GENETIC DIVERSITY, POPULATION STRUCTURE AND MORPHOLOGICAL CHARACTERIZATION OF THE SILVER CYPRINID *RASTRINEOBOLA ARGENTEA* (PELLEGRIN) IN PORT VICTORIA, MBITA AND NYANZA GULF OF LAKE VICTORIA (KENYA)

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Genetic diversity, population structure and morphological characterization of the silver cyprinid *Rastrineobola argentea* (Pellegrin) in Port Victoria, Mbita and Nyanza Gulf of Lake Victoria (Kenya)

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature.....Date.....

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This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

I dedicate this work to my loving parents, siblings and extended family for their immense support, encouragement and always wanting to know my progress which in turn motivated me to work harder, be diligent and not settle for mediocre. You guys rock.

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ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ATPase	Adenine Tri Phosphatase
BLAST	Basic Local Alignment Search Tool
bp	base pair
CH_3CH_2OH	Ethanol
CO1	Cytochrome Oxidase 1 subunit
DNA	Deoxyribonucleic Acid
dNTPs	deoxyribo Nucleotide TriPhosphate
EDTA	EthyleneDiamineTetraacetic acid
FAO	Food and Agricultural Organization
Fst	Fisher‘s Statistical Test
HCl	Hydrochloric acid
HE	Heterozygosity
HWE	Hardy-Weinberg Equilibrium
Kb	Kilobase
MEGA	Molecular Evolutionary Genetic Analysis
mtDNA	Mitochondrial Deoxyribonucleic Acid
NCBI	National Centre for Biotechnology Information
PCR	Polymerase Chain Reaction
RAPD	Rapid Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
rpm	revolutions per minute

SDS	Sodium Dodecyl Sulfate
SET	Sucrose EDTA Tris
SNP's	Single Nucleotide Polymorphisms
SSR	Simple Sequence Repeats
Taq	*Thermus aquaticus*
TE	Tris EDTA

ABSTRACT

Rastrineobola argentea, 'omena' is one of the smallest cyprinid fishes found in the Lake Victoria basin. Currently, *R. argentea* is the most commercially exploited species of Lake Victoria (62.2%) compared to those of *Lates niloticus* (29.9%) and *Oreochromis niloticus* (6.9%). The objective of this study was to morphologically characterize *R. argentea*, determine its genetic diversity and population structure of and test for phylogenetic relationships between three populations in Lake Victoria (Kenya) using SSR, CO1 gene and mtDNA genome. A random stratified sampling was done in a cross-sectional experimental design. A one-way analysis of variance was used to determine whether there was a significant difference between the means of the three populations of fish namely Nyanza Gulf, Port Victoria and Mbita. The results of the one-way ANOVA indicate that the critical *F*statistic, 24.05, was greater than the *F* critical value thereby rejecting the null hypothesis. Comparing values generated using Fisher's exact test confirms that population from Mbita and that of Nyanza Gulf are closely related compared to those of Port Victoria. The Phylogenetic analysis indicated that Mbita population and Nyanza Gulf population were found in the same sub cluster signifying that they are more closely related to each other than to Port Victoria. This signifies that the morphological analysis concurs with the molecular data. Conservation of genetic diversity in species of commercial interest like *R. argentea* fisheries in Lake Victoria is essential to the long-term survival of any species, particularly in light of changing environmental conditions and human pressure.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Background information

Biodiversity has been the central theme of ecology for many years but after the Rio's earth summit in 1992, the development of concepts and tools for sustainable planning and the conservation of biodiversity (Kartavtsev, 2013). Consequently, becoming the focal points for research activities within the biological community, environmentalist, planners and administrators (Potschin & Haines-young, 2006). Measures of biodiversity are seen as indicators of the wellbeing of ecological systems regardless of changing trends and pre-occupation (Khan, 2006). Biodiversity ranging from genetic diversity within population, to species diversity to community diversity across landscapes is now considered an important global change in its own right (Hooft *et al.,* 2003; Somasundaram & Kalaiselvam, 1993). Despite this, global biodiversity is fluctuating at an alarming rate as a result of anthropogenic activities such as over-exploitation of natural resources consequently affecting ecosystem processes (Sala *et al.,* 2000).

Scientist and policy makers are familiar with climate change or concentration of greenhouse gases in projecting the future state of global environment with biodiversity being an important function of ecosystems and the welfare of human beings (Sala *et al.,* 2000). Moreover previous studies have been done to assess extinction threats as an outcome of over-exploitation of natural resource at the global and regional level with freshwater ecosystems being the most affected (Bertorelle *et al.,* 2004). This is because freshwater ecosystems are the focal point for human settlement and extensively modifying the economies, social structure and the riparian zones (Beeton, 2002).

Lake Victoria is the largest tropical Lake in the world with its water shared by three African countries namely Tanzania (51%), Uganda (43%) and Kenya (6%) (Balirwa *et al.,* 2003). It is found 0° 20'N to 3° 00'S and 31° 39'E to 34° 53'E at an altitude of 1134 m (Njiru *et al.,* 2011). It is nearly 68,800km^2 in area and 40 metres deep.

Besides, 30 million people live around the Lake making it one of the world's largest freshwater fishery (Awange *et al.*, 2007). Approximately 1.2 million people with an annual population growth of 3% depend directly or indirectly on Lake Victoria for livelihood (Matsuishi *et al.*, 2006). The Lake is not an isolated water body as its catchment area cuts across national boundaries contributing to the hydrological regime of the Nile basin and the eco-hydrology of the whole region with the vegetated littoral habitat linked to the ecology of the Lake (Williams & Hecky, 2005). Furthermore, the Lake is an important source of food, provision of income, foreign exchange and employment through earnings from exploitation of the fish resources (Tumwebaze *et al.*, 2007). *Rastrineobola argentea* are locally known as 'omena' (Kenya), 'dagaa' (Tanzania) and 'mukene' (Uganda) (Wanink, 1999). This small pelagic fish plays a huge role as the source of animal protein for human population around the Lake Victoria.

1.2 A brief history of Lake Victoria fisheries

The species composition of the catches in Lake Victoria has changed from those which prevailed at the beginning of the 20th Century, when fisheries development started (Ogutu-Ohwayo, 1999). These changes are an outcome of environmental changes, overfishing and introduction of alien species mainly the Nile perch (*Lates niloticus* L) a large predator species and Nile tilapia (*Oreochromis niloticus* L) a detritivore in the Lake (Lysell, 2009). Currently the fisheries in Lake Victoria consist principally of the indigenous Dagaa (*Rastrineobola argentea* P), and the introduced Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*.) (Hecky *et al.*, 2010). Data from the recent past indicate that there are more than 290 species of fish in the Lake (Bille, 2006). By the end of 1940's, fish stocks were under tremendous pressure from robust growth of the international fish markets and the associated transport infrastructure that saw fish being transported down the railway line to the East African coast (Pringle, 2011). Improved fishing technology which saw fleets of boats, outboard motors and the commercial production of artificial-fibre nets and flaxen gill nets being used to catch fish (Balirwa *et al.*, 2003). During the colonial period the more debatable consideration that the fisheries authority had was the enormous amount of *Haplochromis* in the Lake which had little economic

importance and yet comprised of 90% of the Lake's fish biomass (Geheb *et al.,* 2008a). For some, the Lake needed a predator to take advantage of the food source and turn it into an economically more valuable fish meat. To remedy this, a prime candidate the Nile perch (*Lates niloticus*) which was a magnificent predator and had the capacity of growing up to 200kg in weight and more than 2m in length was introduced into the Lake (Kaufman & Victoria , 1992).Besides, the colonial fisheries authority introduced four exotic species of tilapia in the early 1950's to supplement dwindling stocks of the endemic tilapia species (Geheb *et al.,* 2008a). These exotic species are *Tilapia zillii, Tilapia rendalii, Oreochromis leucostictus and Oreochromis niloticus*. Initially, Nile perch was secretly introduced in 1954 from Lake Albert but was officially introduced at Port bell and Entebbe in 1962 and at Kisumu in 1963 by the colonial authorities (Verschuren *et al.,* 2002). This radically transformed Lake Victoria fisheries both ecologically and economically (Hecky *et al.,* 2010; Pringle 2011). At first the alien fish took some time to establish itself and only began to appear in the mid-1970 when trawl surveys and catch landings in the three riparian countries showed an intense population increase (Goudswaard *et al.,* 2002).

This population upsurge also termed as the 'Nile perch boom' was first experienced in the Nyanza Gulf of Kenya in 1979 two to three years later it was also observed in Uganda and four years later in Tanzania (Pringle, 2011). Besides, the boom encouraged a sharp influx of individuals especially women and those involved in traditional fish processing activities into the sector (Thorpe & Bennett, 2004). By mid-70 nearly 80% small scale fishers were exploiting the Lake on a part-time or seasonal basis while the rise in Nile perch population led to creation of new industries and huge export industry for the three countries causing an expansion of the fish-freezing industry (Abila, 2000). Until the 1960s, the native Tilapiine species *Oreochromis esculentus* (Graham) and *Oreochromis variabilis* (Boulenger), *Protopterus aethiopicus* (Heckel), *Bagrus docmak* (Forskåll), *Clarias gariepinus* (Burchell), various *Barbus* species, *mormyrids* and *Schilbe intermedius* (Rüppell) were the most commercially important species (Njiru *et al.,* 2011). However, the *Haplochromis cichlids* and the native cyprinid, *Rastrineobola argentea*, although

abundant, were not originally exploited on a large scale because of their small size (Wanink & Witte, 2000). By 1980's, the catches of *Haplochromis* had crashed whereas an estimated 200 species were driven into extinction; this was described as one of the greatest mass species extinction events in recent history (Seehausen, 2009). *Haplochromis cichlid* was the main prey for the Nile perch and with its rapid population decline the Nile perch shifted its diet to the native fresh water shrimp *Caridina nilotica, L. nilotica* and *R. argentea* which contributes 10-20% of its diet. Freed from competition for food sources by the decrease in *Haplochromis* population, *R. argentea* population had really thrived with 13,000 ton in 1975 to an all-time high of 546,000 ton in 2006 (Abila *et al.,* 2004). This however, is still a challenge since *R. argentea* is currently the main source of food and livelihood of 30 million riparian people found in Lake Victoria region (Abila *et al.,* 2000).

1.3 Morphological characteristics *Rastrineobola argentea*

Rastrineobola argentea is a small zooplanktivorous cyprinid fish which is commonly known as the silver cyprinid (Plate 1). They are somewhat torpedo-shaped which makes it easier to glide through water with a number of fins such as the anal, dorsal, the caudal and a pair of lateral fins comprising of the pelvic and pectoral (Wasko *et al.,* 2003). A fin is considered as a membrane supported by rays and spines that display remarkable locomotors properties in water (Lauder & Drucker, 2004). The bodies of most fish are covered by scales which are used for protection and also in sex discrimination. However, in species such as *R. argentea*, the scales are almost absent and are covered by a thin layer of skin (Requieron*et al.,* 2012). This layer contain the pigment cells which confers colour to fish and produces mucous that enable the fish to be slippery. The mucous contain antibacterial properties that protect the fish from bacteria and toxins in the watery habitat while the colouration enable the fish to determine mate choice and ensure reproductive isolation (Goudswaard *et al.,* 2002). The way of life of a fish is assessed by its body form, different shapes allow fish to be either fast or slow swimmers, bottom dwellers/benthic or live in pelagic zones and in other species it may determine survival in extremes of the oceans (Sharpe, 2012).

The lateral body shape of *R. argentea* is fusiform with a compressed cross-section. A fusiform body shape is compressed at the sides and tapers more at the tail. *R. argentea* has a dorsal head profile which is clearly convex while the position of its mouth is terminal. It has a lateral line which is low on the body and it runs along the lower part of the caudal peduncle. The lateral line has approximately 42-56 scales and two pairs of lateral fins the first being the pectoral fin which is found on the body behind the gills while the pelvic are found on the belly. Its cheek is covered by thin suborbital bones with an overall silver nacreous sheen and a yellow caudal fin (Mu & Leeuwen, 2006).

Plate 1. Fresh 'Omena' collected from Lake Victoria. (Photo taken by Shelmith Wangechi Kigano)

1.3.1 Taxonomy and classification of *Rastrineobola argentea*

Rastrineobola argentea is classified in the following way

Kingdom: Animalia

Eumetazoa: Metazoans

Bilateria: Bilaterally Symmetrical Animals

Deuterostomia: Deuterostomes

Phylum: Chordates

Craniata: Craniates

Subphylum: Vertebrata, Vertebrates

Superclass: Gnathostomata, Jawed Vertebrates

Euteleostomi: Bony Vertebrates

Class: Actinopterygii, Ray-Finned Fishes

Order: Cyprinoformes

Family: Cyprinidae, Minnows or Carps

Genus: *Rastrineobola*

Species: *Rastrineobola argentea,* 'Omena'/ 'Mukene'/ 'Dagaa'.

1.3.2 Adaptation of the *Rastrineobola argentea*

Generally, the abundance and distribution of *R. argentea* in the waters of the earth are the products of the interaction among the fish and their chemical, physical and biological surroundings (Zaccara & Delmastro, 2009). The communities surrounding it are continually changing and are different from one instant to the next. Consequently, like other fishes, *R. argentea* has organically progressed and survived in recognized directions through ecological succession ensuing from morphological adjustment or adaptations that accommodate the pressures of ecological alteration. Therefore, morphometry is an essential ecological character because it can affect reproductive success through the ability to feed, secure territories, avoid predators and attract mates in a species (Hard *et al.,* 1999). Studies suggest that for fish to adapt to local environment selection owing to predation pressure acts on both behaviour and physiology (Archard *et al.,* 2012). For instant, *R. argentea,* has been undergoing a rapid morphological adaptation by shifting its niche due to presence and absence of predator and competitor, respectively in Lake Victoria (Wanink & Witte, 2000). For example, there has been reduction in number of gill rakers by 2.1% while its length decreased by 15% and an increased number of gill filaments which

has been linked to the shift from the pelagic to the benthic zones *(*Ahnelt *et al.,* 2006; Sharpe, 2012). Studies done by Anhelt (2006) from both inshore and offshore specimens indicate that *R. argentea* was able to invade the benthic habitat of the sub littoral zone. The offshore species were predominantly pelagic feeders while those collected from the inshore waters were both pelagic and benthic feeders. Both specimen had four pharyngeal teeth arranged in not more than three rows in ceratobranchial five, which is the last remnant of the gill arch, which is modified to tooth bearing bones an adaptation to food processing (Ahnelt *et al.,* 2006). For prey survivorship, prey must invade deep bottom depth to avoid detection from predator and use the bottom as a refuge habitat considering that predators are distributed near the shore of the Lake (Sass *et al.,* 2006).

