MORPHOMETRIC, GENETIC STRUCTURE AND PHYLOGENETIC STUDIES OF *Barbus altianalis* (Boulenger 1900*)* POPULATIONS IN LAKE VICTORIA WATERSHED

BY

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DECLARATION

Declaration by the candidate

This thesis is my original work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret.

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DEDICATION

To my loving daughter Miss Tiffany Jerono Kipchumba. To my father Mr. Edward Chemoiwa Cherono, my late mother Mrs. Esther Kabon Cherono, all my brothers and sisters, Lengetap Arap Cherono and Kap Rerimoi for your moral support and inspirations. You are a great family.

ABSTRACT

The ichthyofauna of the Lake Victoria ecosystem are some of the most globally threatened biodiversity as a result of anthropogenic and other ecological impacts. The morphological plasticity of barbs (genus *Barbus*) makes them ideal models for studying evolutionary phenomenon in freshwater fishes. *Barbus altianalis* populations have declined in Lake Victoria probably due to anthropogenic activities and hence there is need to identify remaining populations for purposes of conservation and management. In this study, morphological and genetic characterization of the endangered cyprinid a species known to be potamodramous in four main rivers draining Lake Victoria was carried out. Morphological characterization was based on 21 morphometric characters on samples from rivers Nzoia, Nyando, Yala and Sondu-Miriu**.** Principal Component Analysis (PCA) showed partial separation of Rivers Yala from Nzoia, Nyando, and Sondu-Miriu fish populations. Factor loadings established that 11 characters were morphologically informative. PCA1 accounted for 43.25% of the difference while PCA2 accounted for 19.44% of the difference. Mann-Whitney U Test (α =0.05) indicated lack of significanct difference in morphological characteristics between Sondu-Miriu and Nyando, but significant intra-specific morphological difference between all the other pairs of rivers. Growth variability analysis based on 19 characters indicated negative allometric growth of *B. altianalis* in the four populations. The results suggest presence of intra-specisfic morphometric variation between the four populations. To assess genetic diversity, population structure, demographic expansion and phylogeny of this species, 850bp of the mitochondrial control region and 658bp of CO1 were used. The 196 samples yielded 49 mitochondrial DNA haplotypes and 83.7% of these haplotypes were private haplotypes restricted to particular rivers. The overall mean haplotype diversity was high (0.936±0.008) and ranged between 0.566 (Sondu–Miriu) and 0.944 (Nzoia). The overall mean nucleotide diversity was low (0.013±0.001). The *B. altianalis* populations in the Lake Victoria have thus maintained high genetic diversity despite adverse ecological changes in the catchment. Population differentiation tests revealed unexpected strong and highly significant ($P \leq 0.001$) segregation of populations in the four river basins. F_{ST} values for comparison of mtDNA among the four river-based populations ranged from 0.052 to 0.443. The samples formed two main haplotype networks based on a 95% parsimony criterion, each exhibiting a strong signature of past population differentiation. The smaller network was restricted to the river Nzoia and Uganda (secondary data) populations, whereas the larger network contained representatives from all four rivers; within this the central haplotypes were found in more than one river, whereas the peripheral haplotypes tended to be river-specific. Phylogenetic trees from the two markers display two distinct Clades. Clade I having samples from Nzoia and Uganda while Clade II had samples from all the four Kenyan rivers population. River Nzoia and Ugandan populations probably represents phylogenetically different populations or sub-species of *B. altianalis*. The degree of population differentiation and the high number of river specific haplotypes, and the highly supported bootstrap values 100/100/1.0, NJ/MP/Bayesian tree respectively of phylogenetic trees is too high to be explained by recent anthropogenic impacts alone and suggests that the species has probably existed in the Lake Victoria catchment as two populations; the now 'extinct' migratory population and the extant river restricted non migratory population. The decline of migratory populations could have created opportunities for population expansion. The existence of genetically robust population of *Barbus altianalis* in the L. Victoria catchment rivers should provide new impetus for conservation of these species. The four populations should be regarded as Evolutionary Significant Units (ESUs) and river basin specific management measures instituted to protect these populations.

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GLOSSARY OF ABBREVIATION AND TERMS

AMOVA Analysis of Molecular Variance

ANOVA Analysis of Variance

ANCOVA Analysis of Covariance

SM Sondu-Miriu

NY Nyando

NZ Nzoia

YA Yala

KEMFRI Kenya Marine and Fisheries Research Institute

PAUP Phylogenetic Analysis Using Parsimony

DNASP DNA Sequence Polymorphism

MEGA Molecular Evolutionary Genetics Analysis

PAST PAlaeontological STastistics

µg microgram

µl microlitre

5X five times

10X ten times

bp base pair

CO1 Cytochrome c Oxidase I

ddH_2O double distilled water

dNTP deoxynucleotide triphosphate

EDTA ethylenediamine tetraacetic acid

ESU Evolutionary Significant Unit

g gram

h hour

HWE Hardy-Weinberg equilibrium

IUCN International Union for Conservation of Nature

kg kilogram

LD linkage disequilibrium

M metre

DNA Deoxyribonucleic acid

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Lake Victoria is the largest tropical freshwater lake in the world with a surface area of about 69 000 Km^2 and is shared by three East African countries, Kenya (6% of shoreline), Uganda (43%) and Tanzania (51%), with a catchment area of 195 000 Km^2 that also includes Rwanda and Burundi (Shoko *et al.,* 2005). The lake is relatively shallow, with a mean depth of 40 m and supports the world's largest freshwater fishery (Lysell, 2009). Lake Victoria is an important source of food, employment, and earnings for the over 30 million riparian communities (Kayombo and Jorgensen, 2006), and is a source of domestic and industrial water supply (Cowx, 2005). The lake produced about 200,000 tons of harvestable haplochromine fishes in the early 1970s (Shoko *et al.,* 2005). In the last four decades, however, the lake's ecosystem has been severely damaged by overfishing and use of inappropriate net sizes, environmental changes, introduction of exotic species, invasion of water hyacinth (*Eichornia crassipes*), and increased nutrient load from farming activities (Shoko *et al.,* 2005).

The main rivers flowing to Lake Victoria from Kenyan catchments are rivers Nzoia, Sondu-Miriu, Yala and Nyando. Of these, the Nzoia and Sondu-Miriu are the largest with a recognizable fishery (Cadwalladr, 1965a; Cadwalladr, 1965b; Ochumba and Manyala, 1992). River Nzoia has its source in the Cheragany Hills, but has four other major tributaries (Sosio, Ewaso Rongai, Koitobos and Kuywa) that rise from the slopes of Mount Elgon. The Yala drains the central highlands west of the Rift Valley as does the Nyando, which has its sources near Mount Tinderet (0°069 S; 35°219 E)

2640 metres above sea level. The Sondu rises on the slopes of the Mau Escarpment (Ochumba and Manyala, 1992) and has two major tributaries, the Yurith and the Kipsanoi. The upper catchments of all of these rivers experience high and fluctuating annual rainfall causing regular massive floods in the lower reaches, particularly during the rainy season. Rivers Nzoia, Nyando and Sondu-Miriu catchment areas that are under intensive sugarcane and tea plantations and support a number of sugar and tea based factories. In addition there is a pulp mill located in the mid section between Webuye after discharge and Webuye before discharge on the River Nzoia. Nzoia-Mumias Bridge and Nyando-Chemelil Bridge are under direct industrial effluence from Mumias and Chemelil sugar cane factories respectively (Ojwang *et al.*, 2007).

The species composition of the catches in Lake Victoria has changed from those which prevailed in 20th century when the fisheries development started. The lake was home to hundreds of endemic fish species. Amongst them was a potamodromous assemblage of cyprininds and clariids that included *Labeo victorianus* (Boulenger 1901), *Barbus altianalis* (Boulenger 1900), *Schilbe intermedius* (Ruppell 1832), *Synodontis victoriae* (Boulenger 1906), *Synodontis afrofischeri* (Hilgendorf 1888), *Bagrus docmak* (Forsskal 1775) and *Clarias gariepinus* (Burchell 1822). *Barbus altianalis* once the most abundant of these species has virtually disappeared from commercial catches in Lake Victoria (Ochumba and Manyala, 1992; Abila and Jansen, 1997; Balirwa *et al.* 2003; Ojwang *et al.*, (2007). Over exploitation of gravid females during the migratory phase as well as pollution of the major river systems have been cited as major causes of their decline. The loss of the potamodromous cyprinids therefore represents a significant loss of Lake Victoria Basin faunal

biodiversity and there is urgent need to identify and protect remaining populations (Ochumba and Manyala, 1992).

Following ecological changes and the disastrous effects of introduced exotic species in the lake, a number of studies have documented the resultant effects on indigenous fish species (Witte *et al.,* 1992a; Abila *et al.,* 2004). However, there is a dearth of information on the effects of the ecological changes on riverine fish species, given that increased nutrient loading in Lake Victoria is facilitated by inflows from rivers and streams. Most studies have focussed on the effects of agriculture (Mungai *et al.,* 2011; Vuai *et al.,* 2012) industrial wastes (Odada *et al.,* 2004; Raburu and Okeyo-Owuor, 2012), and deforestation (Masese *et al.,* 2012) on river water quality. The four rivers drain some of the most agriculturally rich areas in Kenya (Odada *et al.,* 2004) and agricultural activities coupled with other human activities could be increasing nutrient loads in the rivers (Vuai et *al.,* 2012).

Direct discharge of municipal and untreated sewage into rivers is a serious cause of microbiological pollution in L. Victoria (Odada *et al.,* 2004), and degrades the quality of river and lake habitats (Ntiba *et al.,* 2001). These impacts on river water quality could pose a substantial challenge to riverine fish species, through habitat destruction and alteration, thereby affecting the breeding, feeding and reproduction of fish. According to Ochumba and Manyala (1992), habitat alteration through growth of dense papyrus at the mouth of River Sondu-Miriu hampers upstream migration for spawning among potamodromous fish. Similarly, this vegetation limits the entry of newly hatched fry back into the lake from the river.

In Kenya, *B. altianalis* an important food and game fish is restricted to the Lake Victoria Basin. It was one of the indigenous fish species in the affluent rivers of L. Victoria in the 1950s and 1960s (Corbet, 1961). Overfishing has however reduced the riverine species from annual catches of 2,500 tonnes in the 1950s (Whitehead 1959) to 108 tonnes in the 1980s and 1990s (Ochumba and Manyala, 1992) and habitat changes through damming (Ochumba and Manyala, 1992) and industrial pollution (Odada *et al.,* 2004) could further impact the fish stocks. Like the other rivers of Lake Victoria Basin such as Nzoia, Nyando, Yala, Mara and the others, Sondu-Miriu River fisheries, particularly the lower part played important role as source of fish for the riparian communities in the first half of the last century. However, the significant river fisheries as an important source of fish for the local people gradually waned as a result of reducing fish stocks (Owiti *et al*., 2013). Reduced fishing potential in rivers of the Lake Victoria basin has affected the livelihoods of local communities (Ochumba, 1984), and therefore there is need for urgent conservation and management measures for riverine fish species (Evans *et al.,* 1988).

Recent studies indicate that *Barbus altianalis,* which formed a major component of riverine fisheries, no longer migrate upstream to breed but have stationary populations concentrated at mouths of rivers Nzoia, Sondu-Miriu, Yala and Nyando in the Lake Victoria watershed (Ojwang *et al.,* 2007). Such philopatric behaviour makes the populations prone to genetic differentiation as a result of curtailed gene flow. This study employed morphometric data to assess the level of morphological differences in the four river populations. Mitochondrial Cytochrome c Oxidase I (CO1) and Control region (D - Loop) were analysed to assess the levels of genetic diversity within existing populations of *B. altianalis* from the four river systems and tested for genetic

differentiation between the four populations. Further, it was tested whether *B. altianalis* exists as a single panmictic population in Lake Victoria or whether population structuring has occurred in this species. Elucidating genetic structure and gene flow patterns may contribute towards defining management and conservation units for this Lake Victoria cyprinid.

Issues of genetic diversity have been recognized as critical in management of African inland water fisheries (Mwanja and Fuerst, 2003). Molecular markers can have applications in resource management by providing information on intra- and inter-population genetic diversity, levels of gene flow and demographical and historical factors that explain the observed diversity patterns. Integration of such genetic information with ecological and demographic knowledge may be used to make better informed management strategies. In species of commercial interest like *Barbus altianalis* such knowledge is important as the overexploitation of natural resources could lead to extinctions of locally adapted gene pools. Conservation of genetic diversity is essential to the long-term survival of any species, particularly in light of changing environmental conditions. Current IUCN Guidelines highlight the responsibility of any government to conserve the full genetic diversity of species (Hedrick *et al*., 2000). Conservation efforts for threatened and endangered cyprinids are increasingly dependent on genetic data for guidance in defining conservation units and designing captive propagation plans for endangered taxa (Hedrick *et al*., 2000)

1.2 Statement of problem and justification of the study

While the reproductive biology and dispersal, trophic ecology, population dynamics and socio-economics of *B. altianalis* is well documented in Lake Victoria (Ochumba

and Manyala, 1992) there is a dearth of knowledge on the morphology and genetics of this species in Lake Victoria and its watershed, which has not been documented.

Fish diversity in natural lakes is higher due to the relatively more stable environmental conditions under which fish evolve. In contrast, riverine species have to live under harsher and more variable environmental conditions (Fernando and Holcik, 1991). Studies have shown relationships between environmental and morphological characteristics among taxa (Schaack and Chapman, 2003). However, these relationships have been affected when a given structure used for more than one function is subject to multiple selection or when adjacent structure perform different functions so that one structure influences a second character (Schaack and Chapman, 2003). Such functional morphological versatility have been widely investigated among the cichlids (Fermon and Cibert, 1998), but less so among the Cyprinidae. Due to their migratory nature, potamodromous fishes are particularly prone to effects of environmental perturbation. Recent trophic ecology studies suggest the existence of non-migratory *B. altianalis* populations within the Lake Victoria catchment (Ojwang *et al*., 2007). The morphological changes that may have accompanied such adaptations have not been assessed in this fish community.

