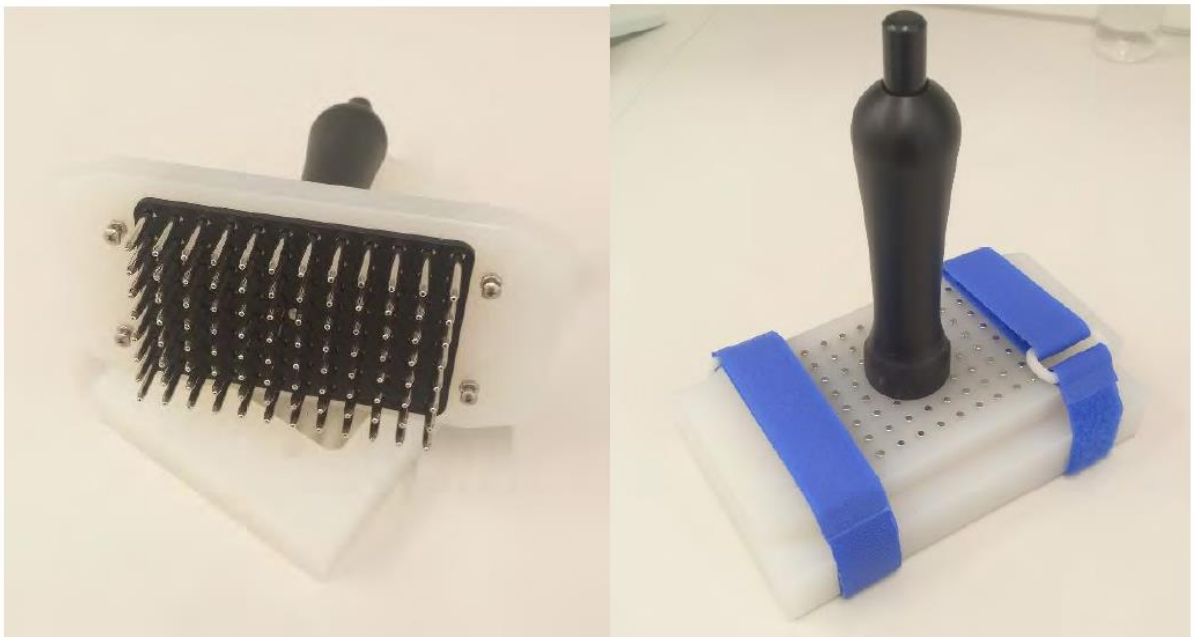


Appendix 3:DNA images visualized under INTAS Imaging Machine; a: Dark run, b: Normal run, C: Light run



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:Evano 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
O. niloticus	TI1_TG	55	326.736	0.000	***
O. niloticus	TI12_TAC	15	103.748	0.000	***
O. niloticus	TI13_ATG	10	73.882	0.000	***
O. niloticus	TI15_TGC	21	397.597	0.000	***
O. niloticus	TI16_AAC	28	496.699	0.000	***
O. niloticus	TI17_GAA	105	733.500	0.000	***
O. niloticus	TI18_ATCT	171	479.817	0.000	***
O. niloticus	TI22_CTAT	351	1052.182	0.000	***

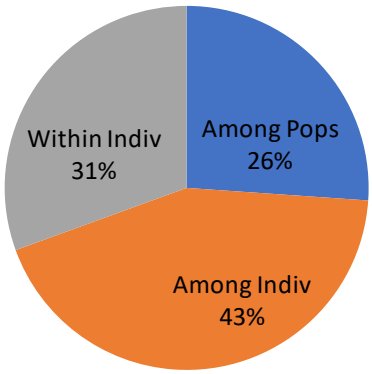
O. niloticus	TI24_TTAC	91	163.327	0.000	***
O. niloticus	TI26_ACAA	28	89.583	0.000	***
O. niloticus	TI27_TTTG	15	140.896	0.000	***
O. niloticus	TI31_CTAAT	105	575.183	0.000	***
O. niloticus	TI32_AAAAT	406	623.252	0.000	***
O. niloticus	TI33_TTCAA	36	35.816	0.477	ns
O. niloticus	TI34_TCTCT	210	701.824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