1.3.3 The socio-economics importance of *Rastrineobola argentea* in Lake Victoria

Once *Rastrineobola argentea* is harvested from the Lake it is sun dried for six to eight hours along the Lake bed (Bille, 2006). It can be eaten directly or transformed into fishmeal and incorporated into livestock and poultry feed thus providing a source of livelihood for the local farmers (Manyala & Ojuok, 2007). This is reinforced by survey done by Abila (2000) who found that six animal feed manufacturing companies in Kenya were using 70% of *R. argentea* to produce fish meal, while two other companies were using 60% Nile perch skeletons for the same purpose (Matsuishi *et al.,* 2006). This commercially produced feed are not readily available and are expensive to the local farmers (Alacs*et al.,* 2010).

Initially, *R. argentea* was the second most exploited fish of the Lake Victoria with catches in 1989 amounting to 20% of the total catch while that of *L. niloticus* formed the larger portion with 60% of the total catch (Manyala & Ojuok, 2007). The Nile perch population boom in the 1970's led to drastic prey population decrease with an almost extinction of the *Haplochromis* in the littoral and offshore areas (Witte *et al.,* 2007). This subsequently led to dietary change of the Nile perch to that of *R. argentea* leading to decline of cyprinid populations (Geheb *et al.,* 2008b). The Nile

perch is in turn harvested and processed into fillet for the export industry therefore making the *R. argentea* a vital player in the food web of *L. niloticus.*

Currently, *R. argentea* is the most commercially exploited species of Lake Victoria (62.2%) compared to *L. niloticus* (29.9%). Nevertheless, there is an annual drop in yields of *Rastrineobola argentea* reported at the Kenyan part of the Lake *(*Jennings & Kaiser, 1997; Wanink, 1999). This is attributed to overfishing, poor fishing practises such as use of mosquito seine, predation and environmental degradation leading to reduced population size of *Rastrineobola argentea* (Wagner *et al.*, 2012). Despite all these challenges, 'Omena' has successfully survived the Nile perch predation and increased fishing pressure yet its commercial exploitation does not meet the demand of the internal market (Tumwebaze *et al.*, 2007). *R. argentea* population persistence is endorsed by the disappearance of the zooplanktivorous *Haplochromis* which reduced competition for food (Shoko, 2005). This led to an increase in their growth rate manifested by reducing their age at first maturity and decrease in size at maturity (Awiti, 2011). This disruption of the ecosystem is seen as a coping mechanism of *R. argentea* in Lake Victoria especially on the Kenyan side (Archard *et al.*, 2012).

The role of fish in the Lake Victoria basin is imperative as a food resource and a commodity that can earn income from its exploitation and trading thus providing a means of livelihood (Minakawa *et al.,* 2008). Regardless of this key role that fisheries plays, Lake Victoria remains a region prone to debilitating and widespread effects of hunger and famine and the fishing communities are grappling with high and increasing poverty levels (Abila, 2000; Matsuishi *et al.*, 2006). According to the Government records and statistics, poverty among the riparian community is as high as 65% with a 50% increase in poverty between 1994 to2005 in Nyanza counties (Olale & Henson, 2012). The main explanation for this rise of poverty in small-scale fishing communities is both exogenous and endogenous. Exogenous poverty is that which rises from lack of an alternatives outside the fishery sector while endogenous poverty is a result of overexploitation of fish resource (Cinner *et al.,* 2008).

It is no doubt that the introduction of Nile perch has led to staggering increases in the fish production of the Lake and changed the entire production system (Goldschimdt et al., 1993). Prior to the colonial administration, fishery was dominated by fishermen who owned their labour and their fishing gear (Kaufman *et al.*, 1997). Traditional fishing methods consisted of basket traps, hooks and seine nets made of papyrus and had a little impact on the fish stock compared to the modern fishing gear (Etiegni *et al.*, 2011). During and after the colonial administration, fishery was reorganized into fleets drawing on hired labour and improved fishing gear (Olale *et al.*, 2010; Geheb *et al.*, 2008b). This led to reduced fishing per person and the local people are continuously edged out of fishing production by pricing, marketing and processing advantages of the factories (Alcala, 1998; Stewart *et al.*, 2010). The increase in human population and the unequal distribution of wealth generated from use of Lake Victoria has led to food insecurity characterized by malnutrition and famine *(*Awange *et al.*, 2007). Other factors contributing to food insecurity include environmental degradation arising from soil erosion, desertification, and biodiversity loss among other factors (Awiti, 2011). Drought in the Lake region has led to decline of water levels in the Lake which has stemmed from supplying the major towns and cities around the Lake with water (Awange *et al.*, 2007). This has led to drying up of some wetlands that are breeding areas for certain fish for example, Usoma wetland in Kenya signifying that the future of artisanal fishery is at stake while that of commercial fishing will continue to flourish (Kiwango & Wolanski, 2008).

Forty years after the introduction of the Nile perch and the Nile tilapia, only a subset of the basin fauna exists and the limnological conditions are being strongly influenced by intense natural resource use. Subsequently, native species that have survived the Nile perch invasion differ in phenotype from the pre-Nile perch fauna (Wanink & Witte, 2000). Originally *R. argentea* was a pelagic/free swimming fish but with overfishing and the introduction of the Nile perch it has shifted its niche to that of a benthic/bottom dweller and can be found deeper into the Lake. This is a form of an adaptation whereby the native prey adopts a predator-avoidance behaviour (Kiesecker & Blaustein, 1997). Moreover, studies carried out have shown that the mean length of *R. argentea* has significantly reduced in parts of Lake

Victoria that experience over-fishing topped up with the presence of Nile perch as compared to unperturbed regions of the Lake *(*Hard *et al.*, 1999; Sharpe, 2012).

1.3.4 Feeding and breeding habits of the *Rastrineobola argentea*

Rastrineobola argentea once considered a pelagic fish mainly fed on macrophytic planktons such as algae and detritus. However, Diane *et al.* (2012) suggested that the niche invasion by *R. argentea* has led to dietary shift and morphological modifications of the silver Cyprinid. This has been an observation feature of the Nile perch ichthyofauna of the Lake Victoria whose day-time diet was composed of novel prey items such as insects, chaoborous larvae and pupae. Samples collected in 1966 showed that *R. argentea* diet consisted of zooplanktons such as copepods, cladocera and rotifers (Taylor *et al*., 2010). The morphological adjustments of these contemporary *R. argentea* include shorter and more tightly packed gill rackers. The gill racker width is larger compared before the introduction of the alien species while the head and the caudal tail have become diminutive (Sharpe, 2012).

Breeding of *Rastrineobola argentea* takes place all year round with peak time being after the long rains in April-May and after the short rains in August-September (Kaufman *et al.,* 1993). These periods are associated with the Lakes turnover where the Lake completely or partially mixes with subsequent high density of zooplankton and insect bloom that provides food for the *R. argentea.* This provides enough food resources to the species encouraging the breeding process. There is increased number of gill filaments which can be directly linked to recent exploitation of the bottom layer by the "omena" (Balirwa *et al.*, 2003).

1.4 Main threats to fish population in Lake Victoria

1.4.1 Predation, over fishing and biological invasions

East Africa is currently experiencing high fishing pressure which has led to changes in the structure of the freshwater habitats and influenced the diversity, composition, biomass and the productivity of the associated biota (Jennings & Kaiser, 1997). This is an area of concern because human beings are the apical predators and their behaviour determines the ecosystem of the Lake besides the rapid loss of fish stock

caused by over fishing with direct result in the loss of genetic diversity (Kitchell *et al.,* 1997). Overfishing which is an anthropogenic stress can impose phenotypic and genotypic changes in natural populations resulting in evolutionary changes in less than a few centuries (Reznick & Ghalambor, 2001). Moreover, molecular data such as parentage and kinship, mating systems, dispersal rates, population structure, gene flow and effective population size make genetic studies. Genetic studies are recognized as an integral part in understanding the biology of any organism by taking a long-term observation on species survival especially when the species environment changes (Bertorelle *et al.,* 2004; Reed & Frankham, 2001). Maintaining high genetic variation is very critical for adaptive evolution, conservation and population persistence to occur (Allendorf *et al.,* 2008). In this case, fishing induces evolutionary changes in the life history traits of *R. argentea* due to the highly selectivity of the fishing gear that targets and removes large species and large individuals within a species (Stergiou & Tsikliras, 2011). The remaining fish are of a particular age and size thus surviving population is composed of species that mature at a reduced size and age and they confer relatively high fitness under fishing pressure but less than optimal under natural selection (Dunlop *et al.,* 2004). Furthermore the size of the fishing gear used is 10mm whilst the average size of *R. argentea* is 40-50mm (Ogutu-Ohwayo*et al.,* 1997). This is a form of exploitative selection analogous to artificial selection used by Darwin for the intentional selection of certain characteristics in natural populations (Allendorf *et al.,* 2008).

Irruption of Nile perch in the 1950's led to rapid decline of cyprinid population since *Rastrineobola argentea* is the now the main prey for *L. niloticus* (Nile perch) especially after the almost extinction of the *Haplochromis* population from the sub-littoral and offshore areas (Geheb *et al.,* 2008b). Introduction and colonization of alien species plays a social aspect determining the interaction among individuals of the same or different species (Reznick & Ghalambor, 2001). These interactions can either be competition or predation. Generally predation in fish does not eliminate the prey due to replacement by growth but through intraspecific competition of the residual individuals (Keller & Taylor, 2008). Predation results in the survival for the most fit in both the predator and the prey making it an agent of divergent selection

driving intraspecific divergence in morphology, life history and behaviour by altering activity level and habitat use especially in the prey taxa (Kiesecker & Blaustein, 1997). Prey may adapt to the non-native species by adopting a predator avoidance mechanism which result in occupation of a different niche. Over a period of time this coping mechanism and adaptation to the new superimposed situations requires genes to differ across individuals and generate variation from selection that retains a deep genetic pool of variants subsequently aid in survival of this species (Loh *et al.,* 2012).

The dynamics of specific environment are reflected in the continual change within the fish populations that inhabit them therefore evolutionary change or response towards selection depends on the heritability of the trait and strength of the selection (Reed & Frankham, 2001). Fishing-induced pressure and biological invasion are potent forms of novel selection driving plastic and/or evolutionary changes in native prey (Sala *et al.,* 2000). Initially *R. argentea* was a pelagic/free swimming fish but with fishing and the introduction of the Nile perch it has shifted its niche to that of a benthic/bottom dweller (Melián *et al.,* 2010). This is a form of an adaptation whereby the native prey adopts a predator-avoidance behaviour (Kiesecker & Blaustein, 1997). This has led to phenotypic change whereby fish develop increased gill filaments to accommodate the low levels of oxygen in the deep waters (Sharpe, 2012). They have also developed shorter caudal fins that enable them to swim faster while avoiding the predator (Sass *et al.,* 2006). Furthermore, *R. argentea* stretched its habitat, diet including its niche component as a result of competitive release of the *Haplochromis* decrease consequently, massive emigration may increase growth and survival but may cause a species to lose its niche (Witte *et al.,* 2007; Chapman *et al.,* 2008). Interestingly, studies carried out have shown that the mean length of *R. argentea* has significantly reduced in parts of Lake Victoria that experience over-fishing topped up with the presence of Nile perch as compared to unperturbed regions of the Lake (Sharpe, 2012a). Previously published values for *R. argentea* standard length at 50% age of maturity from the waters of Lake Victoria as 43-44mm (1998), 42 (1996/7), 41 (2002/3), 40 (2004/5) (Sharpe, 2012).

1.4.2 Ecological factors

Lake Victoria is a huge socio-ecological resource which is a source of drinking and irrigation water and an effective medium for transportation. On a national level it provides electricity through its hydro-power generation, tourism and a convenient disposal site for human, agricultural and industrial waste (Williams & Hecky, 2005). As much as 75% of the riverine nitrogen enters the Lake from agricultural waste and about 80% of the riverine phosphorous comes from municipal and industrial sewage. Moreover, discharge from the urban and agricultural drainage channels and raw sewage from villages and informal settlement are dumped into the Lake (Kiwango Wolanski, 2008). Increased deforestation to create farms, cultivation, urban effluence and the influx of nutrients which in turn has precipitated large algal bloom that increased eutrophication and exacerbated deep water anoxia (Awiti, 2011).

Environmental forces that impinge on the lives of fishes are many, complex and interrelated in their effect. The thick fringe and floating mat of the water hyacinth has reduced light and oxygen levels causing dense phytoplankton production and anoxia causing widespread death of native fish (Williams & Hecky, 2005). Water hyacinth was reported in Lake Victoria in 1989 but problem associated with it became apparent in early 1990's such as affecting fishermen by fouling their nets and traps reducing their catches and impeding navigation thus preventing their access to landing sites (Stager *et al.,* 2009).

In Lake Victoria thermal stratification leads to hypoxia which is restricted to deeper water of approximately 60m deep and for shorter periods during the rainy season, global climatic changes has affected this stratification with the Lake becoming more warmer and stratification being more stable making the Lake less able to mix effectively promoting low oxygen levels in the deep waters especially between September and April (Njiru *et al.,* 2011). Decomposition of algae that sink to the bottom plus the upsurge of temperature in the deep waters increase thermal stratification that would encourage organic matter production in the deep water resulting in low oxygen content subsequently resulting in occasional mass fish kills (Goudswaard *et al.,* 2002;Williams *et al.,* 1993).

Eutrophication has substantially reduced water clarity in Lake Victoria since 1960's narrowing the spectrum of light in the Lake (Muli & Mavuti, 2001; Ormerod *et al.*, 2010). Light affect the vision of the fish, colouration of its integuments, migration and movement, reproduction, the rate and pattern of growth of fish later causes genetic and ecological differentiation among species (Njiru *et al.*, 2005; Balirwa *et al.*, 2003).

Land use changes such as deforestation, soil erosion, desertification, atmospheric pollution has steered the introduction of both organic and inorganic wastes while the loss of vegetation that acted as filters of the Lake are contributing factors to the lives of lentic or lotic fishes (Beeton, 2002). Nutrient input from adjoining catchment causes eutrophication through release of fertilizers and sewage that encourages algal bloom that instigate anaerobic conditions leading to toxic tides and associated mass mortality (Awange & Ong'ang'a, 2006). Besides, Lake Victoria mixes completely owing to its water movements this physical instability tends to trap fish in hypoxic regions killing local fish (Kaufman &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 (Njuru, 2001; Njiru *et al.*, 2005).