The cyprinid genus *Barbus* sensu lato is a polyphyletic assemblage in which a number of unrelated species and/or groups have been included. It is generally acknowledged that this cyprinid taxon requires a complete taxonomic reorganization (Howes, 1987) and several attempts have been made based on morphological characters (De Vos *et al*., 1990; Doadrio *et al*., 2002; Osuka and Mlewa, 2011).

Genetic studies are currently recognized as integral in understanding the biology of any organism since genetic variation is critical for adaptive evolution and population survival. Although the effects of overfishing on species diversity and abundance in Lake Victoria are well documented (Ogutu-Ohwayo, 1990; Ochumba and Manyala, 1992; Mugo and Tweddle, 1999), threats to the genetic diversity and hence the long term evolutionary survival of highly exploited fish populations like *Barbus* have so far been largely neglected.

Current molecular based studies suggest that populations that exist as distinct genetic entities need to be managed, exploited and conserved as different genetic entities (Moritz, 1994). Understanding the population structure and dispersal patterns of commercially important species is therefore considered as an important requirement towards their sustainable management (Duran *et al*., 2004).

While extensive molecular genetic data including phylogeny and population genetics exist for the European and North American cyprinid species (Gilles *et al*., 2002; Turner *et al*., 2004) no published work has addressed the seemingly critical issue of population structure and genetic diversity of these important commercial species in Lake Victoria Kenya watershed. Similar studies have however been carried out for the *Labeobarbus* species in LakeVictoria and Albertine basins in Uganda (Muwanika *et al*., 2012) *B. altianalis* and *B. bynni bynn,* the Nile perch (Hauser *et al*., 1998), the catfish, *Clarias gariepinus* (Galbusera *et al*., 1996), *Labeo victorianus* (Rutaisirea and Boothb 2005), cichlid fishes of Lake Victoria (Meyer *et al*., 1990; Abila *et al*., 2004) and the Nile tilapia *Oreochromis niloticus* (Mwanja *et al*., 2004). Other studies have been carried out by Callejas and Ochando (2000); Kotlik and Berrebi (2001);

Walter *et al* (2001); Frender *et al* (2004) and Fayazi *et al* (2006). There is therefore need for analysis on genetic impacts of ecological changes in the Lake Victoria catchment on migratory fish species.

Results of this study should provide data that may be incorporated into other biological data to create strategies for management of *Barbus altianalis* fisheries in Lake Victoria. The morphology, genetic diversity, population genetic structure and phylogenetics of *B. altianalis* in the Lake Victoria catchment were determined to inform on the status and the need for the conservation and management of the species. Application of genetic tools will contribute to understanding the impacts of habitat loss and fragmentation on the ecology of *Barbus altianalis* in the Lake Victoria catchment for effective conservation management of the remaining populations. It is important to know whether genetically distinct populations exist.

1.3 Objectives

1.3.1 Main Objective

To carry out genetic and morphometric characterization of *Barbus altianalis* populations in Lake Victoria and its watershed.

1.3.2 Specific Objectives

1. To carry out morphometric characterization of four populations of *B. altianalis* and test for morphometric differences between the populations from four major rivers in Lake Victoria watershed, Kenya.
2. To determine growth variability of the morphological characters in populations from the rivers of the Kenyan catchment of Lake Victoria.

3. To determine the genetic diversity, population genetic structure and demographic history of the riverine *B. altianalis* in the Lake Victoria watershed, Kenya using mitochondrial DNA.

4. To determine the phylogenetic position of *B. altianalis* in the Lake Victoria Basin.

1.4 Hypothesis

1. There are no significant morphometric differences between populations from the rivers of the Kenyan catchment area.

2. There is no growth variability of the morphological characters studied between populations from the rivers of the catchment area examined.

3. The four populations of *B. altianalis* are genetically homogenous.

4. Populations of *B. altianalis* in Lake Victoria basin represent one phylogenetic group.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cyprinids: Evolution and adaptive radiation

The Cyprinidae (Cyprinoidea; Cypriniformes) is the most diverse family of freshwater fish with seven subfamilies differing by their recent biogeographical distribution and historical dispersal routes (Winfield and Nelson, 1991). Whereas cyprinidae inhabit predominantly Africa, India and Southeast Asia, Leuciscinae is the most diversified subfamily in Eurasia (Vyskoçilová *et al.,* 2007). Cyprinid is therefore arguably the most species-rich fish family in the world with, over 3000 species distributed on four continents (Froese and Pauly, 2011). The family includes economically and scientifically important freshwater species such as carps, minnows, barbs and zebrafishes (Turner *et al*., 2004). Species belonging to the genus *Barbus* (Cuvier and Cloqueet 1816) constitute a very diverse group. They are considered polyphyletic with about 1146 species (Froese and Pauly, 2011), occupying a wide range of different habitats (De Vos *et al*., 1990; 1999). The African barbs all belong to the genus *Barbus,* usually divided into large and small *Barbus* (Berrebi, 1998). Karyological studies (Oellermann and Skelton, 1990; Golubtsov and Krysanov, 1993; Gue´gan *et al*., 1995) have demonstrated that the small African barbs are diploids (2n = 50), whereas the large African barbs appear to be hexaploids (2n = 150), characterised by an adult size of <10 cm standard length, and by diverging striae on the exposed part of their scales.

A taxonomic revision of the large African species was conducted by Banister (1973), documented extensive intraspecific variation in body shape, pharyngeal teeth, fleshy development of the lips, and robust development of the dorsal spine for several

species. Variation was especially pronounced for the two species represented by large sample sizes, *Barbus intermedius*. The *B. intermedius* complex included *B. intermedius, Barbus altianalis, Barbus acuticeps* and *Barbus rusae* (Dimmick *et al*., 2001). The taxonomic history of *B. intermedius* illustrates the compound problems of limited geographic samples and extensive intraspecific variation faced by systematists investigating the large African barbs. Banister's (1973) synonymy of *B. intermedius* included more than 50 nominal species and subspecies. The latest revision (Nagelkerke and Sibbing, 2000) distinguished 15 biological species composing an endemic species flock of *Barbus* (subgenus *Labeobarbus)* following Berrebi *et al* (1996).

2.2 Diversity and ecology of the genus *Barbus*

The genus *Barbus* is presently restricted to the typical barbel and barbs, and only contains fish from Africa and Europe as well as adjacent Asia. However, even in the reduced version the genus is probably paraphyletic, and many African species (particularly the small ones) do not seem to belong here either (Berrebi *et al*., 1996). The evolutionary history and systematics of the Old World freshwater fish genus *Barbus* Cuvier et Cloquet, 1816 (barbs) are not sufficiently understood (Berrebi *et al*., 1995., 1996). *Barbus* is polyphyletic and comprises a number of diploid, tetraploid and hexaploid lineages (Berrebi *et al*., 1996; Tsigenopoulos and Berrebi, 2000; Machordom and Doadrio, 2001; Tsigenopoulos *et al*., 2002).

Many African species assigned to this genus *Barbus* form two distinct groups-' small ' and 'large' barbels which differ primarily in adult size and type of scale striation (Banister, 1973; Banister, 1987; Skelton *et al*., 1991; Leveque, 1997). Skelton *et al*

(1991), and Tsigenopoulos and Berrebi (2000) assigned the genus state *Labeobarbus* (labeobarbs) to the large hexaploid (Golubtsov and Krysanov, 1993) and Lake Tana barbs (Kotlik *et al*., 2002).

The study species *Barbus altianalis* is commonly known as the ribbon falls barbel or *Barbus*. Native names are Kisinja, Nkuyu, Fwani (Lake Victoria); Ngambwa (Lunyoro, Luruli, Lukenya, and Lunyara); Sanga (Ludope); Changa (Lango). *B. altianalis* belongs to Kingdom – Metazoa, Phylum – Chordatae, Class – Actinopterygii, Order – Cypriniformes, Family - Cyprinidae, Genus – *Barbus*, Species – *altianalis.*

Three subspecies of *Barbus altianalis* were previously recognised according to geographic locations: *B. a. altianalis Blgr*. (found in L. Kivu and Ruzizi River), *B. a. radcliffi Blgr*. (found in Lakes Victoria and Kyoga, and the Victoria Nile) and *B. a. eduardianus Blgr*. (found in Lakes; Edward and George) (Worthington; 1932, Greenwood, 1966). Currently, these subspecies are considered invalid (De Vos *et al*., 1990). *Barbus altianalis* has been reported from Lake Victoria under several synonymous names: *Labeo rueppellii* Pfeffer 1896; *Barbus radcliffii* Boulenger 1903; *B. lobogenys* Boulenger 1906; *B. bayoni* Boulenger 1911; *B. pietschmanni* Lohberger 1929; *B. hollyi* Lohberger 1929; and *B. altianalis radcliffii* Boulenger 1903. Due to the overlapping nature of the water systems, this classification is inadequate and of limited application in the management and conservation programs of any fish species (Muwanika *et al*., 2012).

2.3 Description and uses of *B. altianalis*

The common names barbs and barbels refer to the fact that most members of the genera have a pair of barbels on their mouths, which they can use to search for food at the bottom of the water (Ochumba and Manyala, 1992). Gastropods and molluscs are an important food item for *Barbus* in the lake, while insect larvae are of equal importance in hard bottom areas. *Barbus* also eat plants, fish, and crustaceans, and juveniles consume plant material. Barbs are often fished for food; in some locations they are of commercial significance. The large *Barbus* are also often eaten in their native range (Witte and Van Densen, 1995).

2.4 Morphology of *B. altianalis*

The genus *Barbus* consists of about 400 morphologically diverse species making it an excellent model for studies in ecology and population genetics (Wang *et al*., 2004). A mature 'Barbus' specimens reaches 90 cm total length, and having scales with parallel striae (type A of Boulenger 1911: 2). Scale numbers and locations are as follows 28–37 scales in the lateral line; 5.5–6.5 scales between the origin of the dorsal fin and the lateral line; 2–3.5 scales between the lateral line and the origin of the pelvic-fin; 12–14 around the caudal peduncle. Dorsal fin III–IV/8–11; Anal fin ii–iii/5–6; Mouth sub-terminal and very variably formed, usually horseshoelike, but sometimes with rubberlips (e.g. in the nominal *L. rueppellii* and in '*B.' lobogenys* Boulenger, 1906). There are two pairs of barbels, the anterior barbel reaches to the middle of the eye but usually shorter, posterior barbel reaching to posterior margin of the eye (Seegers, 2008).

Little information is available on the morphology of *B. altianalis*. In particular it is not known what factors are responsible for different specimens developing rubber-lips or 'normal' lips.

2.5 Habitat, feeding and distribution of *B. altianalis*

The food of *B. altianalis* consists mainly of small animals such as molluscs, crustaceans, insect larvae, small fishes and aquatic vegetation (Graham, 1929; Corbet, 1961; Ochumba and Manyala, 1992). *Barbus altianalis* inhabits inshore lake waters and rivers whether they prefer sand and gravel substrate. Juveniles stay in riverine habitats while adults inhabit both riverine and lacustrine habitats. This species is distributed in Lake Victoria and its tributaries, Lake Kivu and tributaries, River Ruzizi, in the middle basin of the Akagera (downstream from Rusumo falls) and in lakes Edward and Albert and Kyoga (Witte and Van Densen, 1995). Most if not all Lake Victoria populations seem to be potamodromous (Whitehead, 1959; Ochumba and Manyala, 1992).

2.6 Reproduction of *B. altianalis*

Large Barbus species spawn on gravel beds within river channels during floods. In continuously flowing regulated rivers, time of spawning is governed by water temperatures. These fish have moderate fecundity, large eggs with an incubation time of several days and larvae which are initially immobile with large yolk sacs. They spawn in the inflowing regulated river (Tormasson *et al*., 1984).

Rainy season spawning is well documented among other *Barbus* species in Africa. Spawning in *Barbus kimberleyensis*, in the Hardap Dam of South West Africa coincided with the peak of seasonal rains (Gaigher, 1976). Spawning *Barbus* have

well-defined seasonal migration up the rivers and streams to spawn at the start of or early into the rainy period.

B. altianalis, *B. nummifer* and *B. doggetti* are anadromous, as their breeding coincided with seasonal flooding while *B. apleurogramma* is non-anadromous since its adults and juveniles occurred in rivers, streams, swamps, as well as in the floodwater pools of Lake Victoria during the dry season (Whitehead, 1959). It has been reported that *Barbus* spawn amongst the grasses in lakeside streams during the dry season (Whitehead, 1959). Adults and juveniles of *B. apleurogramma* occur in the heavily vegetated swamps surrounding Lake Nabugabo in Uganda during both the dry and wet seasons (Sempeski and Gaudin, 1995). This suggests that the reproductive seasonality and migratory tendencies of the population(s) of this *Barbus* species could change depending on their geographical location and environment (Mutia *et al*., 2010).

2.7 Migration of *B. altianalis*

Barbus altianalis is a migratory riverine fish. It has been reported that River Nzoia has larger and more abundant *B. altianalis* within the upper reaches than the lower region of the river throughout the year (Ojwang *et al*., 2007). The occurrence of non-potamodromous populations in the lake region cannot be a recent phenomenon since there are populations in the River Yala that are completely cut off by fringing papyrus swamp from migrating back into the lake (Ojwang *et al*., 2007).

Past studies in the Rivers Nyando and Nzoia generally alluded to the idea that these once migratory fishes are probably confined to particular sections of the rivers (Mugo

and Tweddle, 1999). There is a lack of empirical evidence, however, to show functional dependency of these fishes on specific habitats. It is probable that the lake region had originally both lacustrine and migratory populations but that the former are now rare, with relicts confined to rocky habitats, while those that acquired non-migratory behaviour took refuge and are now thriving in particular sections of the river (Ojwang *et al*., 2007). If these once-migratory species are now sedentary riverine residents, this also casts a different light on their conservation status. Though seemingly abundant at upper river stations, they are now even more vulnerable to human impacts than in the past, when most of the adult population was presumably migratory and susceptible. Subpopulations in these fragmented bodies are forced to differentiate into units where inbreeding is very common with little or no gene flow among them (Wright, 1978).

These environmental effects may often contribute to population bottlenecks which enhance inter-population differentiation. In the individual populations, there is promotion of genetic drift, inbreeding and reduced genetic exchange between sub-populations, resulting in loss of genetic variation (Wright, 1978). The significance of these processes in the generation and maintenance of the fisheries resources is not known.