T. zilli	TI27_TTTG	3	36.416	0.000	***
T. zilli	TI31_CTAAT	28	184.113	0.000	***
T. zilli	TI32_AAAAT	28	76.682	0.000	***
T. zilli	TI33_TTCAA	1	1.000	0.317	ns
T. zilli	TI34_TCTCT	55	140.414	0.000	***
T. zilli	TI35_AAAAG	21	152.716	0.000	***
T. zilli	TI39_ATGG	45	368.121	0.000	***
T. zilli	TI4_GT	3	35.013	0.000	***
T. zilli	TI49_TGT	36	109.876	0.000	***
T. zilli	TI5_CA	3	82.000	0.000	***
T. zilli	TI51_TGT	3	46.250	0.000	***
T. zilli	TI52_TAT	1	32.000	0.000	***
T. zilli	TI55_TCTA	3	6.800	0.079	ns
T. zilli	TI56_TGTT	1	42.000	0.000	***
T. zilli	TI57_TCCA	1	42.000	0.000	***
T. zilli	TI59_AGGA	Monomorphic			
T. zilli	TI6_GA	15	37.013	0.001	**
T. zilli	TI61_TGGA	10	27.000	0.003	**
T. zilli	TI8_AC	21	91.205	0.000	***
O. esculentus	TI1_TG	6	4.759	0.575	ns
O. esculentus	TI12_TAC	6	5.577	0.472	ns
O. esculentus	TI13_ATG	Monomorphic			
O. esculentus	TI15_TGC	Monomorphic			
O. esculentus	TI16_AAC	1	6.644	0.010	**
O. esculentus	TI17_GAA	3	21.320	0.000	***
O. esculentus	TI18_ATCT	15	33.749	0.004	**
O. esculentus	TI22_CTAT	36	57.420	0.013	*
O. esculentus	TI24_TTAC	Monomorphic			
O. esculentus	TI26_ACAA	3	1.009	0.799	ns
O. esculentus	TI27_TTTG	1	0.198	0.656	ns
O. esculentus	TI31_CTAAT	10	31.155	0.001	***

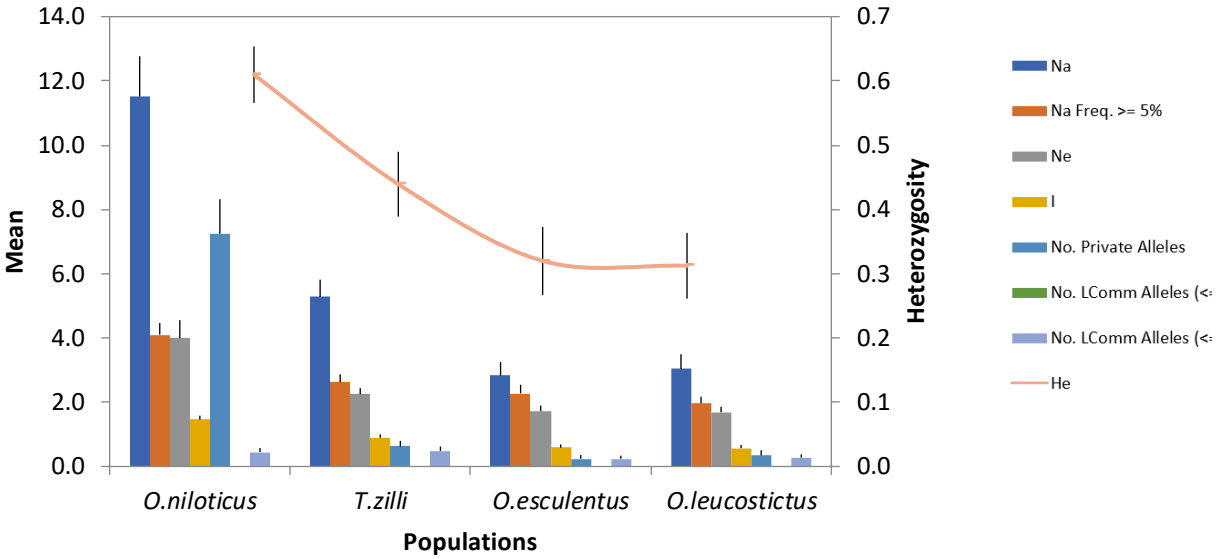
O. esculentus	TI32_AAAAT	6	6.768	0.343	ns
O. esculentus	TI33_TTCAA	Monomorphic			
O. esculentus	TI34_TCTCT	10	15.890	0.103	ns
O. esculentus	TI35_AAAAG	1	0.618	0.432	ns
O. esculentus	TI39_ATGG	1	0.102	0.750	ns
O. esculentus	TI4_GT	1	2.865	0.091	ns
O. esculentus	TI49_TGT	28	42.806	0.036	*
O. esculentus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

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