1.4.3 Social factors

Major constraints on expanded consumption of fish were identified as lack of infrastructure such as roads, refrigerated storage and transport, freezing and processing plants. Other constraints include low education level, ineffective wealth distribution systems, relatively high prices, low product quality and lack of effective quality control and inspection systems (Cinner *et al.*, 2008; Pauly & Froese, 2012). Traditional assessment of fish stock is what is currently being used in the Lake Victoria with the amount being recorded as per the total biomass being used to record as the current stock of the Lake (van der Knaap *et al.*, 2002). There is poor communication between the fishers and the administrative authority which proves to

be a hindrance to the development of fisheries in Lake Victoria considering that fish populations change faster than the policy making process (Kitchell *et al.*, 1997).

1.5 Molecular tools for assessing genetic diversity

Molecular markers have become indispensable in providing valuable data on diversity through their ability to detect variation at the DNA level thus determining the genetic variation and biodiversity with high levels of accuracy and reproducibility. The best molecular markers are those that are highly polymorphic and can distinguish multiple alleles per locus and/or are co-dominant. Several methods have been developed to measure genetic diversity of organisms at the molecular level while in the past protein polymorphism such as Rapid Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism RFLP, Amplified Fragment Length Polymorphism AFLP were the techniques of used for assessing species diversity. However in recent times Simple Sequence Repeats (SSR/microsatellites) and Single Nucleotide Polymorphism (SNP), sequencing are more common genetic diversity measurement (Porth & El-Kassaby, 2014).

1.5.1 Simple Sequence Repeats (SSR)

The simple sequence repeats (also known as microsatellites) are ubiquitous to all nuclear genomes of eukaryotes and to a lesser extent in prokaryotes (Muchadeyi *et al.*, 2008). These are sites in the genome that contain many short tandem repeat sequences and range from one to six nucleotide in length. They are classified as mono-, di-, tri-, tetra-, penta-, and hexanucleotide repeats (Wang *et al.*, 2009). Identification of microsatellite is done by designing specific primers that are complementary to the flanking sequence of either side of the repeat unit array. This are then subjected to Polymerase Chain Reaction (PCR) to target and amplify the highly conserved regions in the genome (Schirtzinger *et al.*, 2012). The easiest way to screen for microsatellite is by developing flanking primers pairs generally 18-24 base 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) The different alleles are identified as differences in repeat length among individual within a population and can be assessed by

separating their respective PCR product using electrophoresis (Tautz, 1989; Wang *et al.,* 2009). Their sizes are scored as co-dominant markers in population studies and are used in developing genetic linkage maps for breeding research. Therefore, they provide important information to identify conservation in motifs used in investigating the genetic process of a population thus inferring genetic diversity, marker-assisted diagnosis/ selection, evolution and phylogenetic process (Tambarussi *et al.,* 2009).

1.5.2 Cytochrome *c* Oxidase (CO1) gene

The level of success in species identification can be endorsed by amplification of the 5' region of the Cytochrome *c* Oxidase 1(CO1) gene which is an acclaimed universal marker (Xia *et al.,* 2012; Arif & Khan, 2009). It can be used to rapidly identify a species using a primer set of 648 base pair region of the CO1 gene and accurately amplify a broad range of unknown biological specimen (Echi *et al.,* 2013).This is because the CO1 gene is a highly conserved region with over hundreds of base pairs allowing for amplification of small DNA fragments moreover, it has lower mutation rates (Dawnay *et al.,* 2007). Although CO1 gene cannot be used in measuring diversity it is most effective in species recognition as CO1 gene variation within species is far less than variation between species making it a good diagnostic molecular marker (Waiho *et al.,* 2013).

1.5.3 The mitochondrial D-loop genome

Mitochondrial DNA (mtDNA) is a haploid genome that is maternally inherited and is used to investigating high evolutionary rates of 1.3% per million years in fish. This is attributed to lower effective population size and rapid rates of evolution (Luhariya *et al.,* 2014). MtDNA of most animals range between 16-18 Kilobase and contain 13 proteins genes, 22 transfer RNA, 2 ribosomal RNA and a regulatory region known as control region in the invertebrate or the displacement loop (D-loop) in the vertebrates (Wilkinson & Chapman, 1991). The D-loop lies between phenylalanine tRNA ($tRNA^{phe}$) and the proline tRNA ($tRNA^{pro}$). It is the most rapidly evolving part of the mitochondrial genome contained in the non-coding control region and plays a role in replication and transcription of mtDNA (Hoelzel *et al.,* 1991). The length of the D-

loop is approximately 1Kb and can be easily amplified by PCR then sequenced to determine the molecular diversity (Arif & Khan, 2009). Mitochondrial genome has been fully sequenced and subsequent publications worldwide have revealed regions of polymorphic sites, population specific sites, haplogroups and haplotypes used in phylogenetic studies especially due to the rich variability within the D-loop region (Hofmann *et al.,* 1997). In cyprinid fish species the ATPase 8 and ATPase 6 genes within the mtDNA is used as a genetic marker and has been utilized in tracking genealogies and variation in progenies of crosses along with analysing both phylogeny and phylogeography in several fish species (Luhariya *et al.,* 2014). Therefore, mtDNA is highly variable hence important in assessment of genetic diversity of species under study.

1.6 Statement of the problem

Rastrineobola argentea is the source of food and livelihood of over 30 million riparian people found around the Lake Victoria region and beyond (Odada *et al.,* 2004). This small pelagic fish plays a huge role as the source of animal protein for these rapidly growing riparian communities (Owaga *et al.,* 2009). It is also transformed into fishmeal and incorporated into livestock and poultry feed thus providing a source of livelihood for the local farmers (Bille, 2006). It therefore plays a pivotal role in food security and poverty alleviation among the Lake Victoria communities (Kayombo & Jorgensen, 2003). The *R. argentea* fishery in Kenya is currently experiencing high fishing pressure which has led to reduced populations (Kizito *et al.,* 2006). This is an area of concern because rapid loss of fish stock caused by over fishing directly results in the loss of genetic diversity. Genetic studies are currently recognized as integral in understanding the biology of any organism by taking a long-term view on species survival especially when the species environment changes. Maintaining high genetic variation is very critical for adaptive evolution and population persistence to occur. This is very evident when a population is subjected to high selection levels for example when there are diseases or environmental pressures. When this occurs an entire species could easily be wiped out. This therefore puts species with little or no genetic variation at a high risk of extinction. Moreover with little genetic variation within species, healthy reproduction

becomes increasingly difficult with and often leads to inbreeding. The reproductive biology, dispersal, trophic ecology, population dynamics and socio-economics of the 'omena' fisheries have been well documented in Lake Victoria (Kenya). However, to date no studies have been conducted on the genetic diversity and characterization of *R. argentea* found in Lake Victoria (Kenya).

1.7 Justification of the study

Sustainable use of natural resources is imperative in the conservation of heavily exploited species such as those of *R. argentea* which is the most commercially harvested species of Lake Victoria with 62.2% of the total catches. Furthermore, its population size and diversity is threatened by introduction and persistence of invasive species and degradation of the freshwater habitats. This study which is a form of biodiversity assessment will expose the impact severity of disturbances accompanying heavily exploited fishery resource. Besides there is rising evidence that overexploitation of species can lead to direct demographic extinction of species/populations of interest or the permanent loss of population biodiversity. Fishing, a form of selective evolutionary stress removes a particular size of fish from the general population tampering with the existing phenotypic and genotypic variation. This in turn leads to reduced genetic diversity within the affected species compromising the evolutionary potential of these populations (Hauser *et al.*, 2002). Genetic diversity is fundamental in species adaptability to change in ecosystem and to short-term environmental strain and permit long-term evolutionary success. The effects of reduced genetic diversity influences the population fitness and persistence by contributing to inbreeding patterns that lead to homogeneous populations that are vulnerable (Pfennig *et al.,* 2010). Elucidating genetic structure and gene flow patterns of *R. argentea* populations is essential towards defining management and conservation strategies for the Lake Victoria cyprinid. Moreover, molecular based studies suggest that populations that exist as distinct genetic entities need to be exploited, managed and conserved as different genetic entities (Nussey *et al.*, 2007). In contrast, *R. argentea* has always been managed and exploited as a single stock in Lake Victoria therefore, identifying and understanding the genetic population structure of *R. argentea* is vital for management of this socio-economically

important species considering that genetic studies in Lake Victoria 's cyprinid are largely lacking (Shitote *et al.,* 2012).

1.8 Null Hypothesis

H_o: There is no genetic diversity between the three population fragments of *R. argentea* in Lake Victoria, Kenya.

1.9 Objectives

1.9.1 General objective

To determine the genetic diversity using microsatellite tools, population structure and morphological characterization of *R. argentea* in Lake Victoria, Kenya.

1.9.2 Specific objectives

1. To morphologically characterize *R. argentea* within the three localities along the Lake Victoria (Kenya).
2. To determine the genetic diversity of *R. argentea* populations in the Lake Victoria (Kenya) using Simple Sequence Repeats (SSR) sequencing of CO1 gene and D-loop of mitochondrial genome.
3. To determine the population structure of *R. argentea* sampled within the three localities along the Lake Victoria (Kenya).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study area

The study was carried out at the three sites of Lake Victoria on the Kenyan side (Figure 2.1). This included the Nyanza Gulf which has a catchment area of 3600km^2 and accounts for 30% of the total Lake Victoria riverine inflow Mbita to the West of Nyanza Gulf, while Port Victoria to the North West of Nyanza Gulf (Njuru, 2001).

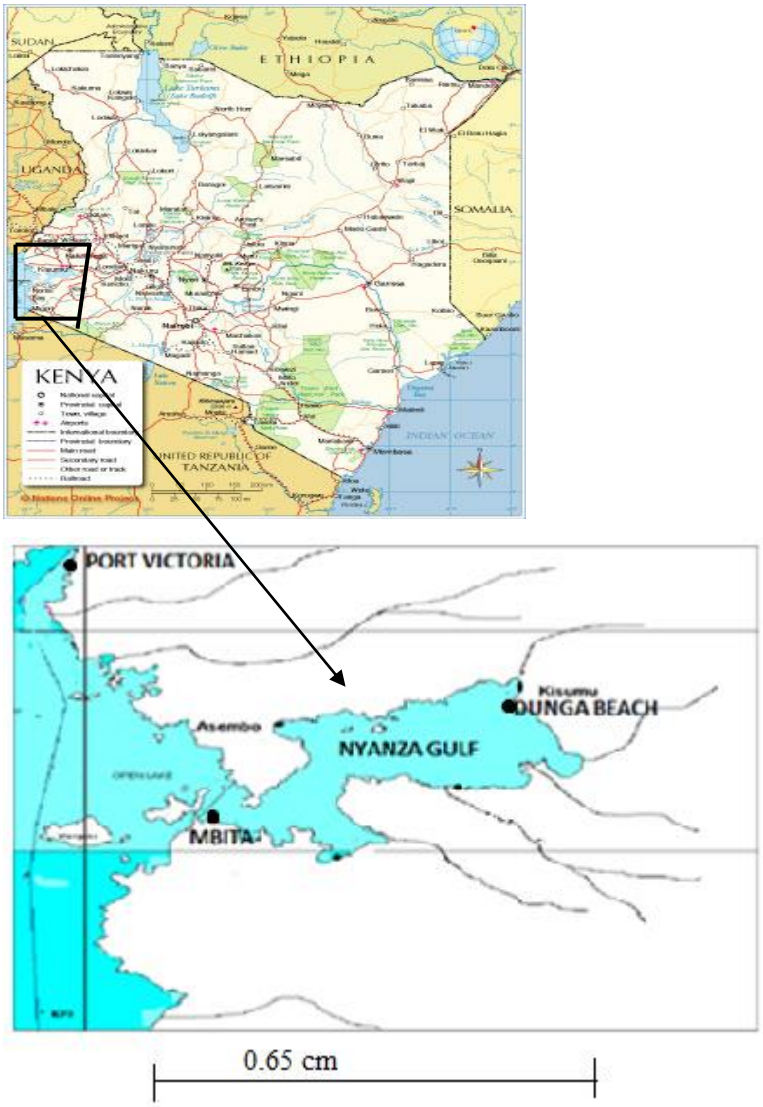

Figure 2.1 Map showing the different sampling sites along Lake Victoria (Kenya)

2.2 Sample collection

Samples of *R. argentea* were collected from each of the three sampling sites namely the Kibuyu beach in Mbita, Senyenye beach in Port Victoria and Dunga beach in Nyanza Gulf in Kenya. Fish collections were made in June 2013, August 2013 and December 2014. In each sampling site ten fishermen were recruited into the study and sent to fish using small-mesh of 3.2-6.4 mm seine. A seine is a fishing net that hangs vertically in the water having floats at the upper edge and sinkers or weights at the bottom. A total of thirty fish samples were collected per site making a total of ninety samples. The samples were sorted to remove unwanted species and later washed with clean distilled water and immediately preserved in 90% Ethanol. The samples were then transported to Institute of Biotechnology Laboratory (IBR) in Juja in a cool-box. Samples that were excluded from the study were juvenile that had a standard length of less than 4cm while the specimens included in the study were mature adults above 3.0cm in length. This study did not discriminate males from females or vice versa.

2.3 Sample size determination

The sample size (*n*) was determined using the following formula by Sereno (2007):

$$n = \left(\frac{ts}{SE}\right)2$$

Where SE is the standard error, *t* is the t-score used to calculate the confidence interval which is dependent on the degree of freedom and the confidence level, whereas *s* is the standard deviation. A total of thirty samples per sampling site were required for both morphological and molecular work.

2.4 Determination of morphological characterization of *Rastrineobola argentea*

The phenotypic characterization of *R. argentea* was performed on the fish. the following nine phenotypic descriptors were used for morphological characterization; standard length, fin height, fin length, mid height, position of the operculum, presence of melanophores, eye colour, eye length and the general colour of the fish.

The fish were measured for each of the phenotypic descriptors described above using a calliper and a ruler except the general and eye colour of the fish. These measurements were then scored and recorded. Fish that recorded relatively distinct characteristics were identified and selected for further genetic characterization using molecular markers.