2.8 Effects of anthropological impacts on populations size and genetic diversity of *B. altianalis* in Lake Victoria basin

The Lake Victoria ecosystem is undergoing unprecedented ecological changes due to anthropogenic and other impacts that threaten long term existence of its biodiversity.

Besides the cichlids, the cyprinids are the other indigenous group of fishes whose populations have severely been reduced.

Many studies have focused on the biology and economic importance of Lake Victoria basin riverine fishes (Okedi, 1969; 1970; 1971; Welcomme, 1969;). Migration of individual species (Whitehead, 1959; Cadwalladr, 1965a; 1965b; Muli and Ojwang, 1996) is governed by the annual flooding pattern (Welcomme, 1979; Beadle, 1981). Combinations of circumstances make these fishes uniquely vulnerable to over exploitation due to habitat degradation or simply poor management. First, freshly caught fish are the most delicious, a treat for royalty among the fishing tribes. Secondly, a strong consumer preference makes them the most valuable and prized sport fishes (Ogutu-Ohwayo, 1988). The fish are harvested with the least cost and effort at the mouths of rivers, where schooling propensity is greatest, weight is at a maximum and physical condition is prime. These characteristics make riverine fishes remarkably easy to catch during this crucial phase of their life cycle (Ogutu-Ohwayo, 1988).

The general decline in riverine fishes in Lake Victoria has also been attributed to anthropogenic activities within the lake basin. For example, past studies ascribed the decline of *B. altianalis* to the use of highly efficient new gear to extract migrating fishes, which began intensively in the late 1950s in the lower reaches of the rivers and targeted mainly the gravid and ripe populations (Whitehead, 1958; Cadwalladr, 1965b). The introduction of the predatory Nile perch *Lates niloticus* in the lake in the 1950's (Welcomme and de Merona, 1988) may have exacerbated the situation, as this species influenced several ecological changes (Ogutu-Ohwayo, 1990; Kaufman, 1992;

Witte *et al*., 1992b), most of which had negative impacts on the integrity of the lake's ecosystem (Balirwa *et al*., 2003). The *Labeo* and *Barbus* fishery was a major regional, cultural, economic and recreational resource. Continued decline in the population over the last 30 years (Cadwalladr, 1965b; Ogutu-Ohwayo, 1990) prompted the Kenyan Government to initiate a hatchery at Sangoro.

2.9 Population genetic studies on *Barbus*

The diverse genus *Barbus*, which is the most species-rich of all vertebrates, has received only limited attention in population genetics studies. In the few studies available on the genus *Barbus* (Cyprinidae), the primary focus has been on a few species that have shared the status of being either commercially important or popular game fish, namely; *B. xanthopterus* populations in Southwest Iran (Fayazi *et al*., 2006), Iberian *Barbus* (Gante *et al*., 2008), *Barbus* of ancient lake systems of the Balkans (Markova *et al*., 2010), tetraploid *Barbus* species from West Europe, North Africa and Iberian Peninsula (Chenuil *et al*., 1999), and *B. intermedius* from Genale River, Ethiopia (Dimmick *et al*., 2001). However, for the great majority of large *Barbus* from East Africa and specifically *Barbus altianalis*, including *Labeobarbus* of the Albertine basin (Muwanika *et al*., 2012) and the large barbine minnows from Lake Tana (Kebede, 2012), very few population genetics studies have been done so far.

2.9.1 Genetic markers

The genetic study of natural populations is dependent on the availability of polymorphic neutral markers. Although electrophoresis of proteins has been widely used for the direct study of genetic variation in fish populations, DNA markers are now more popular for provision of information about gene flow, allele frequencies

and other parameters crucial in population biology (Neigel, 1997). The general goals of population genetic studies are to characterize the extent of genetic variation within and between populations and account for this variation (Weir, 1996). The amount of genetic variation within and between populations can be determined by the frequency of genes and the forces that affect their frequencies, such as migration, mutation, selection and genetic drift (Gall, 1987). The importance of genetic variation to population adaptability in changing environments or under stressful conditions has long been recognized (Allendorf *et al*., 1987).

2.9.2 Mitochondrial DNA

Mitochondrial DNA (mtDNA) of fish is a small circular molecule of 61b-21kb nucleotides with a compact and conserved organization (Wang *et al*., 2002). The mtDNA markers have been shown to be an excellent tools for revealing between-population variability (Presti *et al*., 2012). Mitochondrial mtDNA has many attributes that make it particularly suitable for population genetic studies, including its rapid rate of evolution, accumulation of neutral mutations, lack of recombination, compact size and nearly complete maternal inheritance (Wang *et al*., 2002; Thai *et al*., 2007; Chu *et al*., 2012).

Molecular phylogenetic studies are increasingly being used to investigate cyprinid classification and evolution at a variety of taxonomic levels including the validity of various families and their inter-relationships; these are often based on the mitochondrial Cytochrome b (Cyt b) (Briolay *et al*., 1998, Zardoya and Doadrio, 1998; Durand *et al*., 2002) and D-loop (also called control region) (Gilles *et al*., 2002; Liu and Chen, 2003). Although these studies bring important new insight into

the evolutionary history of the family and its taxonomic classification, most studies have focused on European, Eurasian, North America and East Asian cyprinids with the sampling of species from East Africa neglected so far.

2.9.3 Control region (D-loop)

The control region has been shown to be the most variable region of the mtDNA in both vertebrates and invertebrates. This region is thus an ideal marker for characterizing geographical patterns of genetic variation within and among *Barbus* species (Chu *et al*., 2012), and has been widely used in molecular systematics of fishes (Wang *et al*., 2002). It is possible to reconstruct the distribution pattern of wide-ranging species by using more detailed molecular phylogeographic and morphological studies (Chen *et al*., 1998).

Due to high levels of sequence polymorphism, mitochondrial control region has been of particular importance in the study of closely related populations in various taxa, including the cyprinid *Barbus xanthopterus* (Chen *et al*., 1998; Fayazi *et al*., 2006). Polymorphism in the control region has been found not only to be useful in interspecific studies but has also been shown to be useful for studies of intraspecific phylogeny and phylogeographical reconstruction in fishes (Chen *et al*., 1998). Mitochondrial control region with Cytochrome c oxidase I have been widely used in population genetics studies (Uthice and Benzie, 2003).

2.9.4 Cytochrome c Oxidase I (CO1)

Cytochrome c oxidase I (CO1) has widely been used in barcording to determine the species-level status following taxonomic revision (Kerr *et al*., 2007; Ward *et al*.,

2007; Smith *et al*., 2008). Surveys of CO1 variation already corroborate very well with traditional taxonomic assignments for several animal groups. Most provisional species flagged by CO1 intraspecific thresholds ultimately gain species-level status following taxonomic revision (Hebert *et al*., 2004a; Hebert *et al*., 2004b; Ward *et al*., 2005, Kerr *et al*., 2007; Ward *et al*., 2007; Smith *et al*., 2008).

2.9.5 Implications for conservation and management

For effective management of a freshwater species, it is necessary to have an understanding of the spatial scale of population differentiation. In many cases, common ecological methods such as mark recapture are inappropriate for large freshwater systems (Saccone *et al*., 1999). Genetics and fishery management can interact in several ways. When the genetic population structure of a species is known, the distribution of subpopulations in mixed fisheries can be estimated (Utter, 1991). Regulation of harvests to protect weaker populations can be made based on these distributions. It is important to identify and regulate harvesting, because genetic changes within a population caused by differential harvests may have drastic and long-term effects on a population.

The progressive decline and fragmentation of *Barbus* populations in the Lake Victoria catchment identifies the need for the development of a recovery plan. The relevance of genetic information to species conservation planning has long been documented (Lande and Barrowclough, 1987; Simberloff, 1988), and population genetic information has assumed an important role in conservation biology. Estimates of genetic variation within and between populations can provide important information on the level of interaction between local populations and permit assessment of the

contribution of a metapopulation structure to regional persistence. Molecular markers are also important tools for identifying population units that merit separate management and high priority for conservation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in the Lake Victoria watershed in Kenya between May and August 2011. Some sampling sites identified by Ojwang (2007) were adopted in this study (Figure 1). The selected sites were geo-referenced using a Geographical Positioning System (GPS) to ensure accurate sampling was carried throughout the study period (Appendix V). The four main rivers flowing into Lake Victoria from the Kenyan catchment were sampled. Samples were collected from the following stations; I, Nzoia-Ugunja Bridge and IV, Nzoia-Webuye before discharge on River Nzoia; V, Yala water works and VI, Yala Kakamega Bridge on river Yala; VII, Nyando-Ahero and X, Nyando-Koru on Nyando River and XI, Sondu-Nyakwere and XII, Sondu-Sondu Bridge on River Sondu-Miriu (Figure 1).

River Nzoia, the largest Kenyan river emptying into Lake Victoria, is fed by numerous tributaries arising in the Cherangany Hills and on the slopes of Mount Elgon (Cadwalladr, 1965a, 1965b). At least two *Barbus* species *B. neumayeri* and *B. paludinosus* have been reported from the upper reaches of this river and its tributaries (Mugo and Tweddle, 1999) (Plate 3.1).

River Nyando originates near Mount Tinderet and drains the central highlands west of the Rift Valley, as does the Yala (Plate 3.2). The Nyando, which traverses the sugar belt region in Kenya, is apparently polluted by sugar factory effluent (Mugo and Tweddle, 1999). Species abundance and distribution in this river are strongly dependent on habitat type and prevailing conditions (Fayazi *et al*., 2006) (Plate 3.3).

The Sondu-Miriu rises on the slopes of the Mau Escarpment (Ochumba and Manyala, 1992) and has two major tributaries, the Yurith and the Kipsanoi (Plate 3.4). The upper catchments of all of these rivers experience high and fluctuating rainfall, causing regular massive floods in the lower reaches, expecially during long rainy season. The lower part of the river supports fishing activities (Owiti *et al*., 2013).

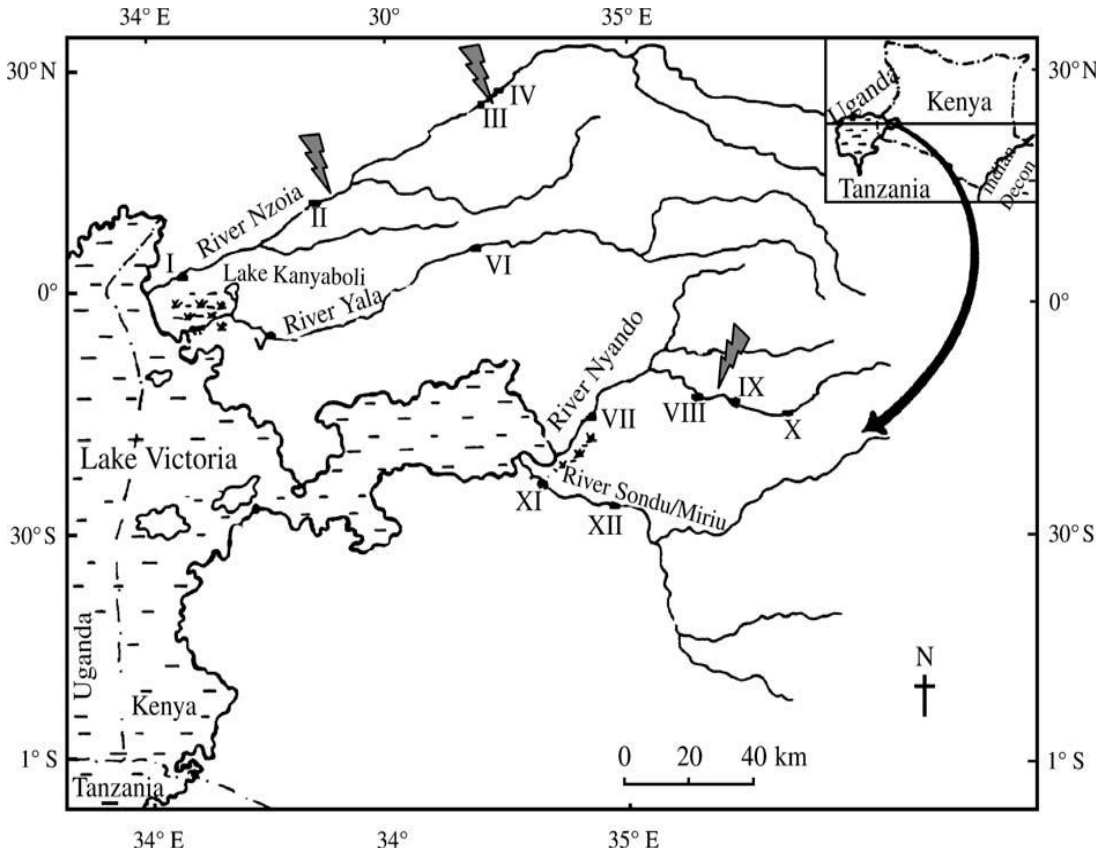

Figure 1: Main rivers draining the Kenyan side of Lake Victoria and stations sampled. Map adapted and modified from Ojwang *et al.*, (2007)

3.2 Sample collection

Samples were collected from each of the four rivers using an electro-fisher model 11. The generator was a Honda GX 240 8 HP that produced a current at 400 V and 10 A. Two sampling sites were selected from each river one near the river mouth and one at the upstream. Sampling was carried out in the months of May and August 2011. A total of 240 samples of *B. altianalis* were collected (Nzoia River, 60; Nyando River, 60; Sondu-Miriu River, 60; Yala River, 60). A total of 30 samples were collected from each sampling sites. Fish were identified to species level using identification keys and photographs (Froese and Pauly, 2011). All other species except *Barbus altianalis* were released back to the river.

Total length (TL) in cm and standard length (SL) in cm were measured using a measuring board, and total weights (TW) in g were measured using a digital balance model ohaus CS200. Data on sex and stage of gonadal maturity in all the 240 individuals were also assessed to confirm their sexes according to Witte and Van Densen (1995). Approximately 0.5cm^2 muscle tissue was clipped from each fish, put in a 1.5m Eppendorf tube and preserved in 95% ethanol for DNA analysis, after which the fish samples were labelled, fixed in 4% formalin, packed in plastic containers and carried to the Zoology Laboratory, University of Eldoret where it was then preserved in 70% ethanol.