2.5 Determination of genetic diversity of *Rastrineobola argentea*

2.5.1 DNA extraction of *Rastrineobola argentea*

Approximately 3mm^2 of caudal fin was clipped and transferred to a labelled eppendorf tube. Genomic DNA was extracted using a salt purification protocol optimized for approximately10-30 mg tissue as described by Lysell (2007). Briefly in order to lyse the cell membranes, the tissue was submerged in 340 µl Sucrose-EDTA-Tris buffer, 12.5 µl Proteinase K (10mg/ml) and 20 µl of 20% Sodium Dodecyl Sulphate and subsequently incubated in 55˚C for two hours. A 300vmicrolitre of 5M NaCl was added, and the sample was vortexed for 20 seconds followed by centrifugation at 13 000 rpm for 10 minutes. 600 microlitre of the supernatant was transferred to a new 1 ml eppendorf tube, 150 µl of Tris HCl (0.01M pH 8.0), and 750 µl freezer-cold 2-propanol was added prior to at least thirty minutes of precipitation. The sample was then centrifuged at 10 700 rpm for 15 minutes. The supernatant was discarded through decantation, leaving the DNA pellet affixed to the bottom of the tube. The pellet was be then washed with 1 ml 70% cold ethanol and centrifuged at 10 700 rpm for 10 minutes. The DNA pellet was dried by inverting the eppendorf on a piece of tissue paper. After drying overnight, the DNA pellet was then dissolved in 100 µl TE-buffer (pH 7.6) and quantified using both 0.8% agarose gel electrophoresis and spectrophotometry. The stock solution was stored at 4˚C.

2.5.2 Determination of DNA concentration

The concentration of genomic DNA was determined by the optical density/absorbance which was generated automatically using a spectrophotometer. The quantification was done by mixing 5 µl of DNA with 495 µl of double distilled water in a microfuge tube and subjecting the diluted DNA sample into 10mm ultraviolet-transparent cuvettes that was loaded into a spectrophotometer. The same

cuvettes were used to set standards using blanks containing double distilled water. The absorbance readings were performed at 260nm (A_{260}) where DNA absorbs light most strongly and the number automatically generated allows one to estimate the concentration of the DNA solution.

2.5.3 Primer selection and design

Simple sequence repeats primer design

Primers were designed for microsatellite characterization, Cytochrome c Oxidase 1 gene identification and for mitochondrial DNA sequencing. Microsatellite primers were developed from homologous sequences of related cyprinid species. They were tested for cross-amplification efficiency. Resource species for the primers used in this study were: *Barbodes goniotus* (Nguyen, 2008), *Campstoma anomalum* (Waits *et al.,* 2008)*Cyprinus carpio* (Crooijmans *et al.*, 1997), *Hypophtalmichtys molitrix* (Schofield *et al.,* 2010) and *Labeo rohit*a (Kumar *et al.*, 2007). Included in this study were 10 microsatellite loci chosen among 24 loci tested for cross species amplification and tentative size ranges was recorded. Details on the primers used in this study are given in Table 2.1.

Table 2.1.: Primer selected for multiplex PCR reactions of samples

Locus code	Primer sequence	Reference
Bgon22	F-TCTTGTTGATCACACGGACG R-GTGACTGTATCAATGAGTCTG	Nguyen, (2008)
Hmo26	F-GATTTCAGGCACATTGCTTATCT R-GAGCGTTTCTCATTTGTACTTATTTT	Schofield, (2010)
Lr3	F-ATCTGGCTGCCTATTCACC R-CATCGGCGACTGCACTGGA	Kumar, (2007)
Lr10	F-GATCTTCAGCGCCAGCGTG R-GAGGACCTGCCCAGCATG	Kumar, (2007)
Lr12	F-CACCGCTGCTGTCCATCA R-AGGTCGGCCAGATACACG	Waits, (2008)
Lr14b	F-TCACATGGGAACAACAAACC R-CCGCCGCTTACCCATCAC	Waits, (2008)
Lr21	F-GATCAGAGGGTCAATGTGG R-CAGCAGAGTACTATGGAAGA	Kumar, (2007)
Lr24	F-CAAGGCCAAAAGTGTCCAT R-AGGAAATTGGTAAAGTGTTTC	Waits, (2008)
MFW15	F-CTCCTGTTTTGTTTTGTGAAA R-GTTCACAAGGTCATTTCCAGC	Croojimans (1997)
MFW26	F-CCCTGAGATAGAAACCACTG R-CACCATGCTTGGATGCAAAAG	Croojimans,(1997)

CO1 primer and mtDNA primer sequence design

Primers were designed from CO1 gene of *Barbus chicapaensis* with 513bp deposited at NCBI database. The primer sequence used in the amplification of both the CO1 and mtDNA sequence are listed in Table 2.2.

Table 2.2 List of CO1 and mtDNA primers selected for PCR optimization and amplification process.

Primer name	Primer type	Primer sequence
Primer 1	CO1	Forward 5' ACCCTCTATCTTGTATTTGG 3'
		Reverse 5' ACGAGGTGTTGAGATTACGA 3'
Primer 2	CO1	Forward 5'GTATTTGGTGCTTGAGCTGGT 3'
		Reverse 5' TGTTACTAAGACGGCTCAGA 3'
Carp-Pro	MtDNA	AACTCTCACCCCTGGCTACCAAAG
Carp-Phe	MtDNA	CTAGGAATAATCTTAGCATCTTCAGTG

2.5.4 Optimization of PCR conditions

A total of 10 polymorphic microsatellite markers, 2 CO1 primers and 2 mtDNA primers were used for optimization and amplification of DNA molecule. PCR amplification was carried out in a total volume of 10 µl containing 1µl (50-200 ng) sample DNA, 1 µl 10X reaction buffer (500 mM KCl, 100 mM Tris [pH 9.0], 10% Triton-X 100), 200 µM of each dNTP, 1-2mM$MgCl_2$, 5 pmols of each PCR primer, and 0.1 µl Taq DNA polymerase. PCR amplification consisted of 25-30 cycles of denaturation ('hot start') at 94°C for 30 seconds, annealing at 56-62°C for 30 s, and extension at 72°C for 30 s, preceded by an initial denaturation step at 94°C for two min. The PCR products were analysed on 3% agarose gels and visualized by agarose gel electrophoresis.

2.5.5 Agarose Gel electrophoresis

The presence of DNA was determined by using gel electrophoresis under the influence of electric current. DNA has a negative charge and therefore migrates towards the anode. High agarose gels concentration inhibit the movement of molecules across the gel while the low gel concentrations separate heavier molecules

thus genomic DNA was ran on 0.8% agarose concentration while the PCR product (amplified DNA fragment) were ran on 0.3% agarose gel concentrations. A 5µl of Ethidium bromide, a fluorescent dye allowed the detection and visualization of DNA fragments and the PCR product in the agarose gel. DNA intercalates with the dye and appears as a bright fluorescent band. One kb ladder was used in sizing the genomic DNA, COI gene while a 500bp ladder was used in determining the microsatellite and mtDNA. The ladder was mixed with 6% blue loading dye. The use of agarose gel electrophoresis reduces the chance of performing a PCR reaction with sheared or absent DNA or genotyping failed PCR reactions.

2.6 Population structure

2.6.1 Sequence editing and alignment

DNA sequences were cleaned and aligned using Bioedit and MEGA ClustalW-2.1-win respectively. Aligned sequences were imported from MEGA Clustal W-2.1-win into MEGA 6 Kumar *et al.,* (2007) for exporting polymorphic sites and multiple sequence alignment was done by Clustal W software. Phylogenetic trees among the *Rastrineobola argentea* mtDNA haplotypes were estimated using MEGA6 software and compared to other species deposited at NCBI using Basic Local Alignment Search Tool (BLAST). The species that closely related to the species *Rastrineobola argentea* were *Salmostoma bacaila* (Hamilton), *Brachydanio rerio* (Hamilton), *Luciosoma bleekeri* (Steindachner), *Cabdio morar* (Hamilton)which were used as out groups for all trees. The Kimura-2 parameter model was used to estimate evolutionary diversity within population, diversity of an entire population, the mean inter-population diversity, the coefficient of differentiation, the evolutionary divergence between sequences, overall sequence pairs, overall sequence pairs within groups and between groups and between groups of sequences (Xia *et al.,* 2012).

2.6.2 Phylogenetic analysis

Evolutionary relationships with taxa and history were determined by using the Neighbour-Joining model (Quinteiro*et al.,* 2000). The evolutionary distances were done using Maximum Composite Likelihood (Tamura *et al.,* 2011).Phylogenetic trees were constructed using heuristic searches, 1000 replicates of random additions

of sequences, equally weighted characters and nucleotide transformation. To increase the robustness of the phylogenetic analysis an evaluation of statistical confidence in nodes were determined by 1000 bootstrap replicates in Neighbour-Joining. A Monte Carlo test (1000 replicates) was used to estimate the (calculated probability) *p*-values (Kumar & Gadagkar, 2001). In this study population relationships were identified and assessed by multilocus allele frequency data and statistical approaches for clustering these populations with a dendogram to identify genetically similar group and subsequently aid in identifying specific management units where appropriate. To examine genetic differentiation between populations, exact tests for population differentiation as well as calculation of pair-wise estimates offixation index (FST)were carried out using MEGA6.

2.6.1 Statistical analysis

The statistical software that was used in this study was MEGA 6 in which ANOVA was used in assessing the morphological differences while Tajima Neutrality test was used in determining sequence polymorphism. Fisher's test was used in determining the number of synonymous and non-synonymous differences between sequences. Chi test was used in estimating equal evolutionary rates between lineages while the Disparity index test was used in analysis the structuring of sub-population

CHAPTER THREE

RESULTS

3.1 Morphological characterization of *Rastrineobola argentea* using phenotypic characters

A total of 90 sampled fish were identified based on the external morphological characters such as the presence or absence of the melanophores, the standard length, mid height, operculum position, diameter of the eye/ eye length, the head length, the fin length and height (Table 3.1).

Based on the morphological characters mentioned above, fish sampled from Port Victoria displayed different characteristics compared to those of the other sites. They were smaller in size compared with samples from Mbita and Nyanza Gulf, exhibited a yellow caudal/tail fin and melanophores (pigmentation) on the upper/ pectoral side of the fish (Plate 3.1 and 3.2). The general cross-sectional body shape of all the fish samples was fusiform and the outer eye composed of an off white lens surrounded by a silver iris and covered by a thin colourless film.

Table 3.1. Multivariant morphometric data from natural populations of *Rastrineobola argentea* along the Lake Victoria

	Nyanza Gulf	**Port Victoria**	**Mbita**
Standard length (mm)	42.9	37.9	39.8
Mid height (mm)	7.1	6.2	7.0
Head length (mm)	4.8	4.1	4.3
Operculum length (mm)	3.2	2.4	2.5
Position of the operculum (mm)	34.4	30	32.6
Eye length (mm)	2.2	2.1	2.0
Fin length (mm)	7.2	6.6	7.2
Fin height (mm)	3.5	3.0	4.2
Melanophores	Absent	Present	Absent

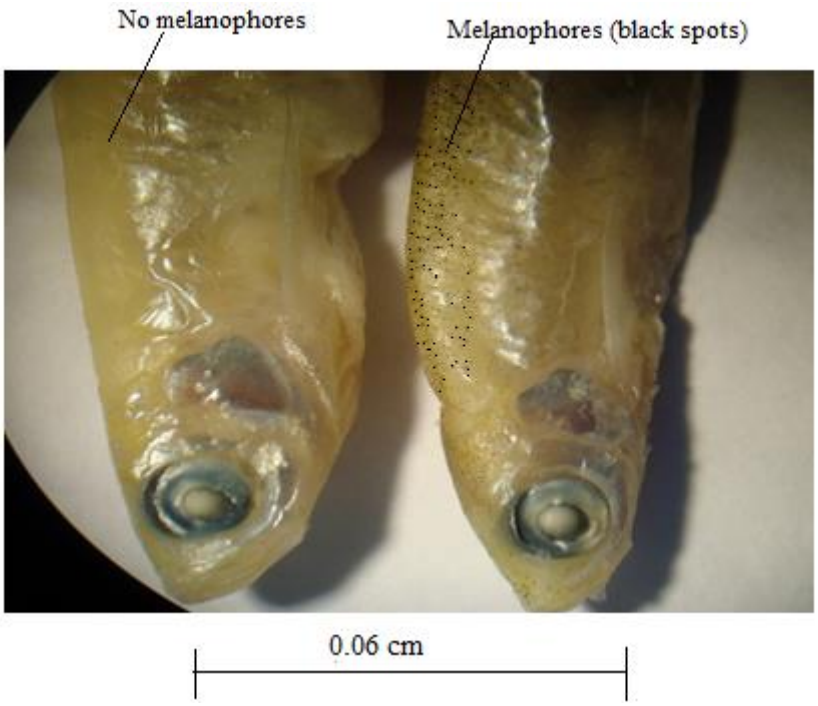

Plate 3.1: Specimen of *Rastrineobola argentea* showing characters used in this study such as eye colour and presence of melanophores.

The melanophores only appeared in samples collected from Port Victoria . Melanophores are black spots or pigmentation found on the body of the fish especially on the pectoral side.

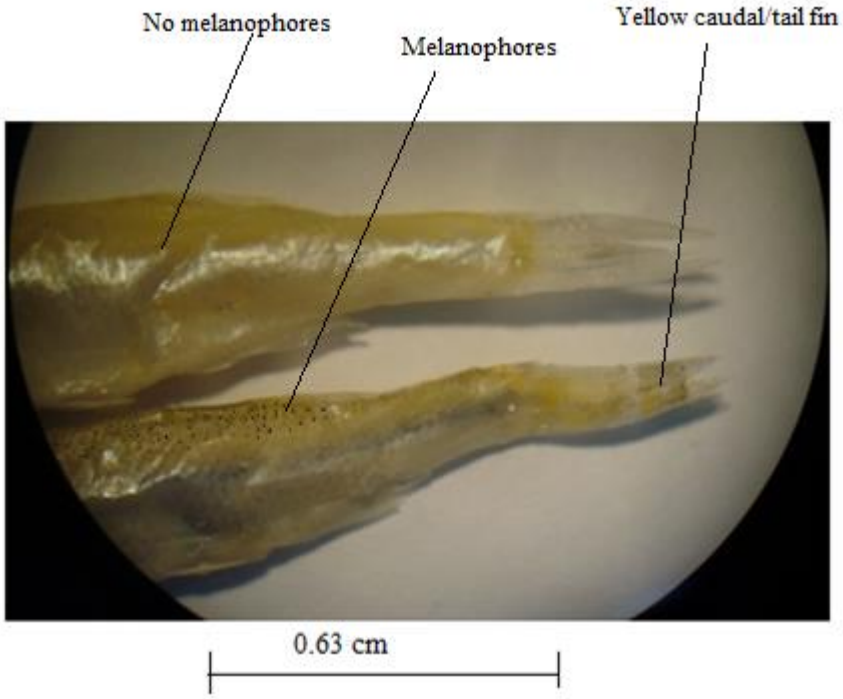

Plate 3.2: Fish specimen of *Rastrineobola argentea* displaying a yellow tail fin and presence of melanophores.

A one-way analysis of variance was used to determine whether there was a significant difference between the mean lengths of the three independent populations of fish namely Nyanza Gulf, Port Victoria and Mbita. The results of the one-way ANOVA indicate that the critical *F* value for α=0.05, and (*k*-1)and (*N*-1) degree of freedom as 2 and 88 respectively is 3.100. Since the *F* statistic, 24.05, is greater than the *F* critical value the null hypothesis is rejected therefore the morphological diversity between the populations is significant (Table 3.2).

Table 3.2. Analysis of variance of the single factor of the standard length of the three population along the Lake Victoria, Kenya.

Sites	***Count***	***Sum***	***Average***	***Variance***
Nyanza	31	1330	42.90323	9.090323
Port Victoria	30	1139	37.96667	5.412644
Mbita	30	1194	39.8	9.2

ANOVA

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	380.7105	2	190.3552	24.05144	0.0465	3.100069
Within Groups	696.4763	88	7.914504			
Total	1077.187	90				

The results obtained from the precise measurement of intra-population and inter-population of morphological markers such as the standard length, allowed interpretation of patterns of distinct phenotypic variation in the three populations.

3.2 Molecular characterization of *Rastrineobola argentea*

3.2.1 DNA isolation of *Rastrineobola argentea*

To obtain the optimum quality of DNA from the sampled fish, two tissues were isolated for their DNA. These tissues were muscle and caudal fin. The DNA extracted from the muscle was of better quality compared to that from the caudal fin as evidenced from the brighter and more fluorescent band it produced. However this muscle DNA degraded really fast especially when left overnight. Due to this fact, DNA isolated from the caudal fin was more reliable for the molecular analysis and used during this study. To evaluate the purity of the DNA, a spectro Ultra violet-Visual spectrophotometer was used to read the samples at a spectrum of 230nm to

320 nm. Good quality DNA has an A_{260}/A_{280} ratio of 1.7-2.0. A reading of 1.8 and 0.7 was obtained from caudal fin and the muscle tissue respectively. The first six lanes (1-6) were loaded with DNA isolated from the caudal fin and did not produce any smears when subjected to electrophoresis while those loaded on lane seven to eleven (7-11) contained DNA isolated from the muscle tissue and produced smears (Figure 3.1).

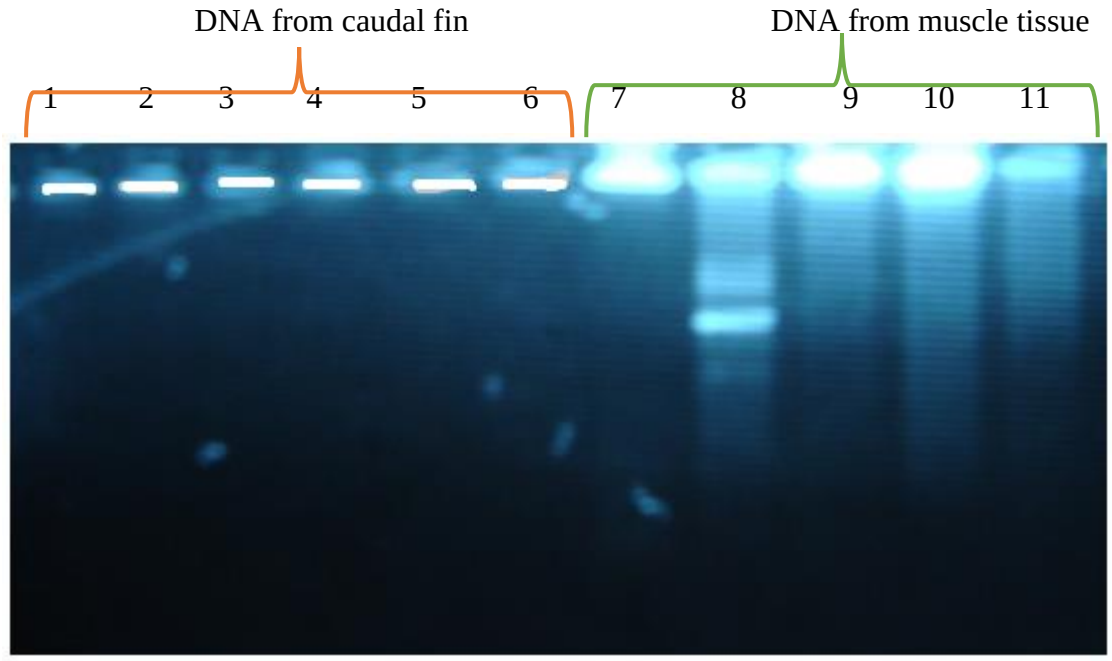

Figure 3.1. Gel electrophoresis of DNA extracted from two fish tissue isolated from *Rastrineobola argentea*.

3.2.2 PCR amplification using Simple Sequence Repeats (SSR)

DNA isolated from all the fish samples from all the three sites were amplified using the primers listed in Table 2.1. Some primers used such as Bgon22 and Lr12 did not amplify any of the DNA isolated from the three sites (see lanes 2 and 6, respectively, Figure 3.2). On the other hand, primer Hmo26, Lr3, Lr10, Lr14 and Lr21 successfully amplified the DNA (see lane 3, 4, 5, 7and 8, respectively, Figure 3.1). Primers Hmo26 and Lr14 amplified DNA isolated from fish sampled from Nyanza Gulf (see lane 3 and 7, respectively, Figure 3.2). Primer Lr3, Lr10 and Lr21 amplified DNA isolated only from fish sampled from Port Victoria and produced polymorphic sites (see lane 4, 5 and 8 respectively, Figure 3.2). A molecular marker of 100bp was loaded in lane M. Primers Bgon22, Lr12, Hmo26, Lr3, Lr10, Lr14 and

Lr21 did not amplify DNA isolated from Mbita. However, primers MFW15, MFW26 and Lr24 amplified DNA isolated from Mbita(see lane 1-5, 6-11 and 12-19, respectively, Figure 3.3). A total of twenty amplicons were sent to Macrogen (Netherlands) for sequencing. Upon sequencing they did not produce any sequencing signals.

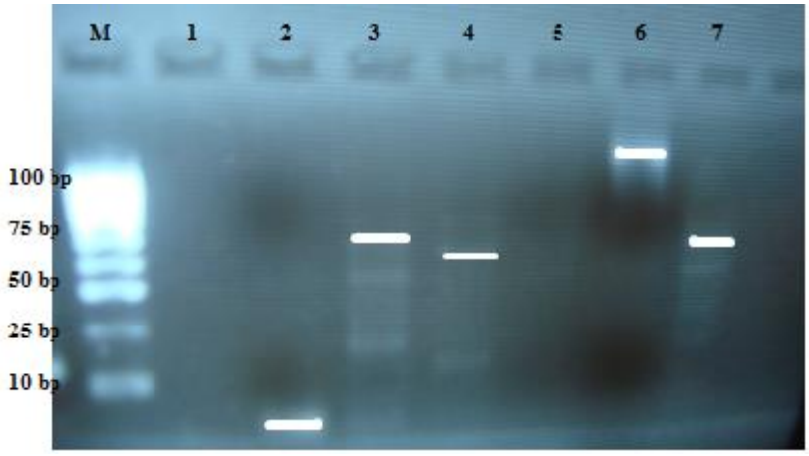

Figure 3.2. PCR product using SSR markers used to amplify DNA isolated from *Rastrineobola argentea* Nyanza Gulf and Port Victoria along Lake Victoria (Kenya).

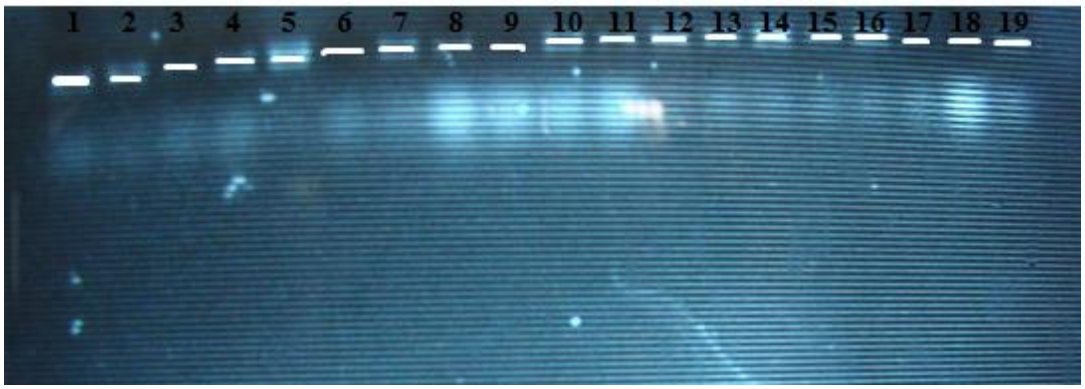

Figure 3.3. PCR product using SSR markers used in amplification of DNA isolated from *Rastrineobola argentea* Mbita beach along Lake Victoria (Kenya).

3.2.3 Cytochrome C Oxidase 1 (COI) gene amplification

The products obtained from the amplification process using the CO1 gene listed in Table 2.2 were loaded on a 0.8% agarose gel as shown in Figure 3.4. Lane 1-8 were loaded with products of amplification from Nyanza Gulf while lanes 8-11 with products of amplification from Port Victoria and lanes 19-27 were loaded with products of amplification from Mbita. Primer set 1 was only loaded on the upper part

of the gel while the primer set 2 was loaded on the lower part of the gel as shown in Figure 3.4. A 1000 bp molecular marker (M) was loaded between 8-11 and 18-19 as shown in Figure 3.4.

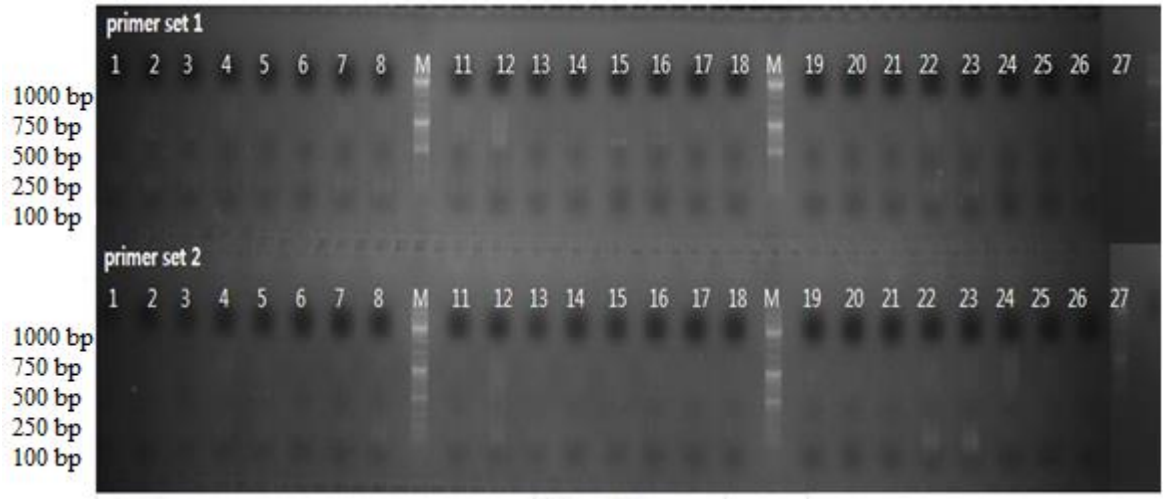

Figure 3.4. No amplification of the CO1 gene from the *Rastrineobola argentea* sampled from the three sites along Lake Victoria (Kenya).

3.2.4 Mitochondrial DNA D-Loop region (mtDNA)

The amplification products from Carp-Pro were loaded on lane 1-8 while those of Carp-Phe were loaded on from lane 10-16 as shown in Figure 3.5 . Lane 0 and 9 contained the molecular ladder 500bp. Eleven band were observed with amplicons on lanes 2, 4, 5, 7 and 8 producing similar polymorphic sites while those amplified with Carp-Phe on lane 12, 13 and 16 produced polymorphic sites. These eleven bands were sequenced and only those which were loaded on lane 1, 4 and 5 produced a sequencing signals.

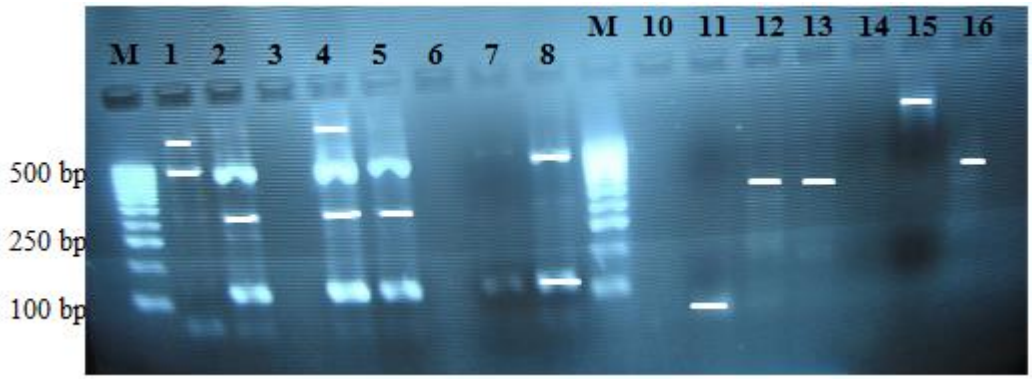

Figure 3.5. PCR amplification of mtDNA D-loop from the *Rastrineobola argentea* sampled along the three sites of Lake Victoria (Kenya).

3.3 Genetic diversity assessment of *Rastrineobola argentea*

3.3.1 Evolutionary diversity

The evolutionary diversity analyses were done using Kimura 2-parameter model involving 9 nucleotides. The highest mean evolutionary diversity was that of the entire population with the number of base substitutions per site as 0.229±0.014 while the lowest mean diversity calculations were that of the inter-population with values of 0.086±0.009 (Table 3.3). This suggests that the mean evolutionary diversity between sub-populations is lower than within sub-population with 0.142±0.01 values while the coefficient of evolutionary differentiation was 0.377±0.028.

Table 3.3 Estimates of Mean Evolutionary Diversity and their corresponding standard errors. Standard error estimates were obtained by a 1000 replicate bootstrap procedure.