Plate 3.1 : River Nzoia (Ugunja) and an inset of *B. altianalis* (enlarged)

(Source: Author, 2011)

Plate 3.2: River Yala (Water works), surrounded by dense Papyrus growth, and inset a Sample of *B.altianalis* (enlarged) collected from river

(Source: Author, 2011)

Plate 3.3: Electrofishing of samples of *B. altianalis* at River Nyando (Koru) (Source: Author, 2011)

Plate 3.4: River Sondu-Miriu (ITEK) during this study and an inset of *B. altianalis* collected using an electrofisher

(Source: Author, 2011)

3.3 Morphometrics

3.3.1 Measurement of morphological characters

All morphometric data were measured on the left side of the fish body: Standard length (SL), Body depth (BD), Head length (HL), Snout length (SnL), Eye diameter (ED), Interorbital width (IOW), Dorsal fin base length (DFB), Anal fin base length (AFB), Predorsal length (PDL), Preanal length (PAL), Prepectoral length (PPL), Preventral length (PVL), Caudal peduncle length (CPL), Caudal peduncle depth (CPD), Pelvic fin base length (PvFB), Pectoral fin base length (PFB), Length of the anterior barbell (LAB), Length of the posterior barbel (LPB), Occipital length (OcL), Total length (TL), Total weight (TW) (Appendix I).

3.3.2 Analysis of Morphometric data

A total of 196 mature fish of *B. altianalis* selected and used to study the morphology. External morphometric characters data obtained from this study were entered in MS Excel spreadsheets for storage and management while analysis was done using PAST (PAlaeontological STastistics version 1.68; http://folk.uio.no/ohammer/past) (Hammer *et al*., 2001) and MINITAB version 14.

Mean values for each morphometric character measured from the *Barbus altianalis* in each of the four rivers in Lake Victoria, Kenya were computed and summarized in a table as mean ± SEM. One - way Analysis of Variance was then performed to test if there were significant variations in each morphometric character between the rivers.

Morphometric data were subjected to a Principal Component Analysis (PCA) especially designed to remove size effects, i.e. the ‘sheared’ PCA (Humphries *et al*., 1981). In the present analysis the Principal components analysis (PCA) was further

done on a covariance matrix to obtain eigenvalues and loadings based on Hammer *et al*. (2001). As a procedure, PCA is used for finding hypothetical variables (components) that account for as much of the variance in a multidimensional data set as possible as per Davis (1986) and Harper (1999). In the present analysis the first principal component (PCA I) integrates size-related variation, whereas the PCA II is theoretically size-free. The nonparametric Mann–Whitney U test was performed on the components identified from PCA loadings for univariate comparisons to evaluate differences between groups on characters contributing most to variation. The sheared PCA was performed using the PAST programme.

3.3.3 Growth Variability

For the purpose of growth variability of all the external morphometric characters studied with respect to standard length, linear regression analysis was done on them and the results summarized in a table. *B*eta (β) values were used to determine the type of growth and the correlation of the relationship was determined using the R^2 value while p value was used to show the significance of the relationship (Verep *et al*., 2006). Analysis of Covariance (ANCOVA) for the four populations was done to test for equality of slopes in the relationships and hence morphometric differences among the populations.

3.4 Genomic DNA extraction

Genomic DNA was extracted from approximately 25 mg muscle tissue from each of the 240 samples of *B. altianalis*. The DNeasy® blood and tissue Kit (QIAGEN Inc., QiagenStraße 1, 40724 Hilden, Germany) protocol was used. The muscle tissue was cut into small pieces to enable more efficient lysis, and placed in a 1.5µl

microcentrifuge tube with 180 µl Buffer ATL. 20 µl proteinase K was added and mixed thoroughly by vortexing, and was incubated at 56°C until the tissue was completely lysed. Vortexing was done occasionally during incubation to disperse the sample. Samples were lysed overnight after which vortexing was done again for 15 s, and 200 µl Buffer AL was added to the sample, and mixed thoroughly by vortexing. Then 200 µl ethanol (96–100%) added and mixed again thoroughly by vortexing. (It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution). The mixture was Pipetted (including any precipitate) into the DNeasy Mini spin column placed in a 2 µl collection tube then centrifuged at 6000 x g (8000 rpm) for 1 min. The flow-through and collection tube were discarded. The DNeasy Mini spin column was placed in a new 2 µl collection tube and 500 µl Buffer AW1 was added then centrifuged for 1 min at 6000 x g (8000 rpm). The flow-through and collection tube were discarded. The DNeasy Mini spin column was then placed in a new 2 µl collection tube, 500 µl Buffer AW2 added, and centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane, then flow-through and collection tube were discarded. It is important to dry the membrane of the DNeasy Mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution. The DNeasy Mini spin column was then placed in a clean 1.5 µl or 2 µl microcentrifuge tube and 200 µl Buffer AE pipetted directly onto the DNeasy membrane. It was incubated at room temperature for 1 min, and centrifuged for 1 min at 6000 x g(8000 rpm) to elute. Elution was repeated once more with 100 µl elution buffer.

Presence and quantity of genomic DNA from the extraction process was checked by electrophoresis using 5 µl of the extract and 2 µl loading dye in a 1% agarose gel stained with ethidium bromide. A standard 100 base pair ladder was co-electrophoresed with the samples to confirm presence of a band of high molecular weight genomic DNA. The DNA concentration was quantified spectrophotometrically using a Nanodrop spectrophotometer.

3.5 Amplification of DNA via Polymerase Chain Reaction (PCR)

The mitochondrial control region (D-loop) and Cytochrome c Oxidase 1 (CO1) gene were sequenced to provide data to infer the genetic diversity, population genetic structure and phylogenetics of *Barbus altianalis* in Lake Victoria.

3.5.1 mtDNA control region (D-loop)

Approximately 850bp of mitochondrial DNA control region was PCR-amplified using common carp primers *Cyprinus carpio,*

Carp-Pro (5'AACTCTCACCCCTGGCTACCAAAG-3') and

Carp-Phe (5'CTAGGACTCATCTTAGCATCTTCAGTG-3') (Chang, 1994). Amplifications were performed in 25 µl volume. Each reaction mixture contained 9 µl of DNA (3 ng µl -1), 0.8 µl of sterile water, 2.5 µl of 10 X reaction buffer (Super-Therm), 4 µl of 25 mM $MgCl_2$ (Super-Therm), 0.5 µl of 10 mM deoxynucleoside-triphosphate mixture (dNTPs) (Roche), 0.2 µl of *Taq* polymerase (5 U µl 1) (Super-Therm), and 4 µl of each primer (6 mM) (forward and reverse) per reaction. Thermal cycling was carried out at 95°C for 2 min, followed by 38 cycles of 95°C for 1 min, annealing at 59°C for 1.5 min, extension at 72°C for 2 min, followed by a final

extension of 72°C for 10 min. The PCR products were then separated usig electrophoresis.

3.5.2 CO1 region

Approximately 658bp of the mitochondrial CO1 gene was amplified using the primers FishF1 (5'TCAACCAACCACAAAGACATTGGCAC-3') and

FishR1 (5'TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward *et al*., 2005). PCR amplifications were performed in 25 µl volumes. Each reaction mixture contained 9 µl of DNA (3 ng µl -1), 0.8 µl of sterile water, 2.5 µl of 10 X reaction buffer (Super-Therm), 4 µl of 25 mM $MgCl_2$ (Super-Therm), 0.5 µl of 10 mM deoxynucleoside-triphosphate mixture (dNTPs) (Roche), 0.2 µl of *Taq* polymerase (5 U µl -1) (Super-Therm), and 4 µl of each primer (6 mM) (forward and reverse) per reaction. Thermal cycling was carried out at 95°C for 2 min, followed by 35 cycles at 95°C for 30 sec, annealing at 54°C for 30 sec, extension at 72°C for 1 min, followed by a final extension of 72°C for 10 min. The PCR products were then separated usig electrophoresis.

3.6 Gel Electrophoresis

PCR products were separated by electrophoresis in 1% agarose gels stained with 0.5% ethidium bromide (for visualization of bands by ultraviolet trans-illumination).

3.6.1 Gel Preparation

To prepare 1% agarose gel, 2g of Agarose was added to 10µl 10X TBE filled up to 100µl with deionised water heated for 2 min. 50µl Ethidium Bromide was added after it was slightly cooled. The tray was set by pouring the gel in the tray and the comb placed. The gel was left for 40 min. 1X TBE running buffer was poured onto the

electrophoretic chamber. The set gel was placed into the electrophoretic chamber. 2 µl loading dye was pipetted into sample plates and about 4 µl of DNA was pipette into the plate and mixed well and pipette into the wells. 1.5 µl of ladder was pipette to the first well. The gel was run at 100 volts for 60 minutes. The DNA was visualised and photographed using a BioRad® GelDoc UV-transilluminator.

3.7 Mitochondrial DNA purification and sequencing

Target fragments were purified from excised gel bands using a Zymoclean Gel DNA recovery kit. The DNA fragments were excised from the agarose gel using a scalpel and transfered into a 1.5 µl microcentrifuge tube. 3 volumes of ADB was added to each volume of agarose excised from the gel (e.g. for 100 µl (mg) of agarose gel slice add 300 µl of ADB). It was then incubated at 37-55 °C for 5-10 minutes until the gel slice was completely dissolved. For DNA fragments > 8 kb, following the incubation step, one additional volume (equal to that of the gel slice) of water was added to the mixture for better DNA recovery (e.g., 100 µl agarose, 300 µl ADB, and 100 µl water).

The melted agarose solution was transfer to a Zymo-Spin™ Column in a Collection Tube. It was then centrifuged for 30-60 seconds and the flow-through discard. 200 µl of DNA Wash Buffer was added to the column and centrifuged for 30 seconds. The flow-through discard and the wash step repeated. 6 µl DNA Elution Buffer was added directly to the column matrix. The column was placed into a 1.5 µl tube and centrifuged for 60 seconds to elute DNA. The Ultra-pure DNA was then ready for eletrophoresis. 0.2µl of the product was electrophoresed in a 1% agarose gel at 60 volts and visualised (Plate 6). Sequencing was carried out at InqabaBiotec laboratory

(Hatfield, Pretoria, South Africa). All fragments were sequenced in both directions to allow for the reconciliation of ambiguous positions.

The program SEQUENCHER 4.8 (Gene Codes, Ann Arbr, and MI) was used to align and edit the forward, reverse and consensus sequences from the DNA fragments. The obtained mtDNA sequences were quality-checked and aligned by eye to confirm the correspondence of the bases in the two complementary sequences. Thereafter, the sequences were assembled in CLC for reconciliation of ambiguous positions. Sequences were aligned using the CLUSTAL W option (Thompson *et al*., 1994) of BioEdit 7.0.9 (Hall, 2005) and by visual inspection. The control region sequences are publicly available under GenBank accession numbers KC860272-KC860467 (Appendix V).

3.8 Determination of genetic diversity and population structuring inferred from haplotype and nucleotide diversity and networks

Because of its higher variability, the mtDNA control region data set was used for population genetic analyses. To determine levels of genetic diversity within the populations and between the four populations, the number of polymorphic sites, number of mtDNA haplotypes, haplotype diversity (h) and nucleotide diversity (π) were calculated for each population using ARLEQUIN 3.01 (Excoffier *et al*., 2005). To investigate the phylogenetic relationships among the mtDNA haplotypes, a haplotype network was constructed using TCS1.21. Control region sequence data were used, with gaps coded as missing and first run at a 95% parsimony connection limit (Clement *et al*., 2000). A single network was then created with a fixed connection limit of 100 steps (Librado and Rozas, 2009). The origins of the haplotypes were identified by differences in shading, where DNA shade represents

Sondu-Miriu, fish scales represented Nyando, Black represented Nzoia and White represented Yala, while the size is proportional to the number of times a given haplotype occurs. The haplotype numbers were written inside the circles while branch labels represent the number of mutational steps between haplotypes. Where no label is given, a single mutational step is assumed. Solid lines represent 100% parsimony connections, whereas dotted lines represent less than 95% parsimony connections.

To examine genetic differentiation between populations, exact tests for population differentiation as well as calculation of pair-wise estimates of F_{ST} were carried out using ARLEQUIN 3.11 (Excoffier *et al*., 2005). The 95% significance levels for pair-wise intra-specific population comparisons were conservatively adjusted using a Bonferroni correction. The fixation index, F, serves as a convenient and widely used measure of genetic differentiation between populations (Wright, 1978) when a single group is defined in the Genetic Structure, and is useful for recognition of the contribution of individual populations to the global F_{ST} measure. Theoretically F_{ST} has a minimum of 0, indicating no genetic difference and a maximum of 1, indicating fixation of alternative alleles/ haplotypes in the sub – populations.

DnaSP 5.10 (Librado and Rozas, 2009) was used to generate haplotype data files for use in Analyses of Molecular Variance (AMOVA). Significant variance in the distribution of control region sequences between individuals, populations and groups of populations from the four rivers were determined by AMOVA as implemented in the program ARLEQUIN (Excoffier *et al*., 2005).

3.9 Demographic history

Several approaches were used to test for past demographic changes in *B. altianalis* populations in the four rivers**.** Mismatch distribution analyses (Harpending, 1994) describing pairwise differences between individuals within populations was carried out on the control region sequences to investigate evidence for demographic changes among the populations of *B. altianalis* (Figure 4-10), using the program DNASP 4.9 (Librado and Rozas, 2009). The shape of the distributions was then used to deduce whether a population has undergone past population growth (Rogers and Harpending, 1992; Rogers, 1995). A smooth uni-modal distribution indicates a past sudden population decline/ a recent population expansion, whereas a multimodal distributions is a characteristic of a long-term stationery/relatively stable population. The plots were drawn according to model of population expansion with the plots representing the observed distribution (dotted lines) and that expected (solid line).

Further analyses were carried out using the coalescent-based neutrality estimators: Fu's *F*s (Fu, 1997) and Tajima's *D* (Tajima, 1989) (Table 2) estimated using ARLEQUIN (Excoffier *et al*., 2005). The above neutrality test is a powerful test to detect historical demographic expansions (Rogers and Harpending, 1992; Ramos-Onsins and Rozas, 2002). The fit between observed and estimated distribution under a sudden expansion model was subjected to two different goodness-of-fit tests (standardized squared differences (SSD) and raggedness index tests. The index distinguishes the unimodal distribution from a ragged distribution.