	Mean diversity	**Standard error**
The mean diversity within sub populations	**0.142**	**0.010**
Mean diversity in entire population	**0.229**	**0.014**
Mean Inter-population diversity	**0.086**	**0.009**
Coefficient of differentiation	**0.377**	**0.028**

3.3.2 Tajima neutrality test

The number of nucleotide position at which a polymorphism is found (also known as the number of segregating sites *S*) was 248. The average per nucleotide diversity (π)(also referred to as the average heterozygosity at nucleotide sites) was found to be 0.191 while the Tajima D statistic was 0.824 (Table 3.4).

Table 3.4. Results from the Tajima's Neutrality Test

M	*S*	p_s	Θ	Π	*D*
9	248	0.449	0.165	0.191	0.824

Abbreviations: m = number of sequences, n = total number of sites, S = Number of segregating sites, p_s = S/n, $\Theta = p_s/a_1$, π = nucleotide diversity, and D = Tajima test statistic.

3.3.1 Test of selection using Fisher's exact test

The numbers of synonymous and nonsynonymous differences between sequences were estimated using the Nei-Gojobori method. Comparing values generated using this method confirms that *R. argentea* 1 and *R. argentea* 5 are closely related with *P* value of 1 while the *P* values between *R. argentea* 4 and both *R. argentea* 1 and 5 are 0.759 (Table 3.5). Therefore the probability (*P*) of rejecting the null hypothesis of strict-neutrality in favour of the alternative hypothesis of positive selection is shown for each sequence pair. Probability values smaller than 0.05 are considered significant at the 5% level.

Table 3.5. Results from Fisher's Exact Test of Neutrality for sequence pairs

	1	2	3	4	5	6	7	8	9
1. Salmostoma_bacaila									
2. Brachydanio_rerio	1.000								
3. Luciosoma_bleekeri	1.000	1.000							
4. R_argentea_1	1.000	0.519	1.000						
5. R_argentea_5	1.000	0.519	1.000	1.000					
6. R_argentea_4	1.000	0.544	1.000	0.759	0.759				
7. Raiamas_guttatus	1.000	1.000	1.000	1.000	1.000	1.000			
8. Raiamas_senegalensis	1.000	1.000	0.484	1.000	1.000	1.000	1.000		
9. Cabdio_morar	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

The equality of evolutionary rate between sequences *R. argentea* 1 and *R. argentea* 5, with sequence *R. argentea* 4 used as an out-group in Tajima's relative rate test and represent sequence A, sequence B and sequence C respectively (Table 3.6). The analysis involved three nucleotide sequences with codon positions included were 1st+2nd+3rd+Noncoding. There were 576 counts of identical sites in all the three sequences while the divergent sites in all the three sequences were 0. Sequences A and B had 0 unique differences in comparison to the 1 unique difference in sequences C .The chi-square χ^2 test statistic was 0.00 (P = 1.00000 with 1 degree of freedom).

Table3.6. Results from the Tajima's test for three sequences from Lake Victoria (Kenya).

Configuration	Count
Identical sites in all three sequences	576
Divergent sites in all three sequences	0
Unique differences in Sequence A	0
Unique differences in Sequence B	0
Unique differences in Sequence C	1

3.3.5 Computed pairwise distance

Table 3.7shows the F_{st} value and their levels of significance for pairs of population. Standard error estimate(s) are shown above the diagonal and were obtained by a 1000 replicate bootstrap procedure. Analyses for *R. argentea* 1/ *R. argentea* 5 were 0.000 while that of *R. argentea* 5/ *R. argentea* 4 were 0.002 which is similar to *R. argentea* 1/ *R. argentea* 4 as shown in Table 3.7. The smallest divergence was between populations 1 and 4 as well as between populations 5 and 4 but there was no divergence between populations 1 and 5. This means that population 1 and 4, and population 4 and 5 are related but not populations 1 and 5.

Table 3.7. Estimates of Evolutionary Divergence between sequences of *Rastrineobola argentea* and its closely related taxa

	1	2	3	4	5	6	7	8	9
1. Salmostoma_bacaila		0.019	0.022	0.026	0.026	0.025	0.025	0.025	0.029
2. Brachydanio_rerio	0.177		0.021	0.025	0.025	0.025	0.025	0.028	0.029
3. Luciosoma_bleekeri	0.213	0.206		0.022	0.022	0.022	0.021	0.023	0.026
4. R_argentea_1	0.289	0.277	0.228		0.000	0.002	0.016	0.019	0.029
5. R_argentea_5	0.289	0.277	0.228	0.000		0.002	0.016	0.019	0.029
6. R_argentea_4	0.286	0.280	0.226	0.002	0.002		0.016	0.019	0.029
7. Raiamas_guttatus	0.264	0.262	0.202	0.145	0.145	0.143		0.018	0.026
8. Raiamas_senegalensis	0.288	0.306	0.241	0.183	0.183	0.186	0.167		0.028
9. Cabdio_morar	0.338	0.338	0.280	0.330	0.330	0.327	0.280	0.318	

The diversity between *Rastrineobola argentea* and its closely related taxa is significant. However between the different fish samples the disparity was not that significant as shown in Figure 3.6

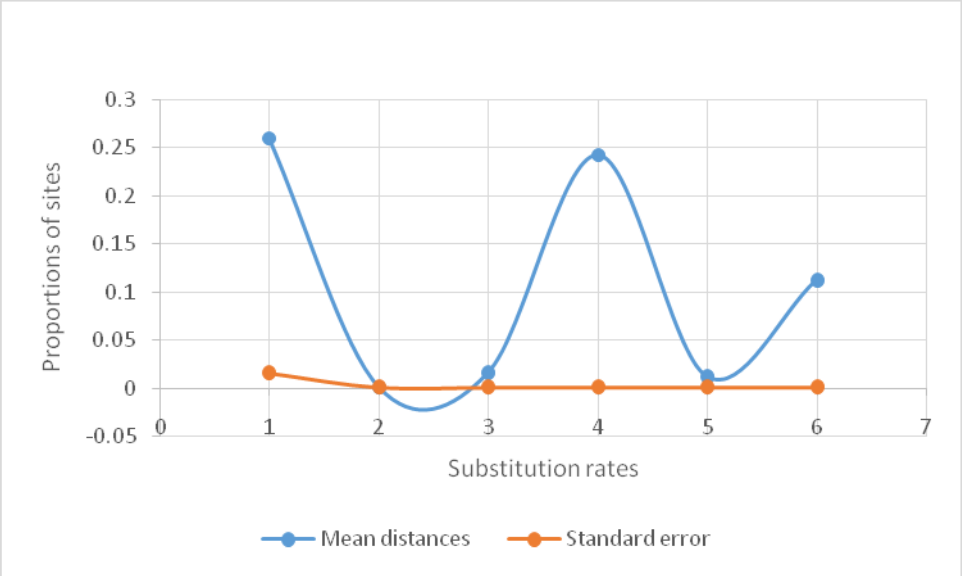

Figure 3.6. Graph showing the mean distances between *Rastrineobola argentea*samples and its closely related taxa.

3.3.5 Disparity Index Test of Substitution Pattern Homogeneity

Computer simulations were used in rejecting the null hypothesis in that the Disparity Index test using the Monte Carlo test (500 replicates) were used to estimate the *p*-values. Analyses for *R. argentea* 1/ *R. argentea* 5/ *R. argentea* 4 were 1.000 involving 9 nucleotide sequences and codon position included were 1st+2nd+3rd+Noncoding (Table 3.8). Values equal to 1 corresponds to a homogeneous process which is shown below the diagonal. The probability of substitution from any state to pre-specified state tallies to a homogeneous process thus sequences that have evolved with the same pattern of substitution, as judged from the extent of differences in base composition biases between sequences. *P*-values smaller than 0.05 are considered significant. The estimates of the disparity index per site are shown for each sequence pair above the diagonal which include analyses for *R. argentea* 1/ *R. argentea* 5/ *R. argentea* 4 were 0.000 as shown in Table 3.8

Table 3.8. Test of the Homogeneity of Substitution Patterns between sequences

	1	2	3	4	5	6	7	8	9
1. Salmostoma_bacaila		0.040	0.000	0.156	0.156	0.118	0.618	0.000	1.482
2. Brachydanio_rerio	0.298		0.040	0.000	0.000	0.000	0.174	0.000	0.993
3. Luciosoma_bleekeri	1.000	0.306		0.246	0.246	0.210	0.770	0.000	1.616
4. R_argentea_1	0.196	1.000	0.080		0.000	0.000	0.000	0.011	0.236
5. R_argentea_5	0.198	1.000	0.086	1.000		0.000	0.000	0.011	0.236
6. R_argentea_4	0.210	1.000	0.106	1.000	1.000		0.022	0.000	0.275
7. Raiamas_guttatus	0.012	0.160	0.000	1.000	1.000	0.336		0.223	0.091
8. Raiamas_senegalensis	1.000	1.000	1.000	0.348	0.374	1.000	0.052		0.955
9. Cabdio_morar	0.000	0.000	0.000	0.130	0.110	0.116	0.226	0.002	

3.4 Population structure of *Rastrineobola argentea*

3.4.1 Phylogenetic trees and analysis

The samples were blasted against other cyprinids of the world and the evolutionary history was inferred using the Neighbour-Joining method. The samples clustered more to *Raiamas guttatus* a freshwater cyprinid fish found in North-eastern India, Myanmar, Cambodia, Thailand and China. *Raiamas senegalensis* also a freshwater cyprinid ubiquitous from Guinea to Tanzania and along the length of the river Nile (Figure 3.7). The optimal tree with the sum of branch length = 0.79223545 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. Simulations have shown that a bootstrap value of greater than 70% correspond to a probability greater than 95% and are considered to be strongly supported.

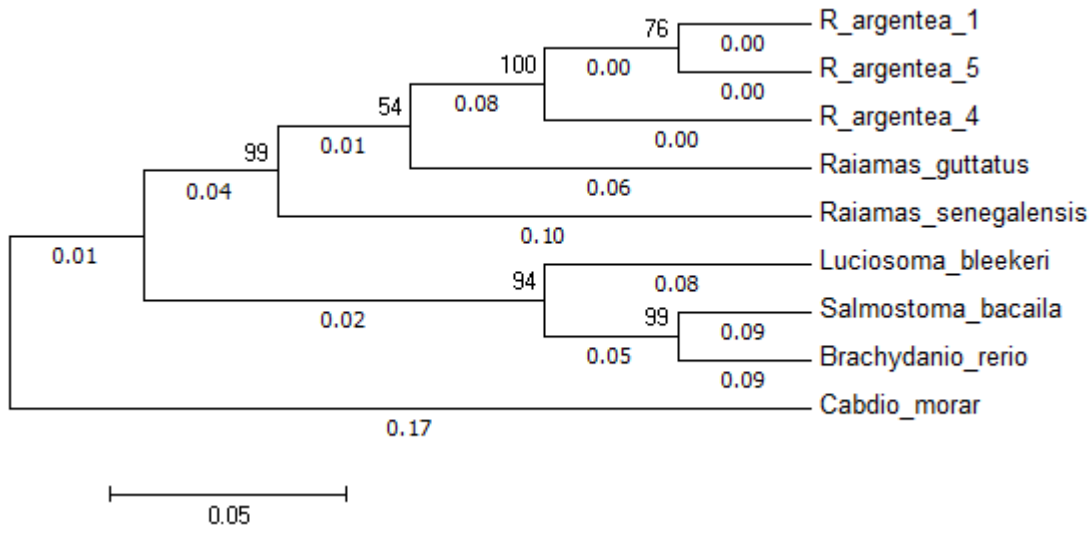

Figure 3.7. Original tree showing evolutionary relationships of taxa.

The evolutionary history deduced using the Neighbour-Joining method suggests that *R. argentea* 1 is more related to *R. argentea* 5 than it is related to *R. argentea* 4. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding and the evolutionary analyses were conducted in MEGA6.

Phylogenetic analysis indicated that there were 2 main clusters and one outlier. The first cluster composed of *R. argentea 1, R. argentea 5,R. argentea 4, Ramaias guttatus and Ramaias* senegalensis. The second cluster comprised of *Luciosoma bleekeri, Salmostoma bacaila* and *Brachydanio rerio.* One species that did not cluster with any of the fish sampled was *Cabdio morar*. *R. argentea* 1 and *R. argentea* 5 were found in the same sub cluster indicating that they are more closely related to each other than to *R. argentea* 4.

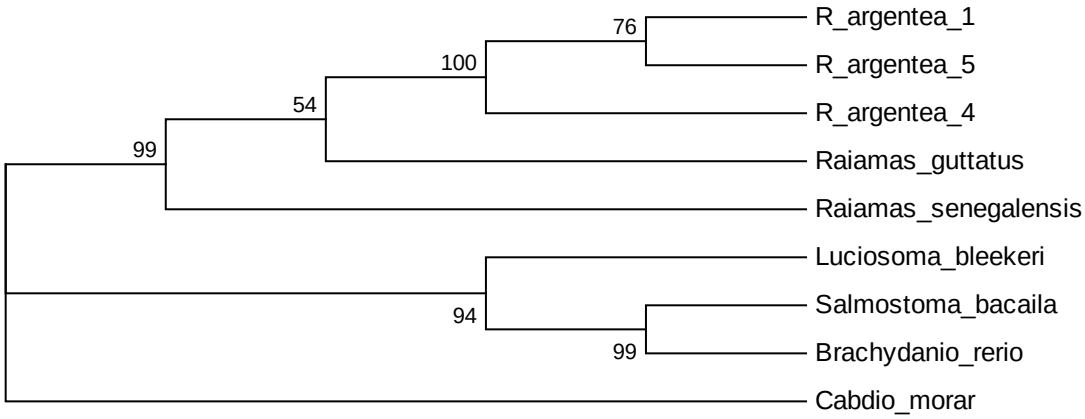

Figure 3.8. Consensus bootstrap tree showing phylogenetic tree displaying relationship between D-loop sequences of *R.argentea* and other species

CHAPTER FOUR

DISCUSSION

4.1 Morphological characterization of *R. argentea*

The morphological assessment carried out in this study identified two distinct phenotypic characters of *R. argentea* with those from Port Victoria having a significantly lower mean length, yellow caudal/tail fin and chromatophores throughout the upper part of their body. According to Sharpe (2012), these morphological differences between the *R. argentea* of Port Victoria to those of Mbita and Nyanza Gulf identify as opportunity for selection.

Port Victoria is located further to the North and has two riverine inflow compared to Mbita and Nyanza Gulf which are geographically closer and have an inflow of five rivers (Njiru*et al.,* 2010). Riverine inflow promotes the proper mixing of the water in the Lake thus regulating temperature, oxygen level and chemical effluent such as nitrogen and phosphorous from farms (Njuru, 2001).Besides, Port Victoria is experiencing varying environmental conditions such as influx of sewage, farm effluents and pollution from small-scale gold mining in Bondo District triggering phenotypic plasticity in fish (Bwathondi*et al.,*2001). This present opportunities for evolutionary changes to act and differentiate the two groups morphologically triggering plasticity (Hard *et al.,* 1999).Studies have shown that adaptive individual phenotypic plasticity is sustained by responses to changing ecological settings explaining the selection of differing patterns among individual fish species (Charmantier *etal.*, 2008). This is linked to environmental pressures that signify allometric changes in fish that alter the size and body shape of the juvenile. This affects the reproductive fitness through the expression of potentially beneficial traits that improve fitness and performance of the fish as an adaptation to novel environment (Agrawal, 2001).

A single genotype can change the chemistry, physiology, development, morphology and behaviour of organisms towards environmental cues leading to mutation, a form of phenotypic plasticity (Browman, 2000;Conover & Schultz, 1995). Various stimuli

in the environment can induce colour change in fish carried out by effector pigments cells called chromatophores. Studies carried out on *Oryzias latipes*, a teleost fish show that changes in chromatophores resulting in increase in melanophores are related to physiological responses associated with nervous and endocrine system induced by environmental stress (Sugimoto, 2002). These mutations force ecological triumph in novel habitats therefore the proliferation of melanophores on *R. argentea* from Port Victoria (Plate 3.1 and 3.2), suggesting a long-term background adaptation to the riverine systems.

Another anthropogenic factor underlying these observed changes in phenotypic traits is fishing. The outcomes of fishing are many and interrelated, it is a form of selective pressure that contribute to selection and compromise the long term survival of fish species (Allendorf *et al.,* 2008; Ormerod *et al.,* 2010). According to FAO (2010), half of the fish stocks are fully exploited and another 32% are over-exploited, depleted or recovering. In this case, vertical migration of *R. argentea* leads to concentration of population at specific sites along the Lake Victoria. Overharvesting of this species may lead to dramatic loss of fish population and genetic diversity (Fryer, 2004). This warrants attention to spatial distribution of fishing efforts by taking into account the density-dependent population, habitat fragmentation, connectivity and shift in species composition detected by declining trends in size related mean levels of the catches (Pauly & Froese, 2012). The *R. argentea* population structure and density is determined by the age composition of the population, moderate migratory losses or gains in numbers though compensated by corresponding changes in mortality rate (Gonzalez-Voyer & Kolm, 2011). When population size is reduced, genetic variation is lost through genetic drift that supersede the benefits of natural selection resulting in reduced reproductive fitness of individuals (Mäkinen *et al.,* 2008). Furthermore, inbreeding occur more frequently in fragmented population whose aftermath lead to reduction in effective population size and ultimately in population extinction (Carson *et al.,* 2014; Cooper *et al.,* 2009). Therefore the main goal of conservation program is to counteract decrease in effective population size (Brook *et al.,* 2002).

4.2 Molecular characterization *R argentea*

4.2.1 Mitochondrial DNA sequencing

Morphological assessment was successful in bringing out the diversity of the *R. argentea* but the technique alone was not sufficient for the accurate identification of *R. argentea*. Three molecular markers were used for molecular characterization of *Rastrineobola argentea*: Simple Sequence Repeats (SSR) Cytochrome 1 gene (Co1) and mtDNA gene (Figure 3.2, 3.3 and 3.4). Only mtDNA sequencing was able to resolve the identity of *R. argentea* (Figure 3.5).

Mitochondrial DNA is strictly maternally inherited and is haploid, permitting the direct sequencing of the PCR product. Moreover, mtDNA is present at high copy numbers per cell ranging from 50,000 mtDNA molecule per oocyte to a few hundred mtDNA molecules in sperm in contrast to a single copy of nuclear DNA (Bender *et al.,* 2000). This high copy number results in an increased sensitivity for detection even when extremely low amounts of DNA or highly degraded DNA is available or samples with little or no genomic DNA (Rousselet & Mangin, 1998). Therefore, DNA typing using mtDNA is more fruitful than typing with polymorphic markers found in nuclear DNA (Budowle *et al.*, 2003).

4.3 Population structure of *R. argentea*

The factors that maintain genetic and phenotypic variation within natural populations have interested evolutionary biologists leading to development of tests that detect life history factors on different timescale (Bürger *et al.*,2006). Parameters such as the Tajima value have been developed to extrapolate statistical information of populations that can be theorized through evolutionary factors such as mutations, population structure, time, genetic drift and selection (Fitzpatrick *etal.,* 2009). In this study, the Tajima value is greater than zero, i.e. Observed theta > Expected theta, suggesting that there are more haplotype or a more average heterozygosity than the number of segregating sites (Table 3.4). This can be interpreted as more alleles are present some at low frequency while others at high frequencies thus balancing selection(Wells *et al.,* 2000).

Balancing selection refers to an assortment of selective systems that maintain genetic diversity within populations (Delph & Kelly, 2014). It is a discriminatory process by which multiple alleles are actively maintained in the gene pool of a population at frequencies above that of gene mutation. Moreover, it inclines that certain phenotypic traits are of selective importance during population growth and balancing selection on other traits may lead to the preservation of a broad phenotypic diversity over time (Alpermann *et al.,* 2010). Therefore, maintaining phenotypes close to the population mean and eliminating extreme phenotypes. This homogenizing allele frequency causes the diversity within population to increase. This happens when the heterozygotes for the allele under consideration have a higher adaptive value than the homozygote consequently sustaining genetic polymorphism (Mäkinen *et al.,* 2008). Genomic regions showing arrays of genetic diversity is considered as candidates incorporating loci involved in evolutionary change (Subramaniam & Rausher, 2000). There are two mechanisms that balances selection. This can either be through heterozygous advantage or frequency-dependent selection. In heterozygous advantage, an individual who is heterozygous at a particular gene locus has a greater fitness than a homozygous individual. This results in a balanced polymorphism. On the other hand, frequency-dependent fitness depends on the occurrence of a phenotype relative to other phenotypes in a given population. Consequently, the possibility of the fitness of a certain genotype depend on the frequency of other genotypes in the populations (Heino *et al.,* 1998). In positive frequency-dependent selection, the fitness of a phenotype increases as it becomes more common while in the negative frequency-dependent selection the phenotype increases as it becomes less common. An example of negative frequency-dependent selection includes prey switching promoting niche partitioning. This is aggrandized by predators which is believed to impose natural selection on various morphs of the prey encouraging fitness in the prey species (Price *et al.,* 2011). Furthermore, several authors have proved that predation has invoked that negative frequency-dependent selection and it is an explanation for the persistence of polymorphism in species (Punzalan*et al.,* 2005; Mäkinen *et al.,* 2008; Melián *et al.,* 2010)

For the application of the Tajima neutrality test, some precautions must be undertaken for example DNA sequences subjected to this method must be picked from a random sample of a population that is either at or not at equilibrium (Johnson & Omland, 2004). The divergent sites of all the sequences are negative Tajima value (Table 3.7). This suggest that the population experienced a bottleneck in recent time and all the different kind of polymorphism either due to comparison between nucleotides or the insertion/deletion polymorphism help deduce bottlenecks (Tajima, 1989; Kiesecker and Blaustein, 1997; Zaccara & Delmastro, 2009; Xia *et al*., 2012). This affects all kinds of DNA polymorphism which signifies an excess of low frequency polymorphism relative to expectation. Moreover, it indicates a population size expansion after a bottleneck or due to selective sweep or purifying selection. The observed Tajima D value is less than the expected value proving that there are fewer haplotypes or lower than average heterozygosity than the number of segregating sites (Sousa *et al.,* 2008). The biological interpretation of this is that there are rare alleles at low frequencies. This is linked to a genetic sweep which increases the fitness of the carrier relative to other member of the population. Immigration and hybridization between two populations may occur more frequently in one direction than in the other thus influencing the genetic structure in fish species. This is said to be directionally bias in gene flow (Rogers*et al.,* 1996). At the population level, species with longer lifespan, delayed maturity, large body size and low rates of natural mortality and recruitment would be highly sensitive to overfishing and once depleted recovery would be slow relative to populations with opposite life history characteristics (Alcala, 1998). Generally, natural selection favours individuals that have a higher fitness and the newly mutated allele will increase in frequency relative to other alleles. This sweep allows for rapid adaptation providing a unifying homoplasty of genetic sequences elucidating a common genetic background. This can be further illustrated by the pressure that *R. argentea* has been experiencing. Initially the population of this cyprinid was composed of both large and small sized populations of fish. With the introduction of Nile perch and the extensive fishing on Lake Victoria using gill nets that selected for larger sized fish population. The population left behind is composed of fish that are small sized (Sharpe, 2012).

The Tajima test tells information about the gene that has been positively selected and the observed Tajima D value is less than the expected value (Table 3.6). This suggests that there are fewer than compared to the number of segregating sites whereby the expected value is zero. This implies that there has been a recent bottleneck that took place leaving behind lower than average heterozygosity. Moreover, population with small value of relatedness are slightly less fit than populations with zero relatedness (Wahl, 2002). Habitat choice may play a role in spatial distribution of different species, predatory risks and competition for resources in fishes which positively correlated with body size (Krause *et al.,* 2013).In addition, assertive shoaling on the basis of body size is advantageous to prey fish in terms of minimizing predatory risks.

4.4 Phylogenetic relationships

The evolutionary trees that were extrapolated in this study linked *R. argentea* with other cyprinids of the world and this include *Luciosoma bleekeri Raiamas guttatus Salmostoma bacaila Raiamas senegalensis Brachydanio rerio* and *Cabdio morar* (Figure 3.7 and 3.8). The common factor in this species is that they are freshwater cyprinid found in some parts of Asia and Africa. *Luciosoma bleekeri* is found in Southeast Asia from the Mae Klong River to the Mekong (Langen *et al.,* 2011). *L. bleekeri* mainly lives in fast moving rivers. The second species was *Raiamas guttatus*, found in North-eastern India, Myanmar, Thailand, Cambodia and China (Wang *et al.,* 2009). Most species are found in shady areas and muddy bottoms in deep hill streams. The adults usually collect in clear water with moderate swift currents while the juveniles in quiet pools further downstream. Its diets is composed of insects and small fishes and are rarely seen in the market. The third is *Raiamas senegalensis,* which is widespread from Guinea to Tanzania and along the length of the Nile (Agnew *et al.,* 2009). It is a demersal (dwelling near the bottom or deep Lake) and potamodromous species (born in upstream freshwater habitat but migrate as juveniles to grow into adults and then migrate back to upstream). The fourth species *Salmostoma bacaila,* is a benthopelagic potamodromous species found in slow running water but also occurring in rivers, ponds, inundated fields in sub-montane regions and brackish water in Afghanistan, Nepal, Pakistan, Bangladesh and

India (Loh *et al.,* 2012). It is an omnivorous surface feeder. The fifth species is *Cabdio morar*, found in streams, rivers, ponds in plain and mountainous regions of Bangladesh, Myanmar, Nepal and India (Hoelzel *et al.,* 1991). It is an oviparous species that scatter their eggs after laying them and fertilise them externally. They grow to a height of 17.5 cm. *Brachydanio rerio* is the sixth species, it is found in Bangladesh, Nepal, India, Myanmar and Pakistan (Chen *et al.,* 2009).

The mtDNA sequence generated provide a correlation of the geographical origin or phenotype/race of these samples investigated (Milošević *et al.,* 2011). Clearly, they share a common phylogeographical pattern suggesting that comprehensive data from more species distributed across these systems would be useful in supporting paleohydrological scenarios in this region. All models of molecular evolution are a simplification of the actual complexities of evolutionary processes. Failure to account for specific features such as misspecification common in analyses of sequence data may imply an assumption on the rate of homogeneity across sampled genes thus creating biases in outcomes of evolutionary inferences. To remedy these analyses, partitioning sequence data by gene and codon position perform well in developing high resolution phylogenetic extrapolations (Wagner *et al.,* 2012). For dating evolutionary time of closely related species synonymous substitution may be used as a molecular clock since its rate of substitution is higher than that of non-synonymous substitution (Nei & Gojobori, 1986).

4.5 Implication for exploitation and management of *R. argentea* fisheries in Lake Victoria

Lake Victoria is a hotspot for fishing for both local and international supply generating revenues for this region will require keen monitoring of *R. argentea* for its population persistence. This is enhanced by the interpretation of Fst values (Table 3.7 and 3.8) which indicate that the structuring between sub-population is weak with little genetic differentiation. This is contributed by the effects of polymorphism which deflates Fst expectation rendering an Fst value of 0.05 an important genetic differentiation (Balloux *et al.,* 2002). The strong historical influence on the genetic population structure of *R. argentea* in Lake Victoria is responsible for the reduction

of population. The significant Tajima's D values are indicative of population bottlenecks followed by expansion, fragmentation of spawning habitat and the capacity for local adaptation provide the necessary prerequisite for population structuring in at least the three sample location (Saura & Faria, 2011).

The phylogenetic trees and the minimum spanning networks are similar to those presented for historical extinction-recolonization models of other fish taxa such as the sardine evolution (Aboim et al., 2005). Therefore, the combination of morphology, nuclear and mtDNA are useful in the reconstruction of phylogeography is suitable in population management, protection and conservation (Salducci et al., 2004). Populations from Port Victoria are morphologically different from those of Mbita and Nyanza Gulf and is in concordance with the molecular data. Therefore, the study has produced genetic data that can be incorporated into the existing extensive biological data for better understanding of in Lake Victoria. This should provide a strong basis for scientifically backed fisheries management and exploitation.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 conclusions

The morphological characterization carried out in this study identified two distinct phenotypic characters of *R. argentea*. Those of Port Victoria had a significantly lower mean length, yellow caudal/tail fin and chromatophores throughout the upper part of their body. This proves that fish communities are shaped by physical and biological characteristics of riverine systems which are sensitive to alterations that result in changes of the native species distribution, fish population and community structure (Welker & Scarnecchia, 2004). The phenotypic and genotypic analytic tools applied here explain the ecological implication of these exposed characters in the context of population and bloom development besides ascertaining environmentally driven directional selection on both the phenotype and genotype (Alpermann *et al.,* 2010). Conservation of genetic diversity and integrity of species relies on the identification of the critical genetic units and then managing this units in a coordinated manner (Kumar *et al.,* 2013).

In the present study, analysis indicate significant genetic variance between and among populations' phylogenetic analyses. The genetic diversity of *Rastrineobola argentea* was determined by D-loop of mitochondrial genome contained in the control region which is the non-coding region (Mesquita *et al.,* 2001). Phylogenetic and population genetic studies provide baseline data required to assignment individuals to their geographic source of origin and identify populations that are most vulnerable to extinction from overexploitation. Since all populations are geographically isolated and the species have decline over the last century it is proposed for the time being all population should be managed and conserved separately and efforts should be directed towards separating the genetic integrity of each population.