3.10 Phylogenetic relationships among haplotypes

The HKY85 model was found to best fit the control region data set and thus was used to construct the neighbour joining (NJ) and maximum likelihood (ML) trees in PAUP 4.1 (Hasegawa *et al*., 1985; Swofford, 2003) as well as in the Bayesian tree searches, carried out using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The trees were visualized using the computer program TREEVIEW 3.2 (Page, 1996). The support values from the neighbour joining analysis and congruent maximum parsimony and Bayesian analyses were presented above nodes; these were represented as neighbour joining bootstrap %/ maximum parsimony bootstrap %/ Bayesian posterior probability. Only values >60%/0.95 were reported. The outgroup sequence used in the control region analyses was that of the cyprinid *Procypris rabaudi* (accession number EU683675.1) (Zhu and Li, 2008). The outgroup used in the CO1 analyses was *Labeobarbus intermedius* (JX066760.1). A neighbour joining tree based on a concatenation of the 2 mitochondrial markers (control region and CO1, 1316 nucleotides) was not rooted owing to the lack of an outgroup for both sequences on the Genbank (Tamura *et al*., 2011).

In order to assess the phylogenetic position of the experimental samples based on the control region, 35 sequences of *Labeobarbus altianalis* from Uganda (Appendix I) were identified by a BLAST search of the NCBI Genebank, downloaded and included in an alignment with the experimental sequences (JN120443 - JN120478). Based on CO1 experimental sequences, closest matches were identified by a BLAST search of the NCBI Genbank, downloaded and included in an alignment with the CO1 experimental sequences. These included members of the cyprinid genera *Labeobarbus, Barbus, Neolissochilus*, *Varicorhinus* and *Tor*. Phylogenetic

relationships were assessed by the neighbour joining, maximum parsimony and Bayesian inference methods. Individual pairwise genetic distances between study samples and outgroup taxa were calculated in PAUP according to the HKY model of nucleotide substitution, determined in jModeltest (Posada, 2008) as best fitting the dataset. In order to assess the significance of the genetic distances observed between the study taxa, mean interspecific distances were calculated between species within the following genera; *Labeobarbus, Varicorhinus, Neolissochilus*.

CHAPTER FOUR

RESULTS

4.1 Morphology

4.1.1. Morphometric Characteristics

The differences noted in external characteristics of 196 *Barbus altianalis* collected from four sites in Lake Victoria, Kenya were summarized in Table 1 49 from each river. The mean values of all external morphometric parameters were highest in the River Nzoia population followed by Sondu-Miriu and Nyando while River Yala population had the lowest morphometric measurements. The mean length and mean weight for example of *Barbus altianalis* from River Nzoia were the largest of all the four populations (20.44 ± 0.78 cm, and 108.12 ± 10.46 g respectively) while that of river Yala were the smallest (13.38 ± 0.65cm and 34.53 ± 8.94 respectively). The length of anterior barbell ($F=1.174$, $p=0.320$) and the length of the posterior barbel ($F=0.165$, $p=0.920$) did not vary significantly among the populations at $\alpha = 0.05$. All the other morphometric characteristics measured varied significantly among populations. The external morphometric characters were further subjected to PCA as percentage standard length to establish the characters variability of the four river populations.

Table 1: Mean ± SEM of the external morphometric characteristics from the *B. altianalis* collected from four sites in Lake Victoria, Kenya

All lengths in cm and weight in g, Significant variation tested at $\alpha = 0.05$, $P<0.0005$.

Traits	Sites				ANOVA	
	Sondu-Miriu N=49	Yala N=49	Nzoia N=49	Nyando N=49	F	p-value
TL	17.21 ± 0.70	13.38 ± 0.65	20.44 ± 0.78	16.67 ± 0.60	17.781	P<0.0005
TW	61.45 ± 7.25	34.53 ± 8.94	108.12 ± 10.46	58.55 ± 7.16	12.972	P<0.0005
SL	12.91 ± 0.57	9.90 ± 0.46	15.72 ± 0.61	12.63 ± 0.49	19.684	P<0.0005
BD	3.12 ± 0.15	2.40 ± 0.14	4.12 ± 0.19	3.10 ± 0.12	22.491	P<0.0005
HL	3.46 ± 0.15	2.81 ± 0.11	3.91 ± 0.15	3.42 ± 0.14	10.805	P<0.0005
ED	0.86 ± 0.03	0.76 ± 0.02	0.91 ± 0.03	0.76 ± 0.02	10.956	P<0.0005
SnL	0.59 ± 0.03	0.46 ± 0.03	0.69 ± 0.03	0.59 ± 0.03	9.540	P<0.0005
DFB	1.79 ± 0.09	1.55 ± 0.09	2.28 ± 0.09	1.85 ± 0.07	13.372	P<0.0005
AFB	0.94 ± 0.05	0.77 ± 0.04	1.14 ± 0.06	0.94 ± 0.04	9.635	P<0.0005
PDL	7.19 ± 0.30	5.83 ± 0.28	8.48 ± 0.32	7.23 ± 0.29	13.100	P<0.0005
PAL	9.79 ± 0.44	7.53 ± 0.37	11.69 ± 0.48	9.43 ± 0.35	17.115	P<0.0005
PPL	3.66 ± 0.14	3.04 ± 0.12	4.29 ± 0.17	3.52 ± 0.12	13.816	P<0.0005
PVL	6.78 ± 0.29	5.25 ± 0.26	7.91 ± 0.29	6.56 ± 0.24	16.385	P<0.0005
CPL	2.19 ± 0.11	1.73 ± 0.09	2.65 ± 0.11	2.11 ± 0.08	15.140	P<0.0005
CPD	1.45 ± 0.07	1.05 ± 0.07	1.98 ± 0.09	1.47 ± 0.06	27.580	P<0.0005
LAB	0.53 ± 0.03	0.57 ± 0.04	0.63 ± 0.03	0.61 ± 0.05	1.174	0.320
LPB	0.71 ± 0.05	0.69 ± 0.04	0.69 ± 0.03	0.72 ± 0.04	0.165	0.920
PvFB	0.59 ± 0.04	0.41 ± 0.03	0.71 ± 0.04	0.62 ± 0.06	8.431	P<0.0005
PFB	0.49 ± 0.03	0.39 ± 0.02	0.64 ± 0.04	0.48 ± 0.03	12.125	P<0.0005
OcL	2.76 ± 0.13	2.41 ± 0.09	3.21 ± 0.11	2.77 ± 0.10	8.889	P<0.0005
IOW	1.05 ± 0.06	0.82 ± 0.05	1.30 ± 0.06	1.12 ± 0.08	9.917	P<0.0005

TL ≡ Total length, TW ≡ Total Weight, SL ≡ Standard length, BD ≡ Body depth, HL ≡ Head length, SnL ≡ Snout length, ED ≡ Eye diameter, IOW ≡ Interorbital width, DFB ≡ Dorsal fin base length, AFB ≡ Anal fin base length, PDL ≡ Predorsal length, PAL ≡ Preanal length, PPL ≡ Prepectoral length, PVL≡ Preventral length, CPL ≡ Caudal peduncle length, CPD ≡ Caudal peduncle depth, PvFB ≡ Pelvic fin base length, PFB ≡ Pectoral fin base length, LAB ≡ Length of the anterior barbell, LPB ≡ Length of the posterior barbell,OcL ≡ Occipital length.

4.1.2. Principal Component Analysis (PCA)

The PCA on the morphometric data showed some partial separation of the polygons of the four rivers (Figure 2). There was slight separation on the first component with most of fish from River Yala being on the positive part of the first principal component, whereas those from River Nzoia were on the negative part. Rivers Sondu-Miriu and Nyando did not show any clear separation in both the first and second components. PCA1 accounted for 43.25% of the variability while PCA2 accounted for 19.44% of the difference (figure 2).

The factor loadings established that a total of eleven variables were morphologically informative. The first principal component was defined mostly by prepectoral length, caudal peduncle depth, lengths of anterior and posterior barbels, whereas the second component was defined mostly by snout length, eye diameter, body depth, preanal length, pelvic fin base length, occipital length and interorbital width (Table 2).

Mann–Whitney U test (Table 3) for variation between pairs of rivers studied showed that all the morphometric traits of *B. altianalis* from River Sondu-Miriu except eye diameter did not vary significantly from those of River Nyando (0.000 (2612.0)). Comparisons between Rivers Sondu-Miriu and Yala showed significantly difference on external morphometric traits except for the length of anterior barbell (0.071 (2575.0)). All the traits tested showed significant variations between Rivers Yala and Nzoia. The length of posterior barbel did not vary significantly between Rivers Sondu-Miriu and Nzoia (0.211 (2114.5)), Sondu-Miriu and Nyando (0.321 (2620.5)) and Yala and Nzoia (0.000 (1608.0)).

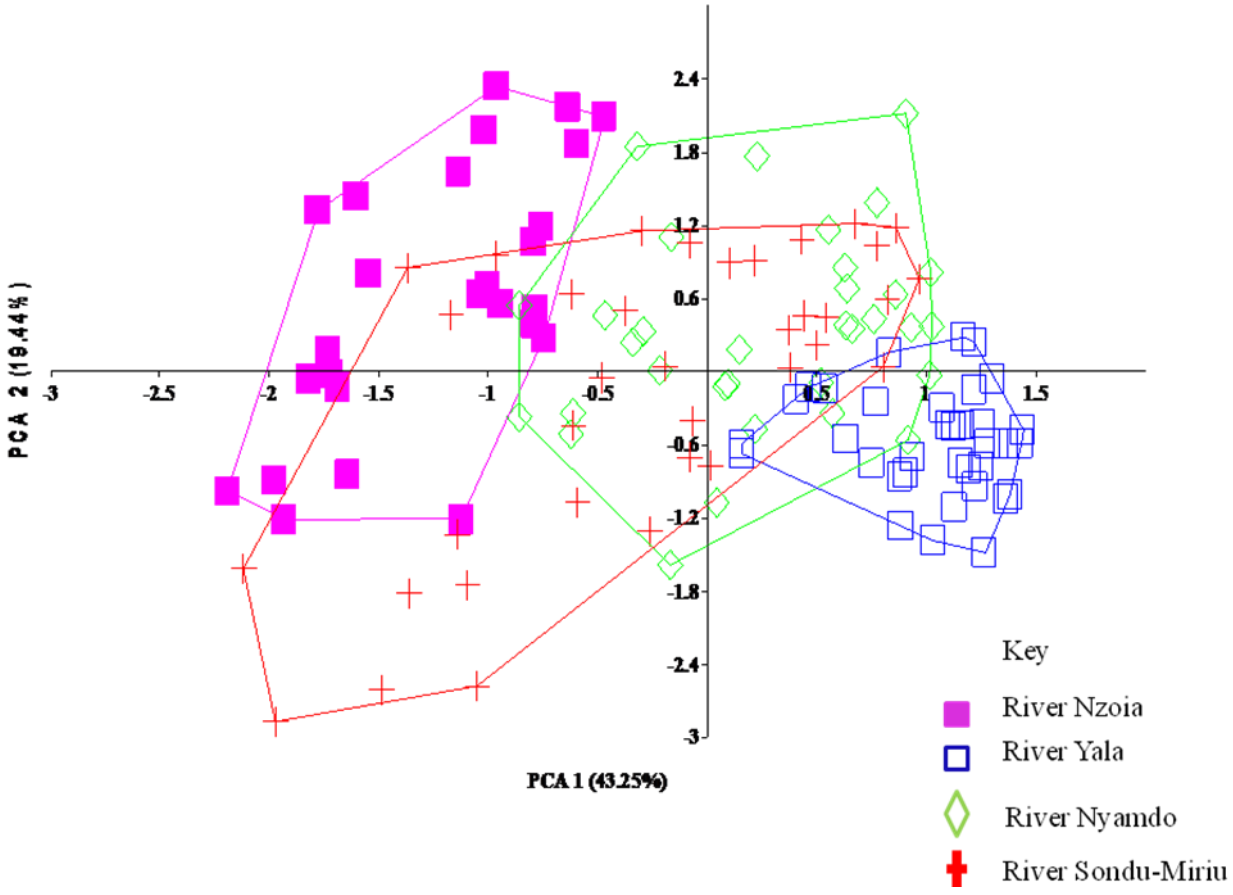

Figure 2: PCA plots showing the variability of the external traits of *Barbus altianalis* specimens from the four rivers draining to Lake Victoria Kenyan basin

Table 2: Loadings of the percent standard metrics on first and second principal components.

Most significant loadings are in bold, SL ≡ Standard Length

	PC1	**PC2**
Head length % SL	-0.118	0.106
Eye diameter %SL	-0.182	**0.357**
Snout length %SL	-0.127	**-0.300**
Dorsal fin base length %SL	0.011	0.033
Body depth %SL	0.004	**-0.334**
Anal fin base % SL	-0.160	-0.194
Predorsal length %SL	0.004	0.085
Preanal length %SL	-0.062	-0.233
Prepectoral length %SL	-0.286	**0.311**
Preventral length %SL	-0.076	-0.104
Caudal peduncle length % SL	0.072	-0.029
Caudal peduncle depth % SL	0.263	**-0.374**
Length of anterior barbell %SL	**-0.960**	-0.113
Length of the posterior barbel %SL	**-0.842**	0.108
Pelvic fin base length %SL	0.119	**-0.958**
Pelvic fin base %SL	-0.178	-0.206
Occipital length %SL	**-0.315**	0.261
Interorbital width %SL	0.040	**-0.426**

Table 3: Mann-Whitney U Test results for selected morphometric traits between the four rivers

(U values are in brackets, ** shows significant difference at both 99% and 95% while * shows significant difference at only 95%)

Variable	Sondu-Miriu versus Yala	Sondu-Miriu versus Nzoia	Sondu-Miriu versus Nyando	Yala versus Nzoia	Yala versus Nyando	Nzoia versus Nyando
ED	0.000** (2823.5)	0.014* (1824.0)	0.000** (2612.0)	0.000** (1274.5)	0.078 (1913.0)	0.0000** (2838.5)
SnL	0.000** (2843.0)	0.001** (1713.0)	0.492 (2227.5)	0.000** (1169.0)	0.000** (1444.5)	0.000** (2641.5)
BD	0.000** (2882.5)	0.000** (1472.5)	0.634 (2200.5)	0.000** (1103.5)	0.000** (1391.0)	0.000** (2902.5)
PAL	0.000** (2876.5)	0.000** (1564.0)	0.343 (2261.0)	0.000** (1116.0)	0.000** (1400.5)	0.000** (2819.5)
PPL	0.000** (2809.5)	0.000** (1587.0)	0.371 (2254.0)	0.000** (1167.0)	0.000** (1522.5)	0.000** (2773.0)
CPL	0.000** (3182.0)	0.000** (1566.0)	0.755 (2371.0)	0.000** (1199.5)	0.000** (1424.5)	0.000** (2985.0)
LAB	0.071 (2575.0)	0.012* (1947.0)	0.638 (2542.5)	0.000** (1608.0)	0.247 (2299.0)	0.002** (2762.0)
LPB	0.026* (2632.0)	0.211 (2114.0)	0.321 (2620.5)	0.000** (1748.0)	0.433 (2354.5)	0.008** (2702.0)
PvFB	0.000** (3112.5)	0.000** (1804.0)	0.089 (2725.5)	0.000** (1281.5)	0.000** (1737.0)	0.000** (3113.0)
OcL	0.000** (3056.5)	0.000** (1814.5)	0.134 (2696.0)	0.000** (1246.5)	0.000** (1892.0)	0.000** (3051.0)
IOW	0.000** (3092.5)	0.000** (1692.5)	0.365 (2607.5)	0.000** (1231.5)	0.000** (1679.5)	0.000** (3037.0)

BD ≡ Body depth, SnL ≡ Snout length, ED ≡ Eye diameter, IOW ≡ Interorbital width, PAL ≡ Preanal length, PPL ≡ Prepectoral length, CPL ≡ Caudal peduncle length, PvFB ≡ Pelvic fin base length, LAB ≡ Length of the anterior barbell, LPB ≡ Length of the posterior barbell, OcL ≡ Occipital length.

4.1.3. Growth Variability of Morphometric Traits

The analysis of growth variability of the external morphometric characters was carried outwith respect to standard length (SL) and several correlations were observed (Table 4). Based on the beta (b) value, all the external parameters displayed negative allometric growth for the samples from Rivers Nzoia, Yala, Nyando and Sondu-Miriu. Based on the regression, all external parameters except LAB (R^2 = 47.7), CPL (R^2 = 32.8), LPB (R^2 = 49.3) and ED (R^2 = 26.1) showed strong positive relationships with the SL ($R^2 > 0.5$) which were statistically significant ($p < 0.001$).

4.1.4 Analysis of Covariance (ANCOVA)

Analysis of covariance for the four populations was carried out to test for equality of slopes in the relationships and hence show morphometric differences among the populations. All the morphometric variables showed significant variation ($P<0.05$) between the populations (Table 5) except in Anal Fin Base, Predorsal Length, Preanal Length, Prepectoral Length, Preventral Length, Caudal peduncle length, Pelvic fin base length, Pectoral fin base length and Interorbital width were the variation was not significant. Based on R^2, all the external parameters showed strong positive relations with the SL ($R^2>0.5$).

Table 4: R^2 and beta (β) values for the morphometrics measured against the standard length

(Bold values shows metrics that do not have strong relationships)

	Sondu Miriu		**Yala**		**Nzoia**		**Nyando**		**Overall**	
Dependent Variable	**R^2**	**β**	**R^2**	**β**	**R^2**	**β**	**R^2**	**β**	**R^2**	**β**
Body Depth	91.2	1.018	86.6	1.256	91.5	1.144	86.7	0.900	93.3	1.102
Head Length	89.6	0.858	87.0	0.920	49.7	0.991	96.1	0.969	85.2	0.843
Eye Diameter	62.2	0.452	73.6	0.668	**26.1**	0.444	83.2	0.594	66.6	0.493
Snout Length	92.1	1.164	74.7	1.391	81.7	1.152	80.4	1.120	88.4	1.108
Dorsal Fin Base	70.3	0.988	60.3	1.282	66.4	0.717	74.9	0.859	78.4	0.945
Anal Fin Base	88.2	1.161	72.0	1.277	57.6	1.091	81.2	0.985	83.6	1.024
Predorsal Length	97.8	0.923	93.1	1.078	96.3	0.929	58.0	0.868	87.3	0.907
Preanal Length	96.1	1.007	92.4	1.091	96.5	0.957	97.1	0.976	97.7	1.005
Prepectoral Length	94.4	0.865	72.2	0.901	91.4	0.844	92.7	0.886	83.0	0.826
Preventral Length	85.3	0.915	71.6	1.177	90.1	0.809	91.7	0.865	90.4	0.926
Caudal peduncle length	70.3	0.887	**32.8**	1.044	73.4	0.958	58.1	0.709	72.0	0.913
Caudal peduncle depth	88.8	1.050	64.3	1.247	93.9	1.142	84.6	0.989	90.3	1.204
Length of the anterior barbell	67.9	1.248	**47.7**	1.068	68.8	1.306	74.2	1.500	59.2	0.952
Length of posterior barbell	78.2	1.388	**49.3**	0.855	65.8	1.259	67.2	1.037	55.0	0.795
Pelvic fin base length	79.8	1.149	64.5	1.790	89.4	1.098	77.8	1.033	83.1	1.215
Pectoral fin base length	81.8	1.220	55.2	1.049	86.7	1.130	80.9	0.976	84.6	1.041
Occipital length	83.8	0.967	78.3	0.740	84.9	0.672	90.8	0.879	87.3	0.766
Interorbital width	90.9	1.124	70.4	1.268	77.0	1.079	88.4	1.168	90.6	1.149

Table 5: R^2 and F values for the morphometrics measured against the standard length

(Bold values shows metrics that do not have strong relationships) P<0.05 showed significant variation in morphometrics between populations.

Morphometric Traits	**F**	**P**	**R^2**
Body Depth	13.82	P<0.005	94.25
Head Length	10.19	P<0.005	91.74
Eye Diameter	8.31	P<0.005	67.54
Snout Length	3.86	0.010	83.32
Dorsal Fin Base	2.93	0.035	83.39
Anal Fin Base	2.19	**0.091**	83.56
Predorsal Length	1.90	**0.131**	92.07
Preanal Length	1.11	**0.347**	97.59
Prepectoral Length	2.21	**0.089**	93.11
Preventral Length	1.75	**0.159**	90.36
Caudal peduncle length	0.88	**0.450**	72.48
Caudal peduncle depth	13.82	P<0.005	93.75
Length of the anterior barbel	16.64	P<0.005	64.44
Length of posterior barbell	21.78	P<0.005	65.12
Pelvic fin base length	2.20	**0.089**	77.79
Pectoral fin base length	1.38	**0.250**	82.34
Occipital length	5.31	0.002	88.87
Interorbital width	0.71	**0.547**	88.35

4.2 Genetic characterization of *Barbus altianalis*

4.2.1 Electrophoresis

PCR products appeared as sharp bands (Plate 5 control region and plate 7 CO1**)**, and were selected for further purification based on their migration relative to marker bands**.** The purified fragments visualised (Plates 6) were sequenced.

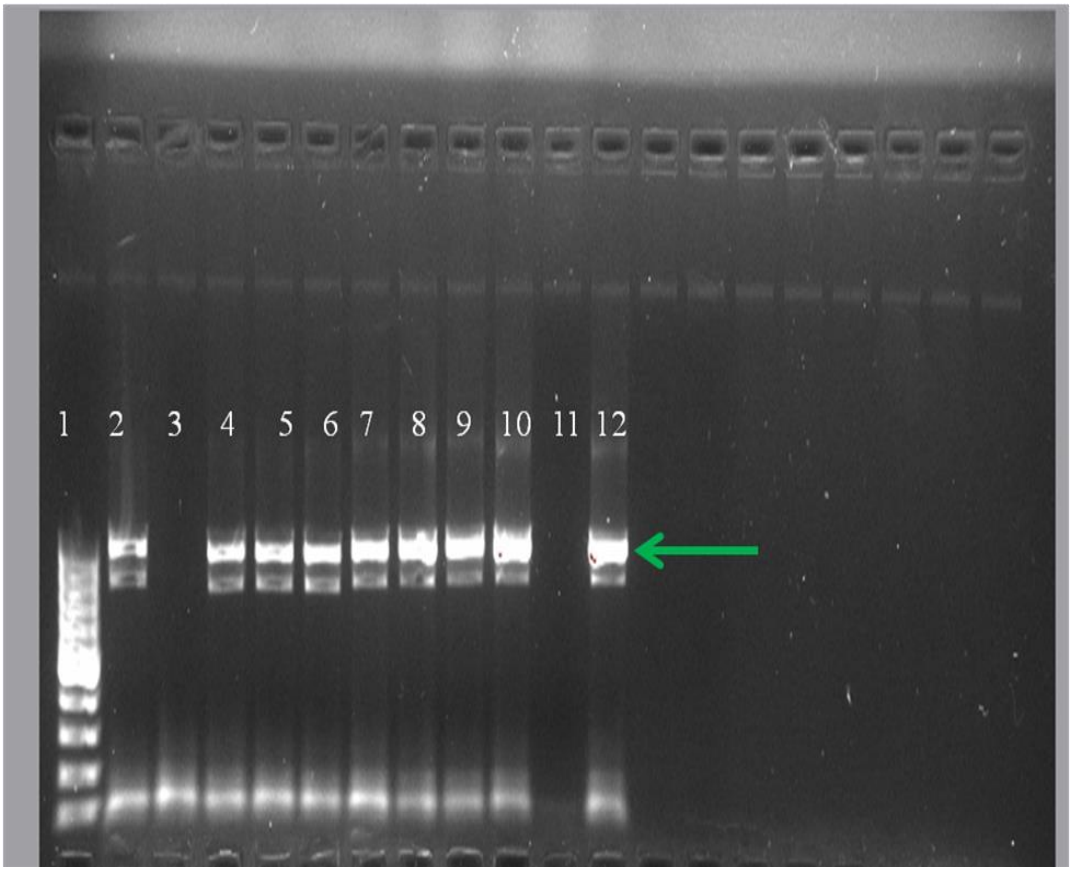

Plate 4.1: Electropherogram showing separation of products of PCR amplification using the control region primers

Lane 1, 100 bp ladder. Lanes 2-11, PCR products. The target control region fragment (major product selected for sequencing) is indicated with an arrow.

(Source: Author, 2012)

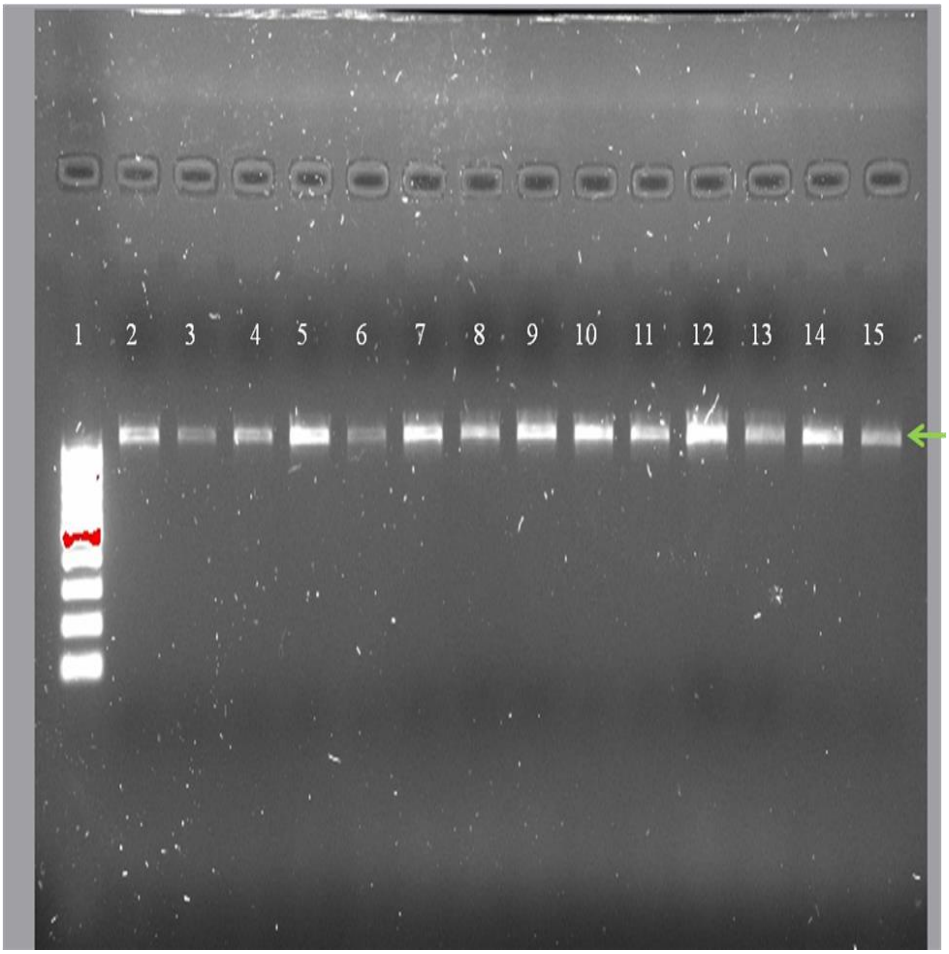

Plate 4.2: Electropherogram showing separation of purified control region fragments after excision from the gel

Lane 1, 100 bp ladder. Lanes 2-14 purified control region amplicons. Purification using Zymoclean Gel DNA extraction kit

(Source: Author, 2012)