Increase in human population produces pressure on the environment for food and livelihoods thus challenging the integrity and functions of the ecosystem (Stewart *et*

al., 2010). Biological biodiversity appears to enhance ecosystem resilience of desirable ecosystem state in the face of change. In the absence of comprehensive conceptual framework it will remain difficult to assume underlying mechanism and their interaction from simple empirical patterns near fishing boundaries (Kellner *et al.*, 2015). Therefore, income diversification is a potential way out of poverty for fishing communities in developing countries which can be stimulated by providing adult education, improving access to loans and financial assistance for alternative income generating activities (Olale *et al.*, 2010).

5.2 Recommendations

1) Future studies should include a large range of morphological parameters and employ more robust analytical techniques to discern the particular morphological characters contributing to the differences.
2) The genetic basis of the chromatophores should be investigated
3) A Lake wide sampling should be undertaken and compared to Ugandan and Tanzanian populations
4) Similar studies should be initiated on other heavily exploited stocks such as *Lates niloticus* and *Oreochromis niloticus* of Lake Victoria.

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APPENDICES

Appendix I. Laboratory solutions

SET buffer

50 mM Tris HCl, pH 8 (0.394 g)

40 mM EDTA pH 8 (0.744 g)

0.75M Sucrose (12.836 g)

1 L Nuclease-free water

50 X TAE buffer

242g of Tris borate

57 ml of glacial acetic acid

100 ml of 0.5 M EDTA

Dilute to 1litre with double distilled water.

0.5 M EDTA (pH 8.0)

336.2g anhydrous EDTA. For 500ml start with 450ml double distilled water and adjust pH using NaOH pellets and autoclave .

TE buffer

10 ml of 1M Tris HCl pH 8.0

2 ml of 0.5M EDTA

Bring to total volume of 1L with double distilled water.

0.5 M EDTA

186.12 g EDTA

700ml water

Adjust pH to 8 by adding EDTA pellets to a total volume of 1 L.

1M Tris HCl pH 8.0

121.12 g Tris

Adjust pH to 8 by adding concentrated HCl to a volume of 1L.

5 M NaCl

292.2 g of NaCl

700 ml of double distilled water and dissolved completely to 1 L.

Ethidium bromide

10 mg/ml of ethidium bromide to nuclease free water. Store in dark bottle

Agarose gel

1.8% for Genomic DNA visualization

1.8 g of electrophoresis grade agarose

100ml 1X TAE buffer

Heat to dissolve and pour to an electrophoresis plate containing combs to solidify.

3% for PCR product visualization

3.0 g of electrophoresis grade agarose

100ml 1X TAE buffer

Heat to dissolve and pour to an electrophoresis plate containing combs to solidify

Running buffer

1 X TAE

Dilute 10 ml of 50X TAE with 490 ml double distilled water

1 kb ladder (1 µg/ml)

5µl ladder stock

44µl of 1M HCl

7.5µl of glycerol loading dye (6X)

Glycerol loading dye (6X)

0.26g of bromophenol

30ml glycerol

100ml nuclease free water

Appendix II: DNA sequences of *R.argentea* blasted against other species

Upon sequencing of the eleven amplicons the phylogenetic dataset consisted of only three outcomes while the other eight products produced ambiguous sequences which could not be scored. The sequences below was the most common repeats in all the three samples which were blasted on NCBI database revealed seven other species that shared the same sequence repeat.

R.argentea 1

ATGAAGTACATACTATGATTAATAACCATTCATCTATCTTAAACATAAAGCAAGTAATAACAGACCTTTATCCAC
CACCTAGTGAGAGACCACCAACGATTTTAGCAGGAACATATTATCCATGATAGAACCAGGGACACATTAATAAG
GGTTGTTAACAATGAATTATTCCTGGTATCTGGTTAGGAATCTCACGTACATCGCTATAAGAACCACAAACAATC
TAGTAGTAAACGGCATCTGATTAGTCAGATGTGTTAAACATTTCATTCATTACCCCACATGCCTAGCGTTCTTTTA
TATGCAAGGGGTGATCTTTATTTGGTTCCTTTTCATCAACATCCCAGAGTGCAAGCTCAAATGTTAATTAAGGTAG
TTCATTTTCCTTGTTTGTGATAAAGTAAGTTAATTATTGGAAGACATAACTGAAGACTCATACACTTTTAAGTCAT
GTACATAACGTATCTGCACTATTCCTCTATTATTGCTGCTTCCGCCCCCGGCTTTCGCGCGACAAACCCCCTTACC
CCCTTAACGTCCTAAAAGTCCTATTTATCCTTGTCAAACCCCCGAAAGC

R.argentea 4

ATGAAGTACATACTATGATTAATAACCATTCATCTATCTTAAACATAAAGCAAGTAATAACAGACCTTTATCCAC
CACCTAGTGAGAGACCACCAACGATTTTAGCAGGAACATATTATCCATGATAGAACCAGGGACACATTAATAAG
GGTTGTTAACAATGAATTATTCCTGGTATCTGATTAGGAATCTCACGTACATCGCTATAAGAACCACAAACAATC
TAGTAGTAAACGGCATCTGATTAGTCAGATGTGTTAAACATTTCATTCATTACCCCACATGCCTAGCGTTCTTTTA
TATGCAAGGGGTGATCTTTATTTGGTTCCTTTTCATCAACATCCCAGAGTGCAAGCTCAAATGTTAATTAAGGTAG
TTCATTTTCCTTGTTTGTGATAAAGTAAGTTAATTATTGGAAGACATAACTGAAGACTCATACACTTTTAAGTCAT
GTACATAACGTATCTGCACTATTCCTCTATTATTGCTGCTTCCGCCCCCGGCTTTCGCGCGACAAACCCCCTTACC
CCCTTAACGTCCTAAAAGTCCTATTTATCCTTGTCAAACCCCCGAAAGC

>R.argentea 5

ATGAAGTACATACTATGATTAATAACCATTCATCTATCTTAAACATAAAGCAAGTAATAACAGACCTTTATCCAC
CACCTAGTGAGAGACCACCAACGATTTTAGCAGGAACATATTATCCATGATAGAACCAGGGACACATTAATAAG
GGTTGTTAACAATGAATTATTCCTGGTATCTGGTTAGGAATCTCACGTACATCGCTATAAGAACCACAAACAATC
TAGTAGTAAACGGCATCTGATTAGTCAGATGTGTTAAACATTTCATTCATTACCCCACATGCCTAGCGTTCTTTTA
TATGCAAGGGGTGATCTTTATTTGGTTCCTTTTCATCAACATCCCAGAGTGCAAGCTCAAATGTTAATTAAGGTAG
TTCATTTTCCTTGTTTGTGATAAAGTAAGTTAATTATTGGAAGACATAACTGAAGACTCATACACTTTTAAGTCAT
GTACATAACGTATCTGCACTATTCCTCTATTATTGCTGCTTCCGCCCCCGGCTTTCGCGCGACAAACCCCCTTACC
CCCTTAACGTCCTAAAAGTCCTATTTATCCTTGTCAAACCCCCGAAAGC

>Raiamas guttatus

ATGAGTACATACTATGTTTAATAACCATTCATCTATTTTAAACATAAAGCAGGTAATAGCTTATATTTATTCACCA
TTCAAGTGAGAGACCACCAACCGGTCCAATCAAGAACATATCATTAATGATAGAATCAGGGACATTTTATCCAA

GGGTCGTTATTAGTGAATTATTCCTGGTATCTGATTCGGAATCTCAGGTACATCGCTACAAGAACCACTAACAAT
CTAGTAGTAAACGGCATCTGATTAGTCAGATGTGTTAATCATTCGATTCATTACCCCACATGCCTGGCGTTCTTTT
AAATGCAAGGGGTGAATTTTTATTGGTTTTCCCTTCACTAACATTTCAGAGTGCAGGCCCAAATGTTAAATTAAG
GTTGTACATTTTCCTTGCATGTGACAAAGTATATCCATTATTGAAAGACATAACCGAAGACTCATACACTTCTAAT
TCATGTACATAACGCCTATTGCACTTTCCTCTATAATTGCTGCTTCCGCCCCCGGCTTTTGCGCGACAAACCCCCT
TACCCCCTTAACGCCCCGGAAGTCCTGTTTATCCTTGTCAAACCCCGAAACC

>Raiamas senegalensis
ATGTATAAGGACATACTATGTATAATCCCCATTCATCTATTTTAAACATAAAGCAGGTACTAACAGTCCTTTATCT
ACCATCTGTGTGAGAGATCACCAACGATTTAAGCAGGAATACAATATTAATGATAGAATCAAGGACATATATAC
TAAGGGTCGTTATTAGTGAATTATTCCTTGCATCTGGTTAGGAATCTCAAGCACATCATTATAAGAACCATTAAC
AATCTAGTAGTAAACGGCATCTGATTAGTCAGATGTTTTAACCATATTATCCTTTACTCCACATGCCTAGCGTTCA
CTTATATGCAAGGGGTGAATTTTTATTGGTTTTCCATTCACCAACATTTCACAGTGCAAATTCAAATGTTTAATTA
AGATTGTACATTTCCCTTGCATTCATGAATGTTTATTCATTATTGAATGACATAACTGAAGATCCATTAACTTCTA
ATTCATGTACATAACATATTTGCACTTTCCTCTATTATTGCTGTTTCCACTCGGCTTTTGCGCGACAAACCCCCTTA
CCCCCTTAACGCCCCTAAAAGTCCTGTTTGTCCTTGTCAAACCCCGAAACC

>Luciosoma bleekeri
ATTAGTTCATATTATGTATTATCCCCATTTCATTATTTTAAACATAAAGCAAGTACTAAATGTTATTTATTTAACAT
TCAGATGAGAGACCACCAATGAACTATCTAAAAACATATTATTCATGATAGAATCAGGGACATTAACCTAAGGG
TCACTATTATTGAATTATTCCTGGTATCTGATTCAAATCTCAGGTCCATCGCTACAAGAACCATTAACAATCTAGT
AGTAAACGGCATCTGATTAGTCAGATGTGTTAATCATAAATTTCATTACCCCACATGCCGAGCGTTCTCTTATATA
CAAGGGGTGAATCTTTTTATGGTTTCCTTTCATCAACATTTCAGAGTGCAAATATAAATGTTAATTAAGATTGTAC
ATTTTCCTTGTATGTGATAATATAAGTGAATTATCGTAAGACATAATTATAAGATTCATACAAGTTTAACTCAAGT
ACATAACATATCTGTATATTCCTCAATGATACCTGTTTCACCCGGCTTCTTCGCGACAAACCCCCTTACCCCCTTA
ACGCCCAGAGAATCCTGTTTATCCTTGTCAAACCCCGAAACC

>Salmostoma bacaila
ATGTAAGACATATTATGTATTATCACCATTTCATTATTTTAACCATAAAGCAGGTACTATCGTTCATATAATTACA
TTTAAGAGAGAGATCACCAATGATTTATATAAAGATACATTATGCATGATAGAATCAGGGACATTAATTGAAAA
GTTGTTAAAAAATGAATTATTCCTGGTATTTGATTCTACATCTCATGTTCATCGCTACAAGACCCACACACAATCT
AGTAGTAAACGGCATCTGATTAGCCAGTTGTGTTAATCACTCATTTCATTACCCCACATGCCGAGCGTTCTTTTAT
ATACAAGGGGTGATCTTTCTCTTTTTTCCTTTCAACGTGCATTTCATAGTGCAAATACAAATGTTCAATAAGGTGG
TACATTTTCTTGAATATAACAAAGTTAGTTAATTATTGAAAGACATAACCGAAGAATCACTAACTTTTAATTCAG
GTGCATAAAATACTTATCACTTCCTCAACATCTAGATATAATACTCACCCCCTGTCTTTTTTTGCGCGACAAACCC
CCTTACCCCCTTACGCTCAGTAAATCCTGTTTGCCCCCGTCAAACCCCAAAACCG

>Brachydanio rerio
ATTAGGACATACTATGTATTATCACCATATCATTATTTTAACCACAAAGCAGGTACATAATGTTTATATTATTCAC
CATATCAAGTGAGAGACCACCAATAATTTATATAAATGCATATTATGCATGATAGAATCAGGGACATCGATTTAA
ACGTTGTTAAAAAATGAACTATTCCTGGTATCTGGTTCAAATCTCACGTTCATCGCTACAAGACCCACCCACAAT
CTAGTGGTAAACGGCATCTGATTAGTCAGATGTGTTAATCATTTAGTCCTTTACTCCCCATGCCGAGCGTTCTTGT
ATATACAAGGGGTGATCTCTTATTTTTTTCCTTTCGCTGGCATTTCATAGTGCAAATATAAATGTTAAATTAAGGT
GGTACTATTCCTTGCATGTGATAATATATATTAATTATCTAAAGACATAATATTAAGACTCACTAACTTTTAATTC

AAGTGCATAAAGTACTTATTATTTCCTTGATAAACTATATATTATTATCTCCCCCTTTTGGTATACGCGCGACAAA
CCCCCTTACCCCCTTACGTCCAGCGATTCCTGTTATCCTTGTCAAACCCCGAAACCA

>Cabdio morar
TTGAAGTTCAGGGTATGTATTATCACCATTGGCTTATTTTAACCATGAAGCAGGCACTAACAGTCTCTAGCCGAC
CATTGAGTGAGAGACCAGCAATGATCCAGGCGAAGAATACATGGCTAATGATAAGATCAGGGACATGAGACCA
AGGGTTGTACTAGTGAACTATTACTTGCATCTGATTCAAATCTCACGGCCATGAATACAAGAGCTCTAACAATCT
AGTAGTAAACGGCATCTGATTAGTCCGATGTGTTAATCATAAAGTCCATGTCCCCACATGCCTAGCATTCTTTTAT
ATGCATGGGGTTCTTCTTTTTTGGTTTTCCTTTCATTAACATATCAGAGTGCAAGCGTCAACGACTATCAAGGTGG
TACTACTTCTTGAATGAGACAAGTGAACCAATGATTGAAAGACATAATACAAGACTCACACATATTTAATTCAGG
TGCATACACTCATGTACTATTCCTCAATGGCTTCTGTTTCCACCCCGGCTTCTACGCGACAAACCCCCTTACCCCC
TTAAATGCTCAGAAAGTCCTGTTTACCCTCGTCAAACCCCGAAACC