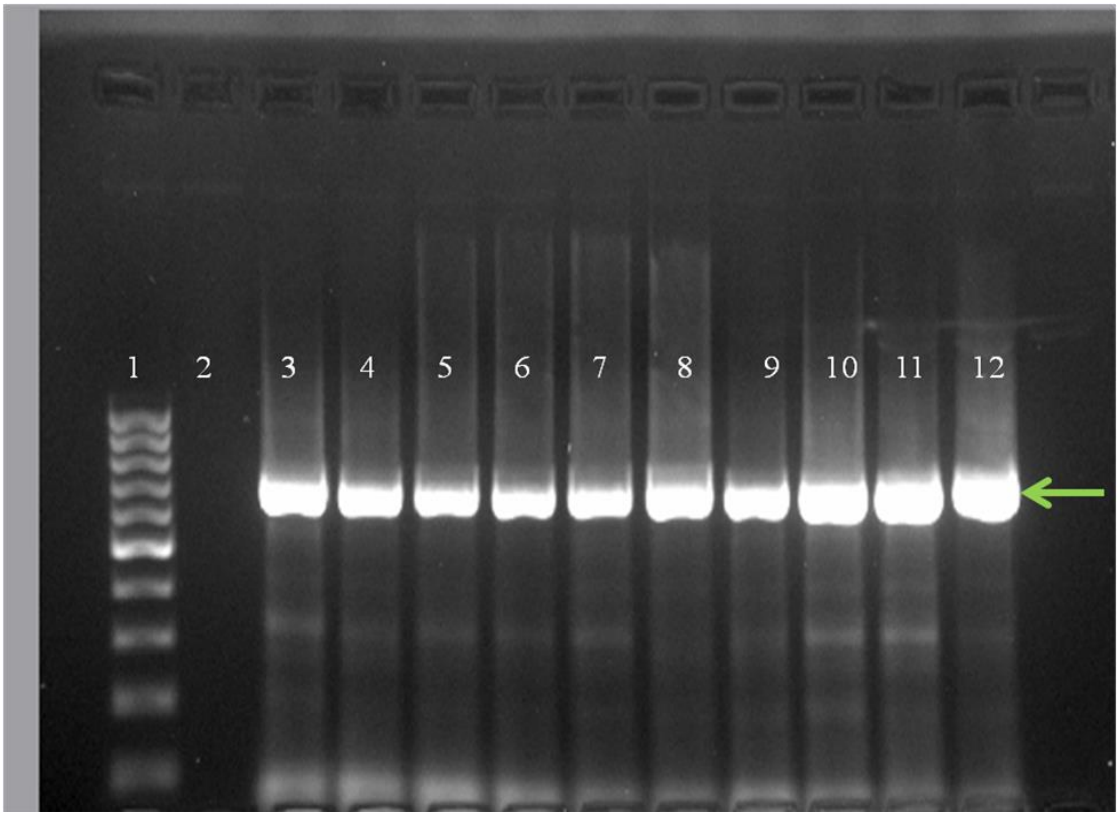

Plate 4.3: Electropherogram showing separation of products of PCR amplification using the control region primers

Lane 1, 100 bp ladder. Lanes 2-11, PCR products. The CO1 fragment (major product selected for sequencing) is indicated with an arrow.

(Source: Author, 2012)

4.2.2 Genetic diversity inferred from haplotype diversity, nucleotide diversity and haplotype network structure

The 196 individuals sampled from the four rivers yielded 49 different haplotypes (Table 6). Overall mean haplotype diversity (*h)* was 0.937±0.008 and ranged between 56.6% in Sondu–Miriu and 94.4% in the Nzoia while the overall mean nucleotide diversity, π was low (0.013±0.001), ranging between 0.003 in the Sondu–Miriu and 0.027 in the Nzoia (Table 7).

Each river-based population group was characterized by the presence of a high number of private haplotypes (those occurring only in samples from a particular river) (Table 7). Haplotype 6 was most widely distributed and was found in all four rivers; haplotype 3 was found in the rivers Nyando, Nzoia and Yala. Haplotype 4 was restricted to the Nyando and Nzoia Rivers, haplotypes 1, 2, and 9 were restricted to the rivers Nyando and Yala, whereas haplotypes 20 and 22 were restricted to the Nzoia and Yala. Overall, 83.7% of the mitochondrial diversity was restricted to respective rivers. This skewed haplotype frequency is thought to reflect a large population size that allows for the retention of numerous unique haplotypes, and suggests that 'private alleles' could be used as indicators for stock identification.

Table 6: Aligned mtDNA haplotypes from 4 riverine *Barbus altianalis* populations from Lake Victoria basin, Kenya.
(Dots indicate identity with the *Barbus altianalis* haplotype I sequence and dashes indicate gaps)

HAPLOTYPE

```
Hap_1   ATTCTCTTTAGATATTAAAATATACTGATGTACAAAATCCCGCGGCAGTTAGTTCACAAAGCTATGCACTCTTATTCTGTTTATCTATTCGCAACCACCCATCACAGAGGATTGGATT
Hap_2   .............................................C.....................................................................
Hap_3   .............................................C............G.....................................................................
Hap_4   ............C.....................................C.....................................................................
Hap_5   ............C....G.....................T.................T.....................................................................
Hap_6   .....................................T.................T.....................................................................
Hap_7   ............C.....................................C............G............A.....................................................
Hap_8   T.....................................................................................................................
Hap_9   ............C.....................T.................T.....................................................................
Hap_10  ............C.....................................................T.G.....................................................
Hap_11  .........................................A.....C....T........G........C..........C.....................................
Hap_12  ..............................G..............C............G.....................................................................
Hap_13  ..C..T.C....C.........CG....C.................A...CCG...T.T..............C..C...AC...C.............T....T...............
Hap_14  .....................................T.................T.....................................TGG.....................
Hap_15  ....................................C.....................................................................
Hap_16  ..............................G..............C............G..................G..............TGG...C.G........CCC.
Hap_17  ............C.......C.....................C.....................................................................
Hap_18  T.GA..C.CG.GCC.CG.CT.............................C.....................................................TC.....................
Hap_19  T.CT.....................................C...C.........G.....................................................................
Hap_20  T.....................................C............G.....................................................................
Hap_21  T.A...................................C............G.....................................................................
Hap_22  ..................G.....................T.................T.....................................................................
Hap_23  ............C.....................................C............G.....................................................................
Hap_24  ............C.....................................C............G.............C.....................................................
Hap_25  .....................................TT.................T.....................................................................
Hap_26  ..C..T.C....C.C.......CG....C............T...A...CCG...T.T....T.....G.C....C...AC...C.............T....T.........C.....
Hap_27  CA....................................TT.................T.....................................................................
Hap_28  ............C.....................T.................T......G.....................................................................
Hap_29  ......................G.....................C............G.....................................................................
Hap_30  ............C..................A.............T......C............G.....................................................................
Hap_31  T.....................................TT.................T.....................................................................
Hap_32  ....C.....................................C............G.....................................................................
Hap_33  C.....................................T.................T.....................................................C.....
Hap_34  ............C...................T.................C....T........G.....................................................................
Hap_35  ........A.....................................C.....................................................................
Hap_36  T.....................................T.................T.....................................................................
Hap_37  T.CT..................................T.................T.....................................................................
Hap_38  ............C........G...CAGC.CTT.GCGCT.TA...T.A.C....T.T...A....A....T..G....A.CC..T.GC.....................G.C.....
Hap_39  ..........A.C........G...CAGCACTT.GCGCT.TA...T.A.C....T.T...A....A....T..G....A.CC..T.GC.....................G.C.....
Hap_40  ............C........G...CAGCACTT.GCGCT.TA...T...C.A..T.T..GA....A....T..G....A.CC..T.GC.....................G.C.....
Hap_41  ..........A.C........G...CAGCACTT.GCGCT.TA...TCA.C..GGTTTCC.A.G.GAT.T.T..G.C..A.CC..TAG.CGAG...AC.......T.AGA.G.C....A
Hap_42  ............C........G...CAGCACTT.GCGCT.TA...T.A.C....T.T...A....A....T..G....A.CC..T.GC.....................G.C.....
Hap_43  ............C........G...CAGCACTT.GCGCT.TA...T.A.C....T.T...A....A....T..G....A.CC..T.GC..................A...G.CA...A
Hap_44  ............C........G...CAGCACTT.GCGCT.TA...T.A.C....T.T...A....A....T..G....A.CC..T.GC.....................GGC.....
Hap_45  ............C........G...CAGC.CTT.GCGCT.TA...T.A.C....T.T...A....A....T..G....A.CC..T.GC.....................AG.C.....
Hap_46  ............C........G...CAGCACTT.GCGCT.TA...T.A.C.A..T.T..GA....A....T..G....A.CC..T.GC.....................G.C.....
Hap_47  ............C........G...CAGC.CT..GCGCT.TA...T.A.C....T.T...A....A....T..G....A.CC..T.GC.....................G.C.....
Hap_48  ............C........G...CAGCACTT.GCGCT.TA...T.A.C.A..T.T...A....A....T..G....A.CC..T.GC.....................G.C.....
Hap_49  ............C........G..TCAGC.CTT.GCGCT.TA...T.A.C....T.T...A....A....T..G....A.CC..T.GC.....................G.C.....
```

Table 7: Indices of genetic diversity based on 850 nucleotides of the mitochondrial control region of four *Barbus altianalis* populations

(Nyando, Nzoia, Sondu-Miriu and Yala Rivers) and Clade I and Clade II (see Figure 12) derived from the Lake Victoria catchment, Kenya.

INDEX	NYANDO	NZOIA	SONDU	YALA	Clade I	Clade II
Sample size	60	48	47	41	25	171
No. haplotypes (%)	16 (26.7)	23 (38)	8 (13)	13 (21)	-	-
No. private haplotypes	10 (20.5)	18 (36.7)	7 (14.3)	6 (12.2)	-	-
Haplotype diversity	0.868	0.944	0.566	0.887	-	-
Nucleotide diversity (π)	0.006	0.027	0.003	0.005	-	-
No. segregating sites	44	90	34	20	-	-
No. in Clade I	0	25	0	0	-	-
No. in Clade II	60	23	47	41	-	-

AMOVA revealed 66.96% of the variation occurred within populations (Table 8). When the populations were grouped as per the two main clades (clade I and clade II, Figure 11), AMOVA revealed 82% of the variation was due to variance within regions (Figure 3). The ΦPT value for this grouping per clade was 0.857, which was significant at a probability of 0.01.

The population differentiation test based on control region sequences was highly significant ($P<0.001$) and reveals strong segregation of the four populations derived from the four river basins. For comparison of mtDNA among the four populations F_{ST} values ranged from 0.052 to 0.443 (Table 10). The Nzoia river population showed the highest F_{ST} values when compared to the other three populations. Population differentiation F_{ST} values (Table 9) revealed the two most distant sites (River Nzoia and River Sondu-Miriu) to be the most genetically distinct. Samples in clade I were significantly distant from all populations. When compared per sampling site, the two sub-populations of River Nzoia, NZB and NZU and Sondu-Miriu SMW, were most genetically different from other population combinations, supported by significant pairwise ΦPT values between some sub population groupings (Table 10).

AMOVA

Table 8: Analysis of molecular variance populations of *B. altianalis* in Lake Victoria watershed, Kenya

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation index	p value
Among groups	3	333.324	2.138 Va	32.52	FCT: 0.325	0.009
Among populations within groups	4	20.906	0.033 Vb	0.51	FCS:0.007	0.261
Within populations (Regions)	191	841.171	4.404 Vc	66.96	FST:0.330	0.000
Total	196	119.402	6.576			
Fixation Index					FST : 0.327	

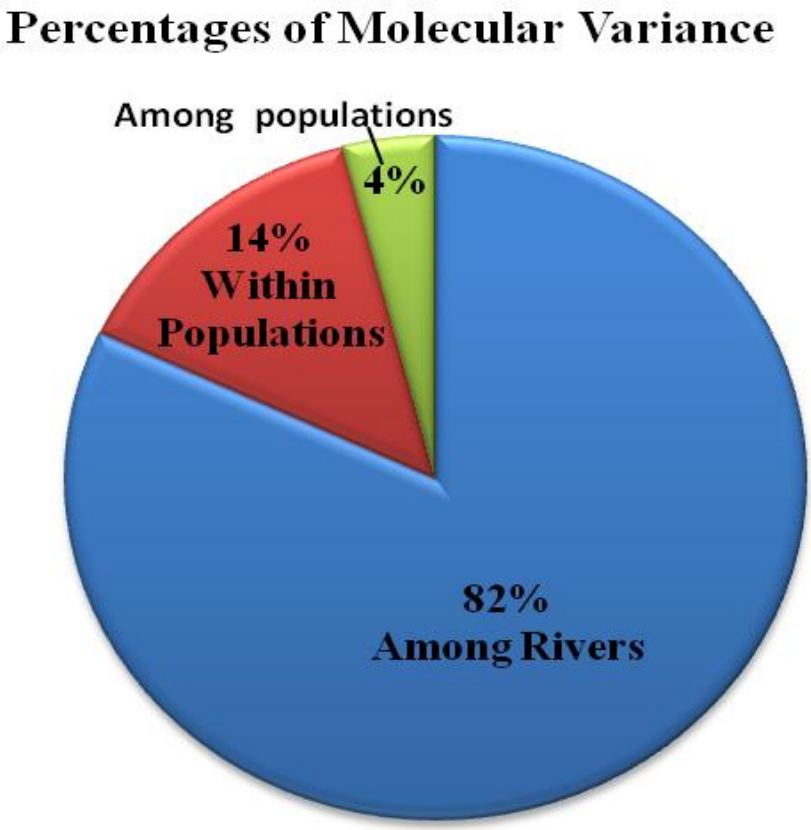

Figure 3: AMOVA chart based on 196 experimental samples showing variation within and among the samples belonging to clade I and clade II

Table 9: Population differentiation (F_{ST} values) between four rivers based populations and two clades of *Barbus altianalis*

* = significant, P< 0.001.

	Nyando	**Nzoia**	**Sondu-Miriu**	**Yala**	**Clade I**	**Clade II**
Nyando						
Nzoia	0.441*					
Sondu-Miriu	0.332*	0.455*				
Yala	0.068*	0.409*	0.218*			
Clade I	0.896*	0.324*	0.912*	0.904*		
Clade II	0.037*	0.410*	0.109*	0.011	0.804*	

Table 10: Population subdivision (FST) values among eight sub-populations of *B. altianalis* (two sampling sites per river)

The populations with significant differences are indicated by ** with their P values <0.001 and * with values P<0.05

	NYK	NYM	NZB	NZU	SMB	SMW	YAB	YAC
NYK								
NYM	0.003							
NZB	0.402**	0.388**						
NZU	0.388**	0.375**	0.000					
SMB	0.107*	0.082 *	0.300*	0.288*				
SMW	0.382**	0.323**	0.521**	0.516**	0.124*			
YAB	0.049*	0.041*	0.303**	0.287 **	0.000	0.166**		
YAC	0.021	0.015	0.360 *	0.353**	0.048*	0.360**	0.000	

NYK ≡Nyando Koru, NYM ≡Nyando Ahero, NZB ≡ Nzoia Webuye before discharge, NZU ≡ Nzoi Ugunja Bridge, SMB ≡ Sondu-Sundu Bridge, SMW ≡ Sondu Nyakwere, YAB ≡ Yala waterworks, YAC ≡ Yala Kakamega Bridge

4.2.3 Demographic history

The overall observed mismatch analysis for the four *Barbus altianalis* populations showed a bimodal distribution, suggestive of the presence of two genetically distinct populations, and inconsistent with the pattern expected for a single stationary population (Figure 4). The mean Fu's Fs-test and Tajima's D test of neutrality, were negative (Table 11) but not significant, suggesting a tendency for population growth (Fu, 1997).

Each of clades I and II showed an essentially unimodal mismatch distribution, inconsistent with the pattern expected of a stationary populationa and consistent with the bell-shaped distribution expected of an expanding population (Figures 5 and 6).

None of the mismatch distributions for the four river-based *Barbus altianalis* populations were consistent with the pattern expected of a stationary population. These distributions were bimodal (Nzoia, Figure 7), skewed unimodal (Nyando, figure 8), and somewhat ragged (Sondu-Miriu, Figure 9; Yala, Figure 10). For all, the raggedness indices were low and non-significant (P rg >0.05). Fu's tests were negative for each population except Sondu–Miriu. Tajima's D test was negative and significant for the Sondu–Miriu and Nyando populations but not for Yala and Nzoia populations, (Table 11) showing that the Sondu–Miriu and Nyando populations may have experienced a past population expansion. The goodness of fit between the observed and expected distributions under a sudden expansion model was tested. The Standardized Squared Differences (SSD) was significant for the Nzoia and Sondu-Miriu groups (Table 11). Observed distribution curves for Sondu–Miriu and Nyando populations did not significantly differ from the simulated distribution curves of demographic expansion.

Table 11: Neutrality tests based on the pairwise comparison of control region sequences

All pairwise comparisons from mtDNA were significantly differentiated (*P < 0.05; **0.01; ***P < 0.001). (Tajima' *D* and Fu's *Fs*, standardized squared differences (SSD) and raggedness).

Statistics	**NYANDO**	**NZOIA**	**SONDU**	**YALA**	**Clade 1**	**Clade II**	**Mean**	**s.d.**
Sample size	60	48	47	41	25	171	32.666	13.506
Tajima's D	**-1.691***	0.181	**-2.154****	0.078	**-2.255****	**-2.066****	-0.401	1.250
Fu's FS test	-3.885	-0.182	0.699	-4.292	-3.037	**-17.546*****	-1.185	2.705
SSD	0.006	**0.945****	**0.397****	0.004	**0.006****	**0.001****	0.130	0.279
Raggedness index	0.022	0.007	0.170	0.007	0.063	0.011	0.052	0.079
No. of Clade I	0	25	0	0				
No. of Clade II	60	23	47	41				

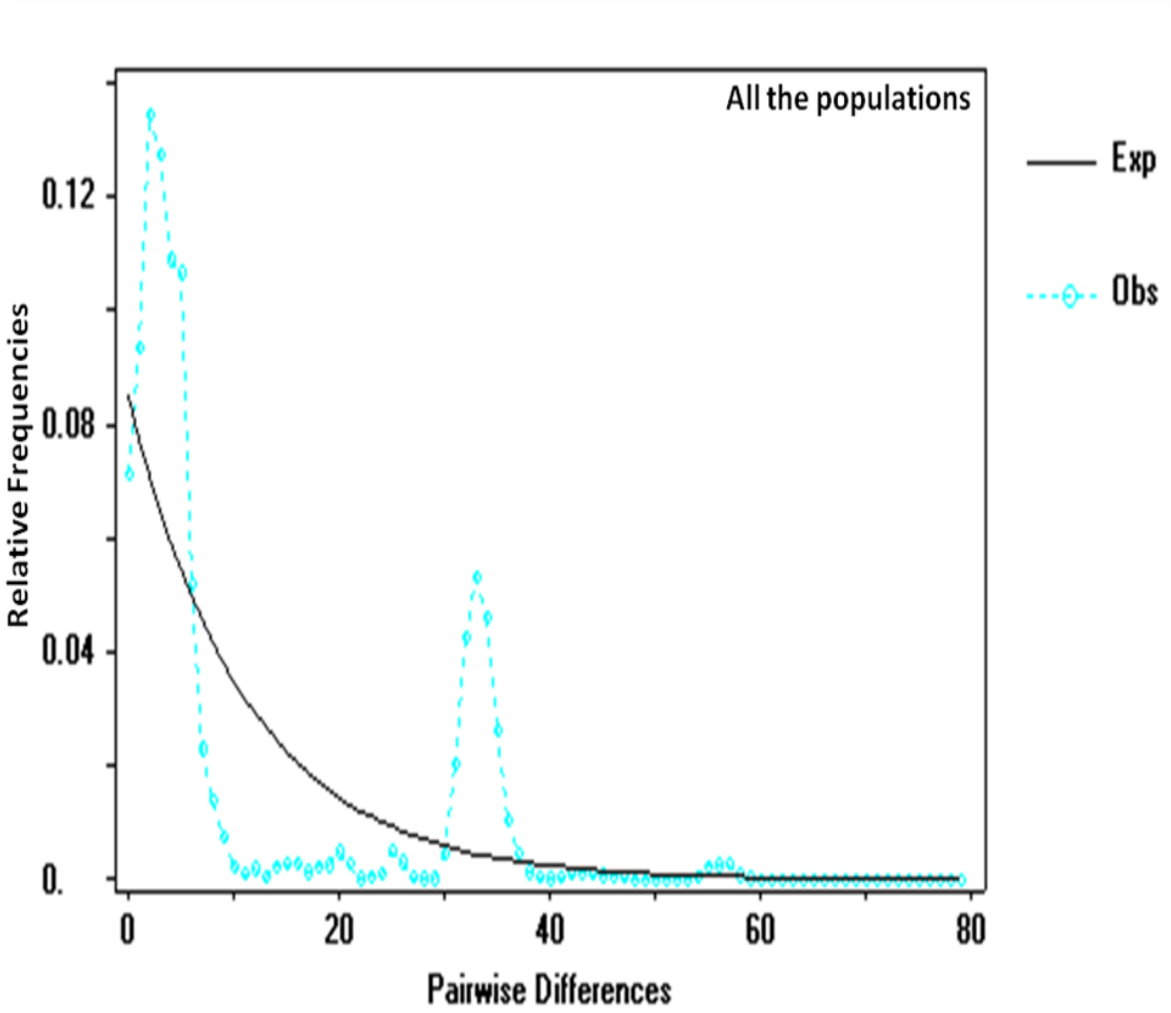

Figure 4: Mismatch distributions based on the control region sequence for the entire sample

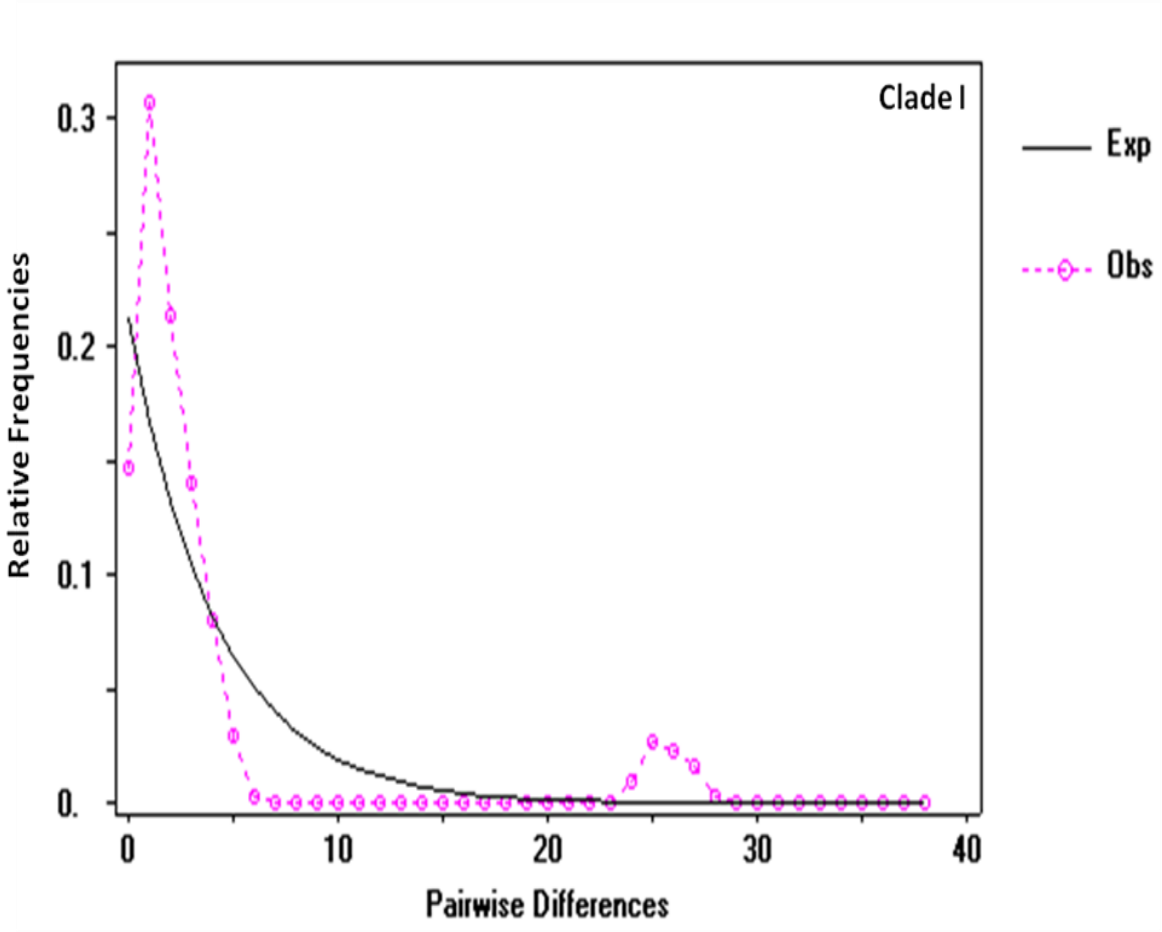

Figure 5: Mismatch distributions based on the control region sequence of the Clade I populations

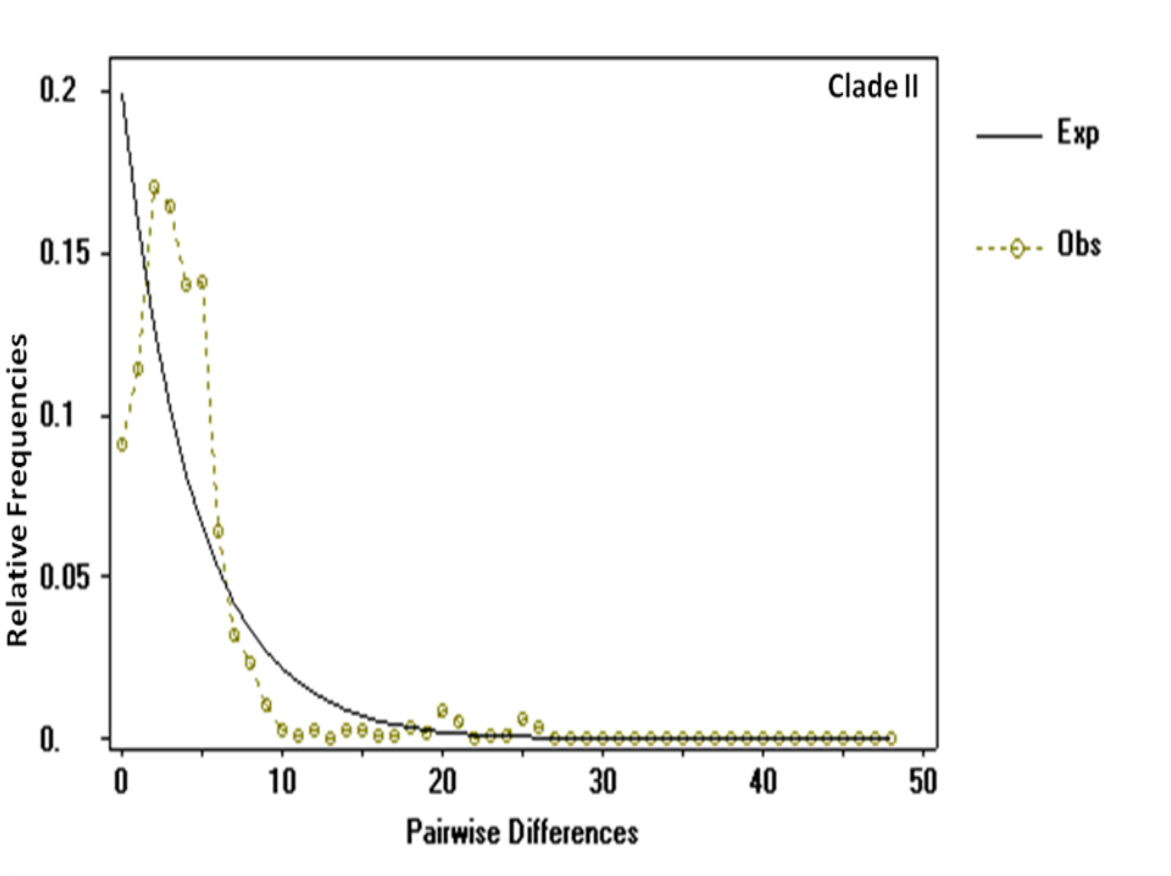

Figure 6: Mismatch distributions based on the control region sequence of the Clade II populations

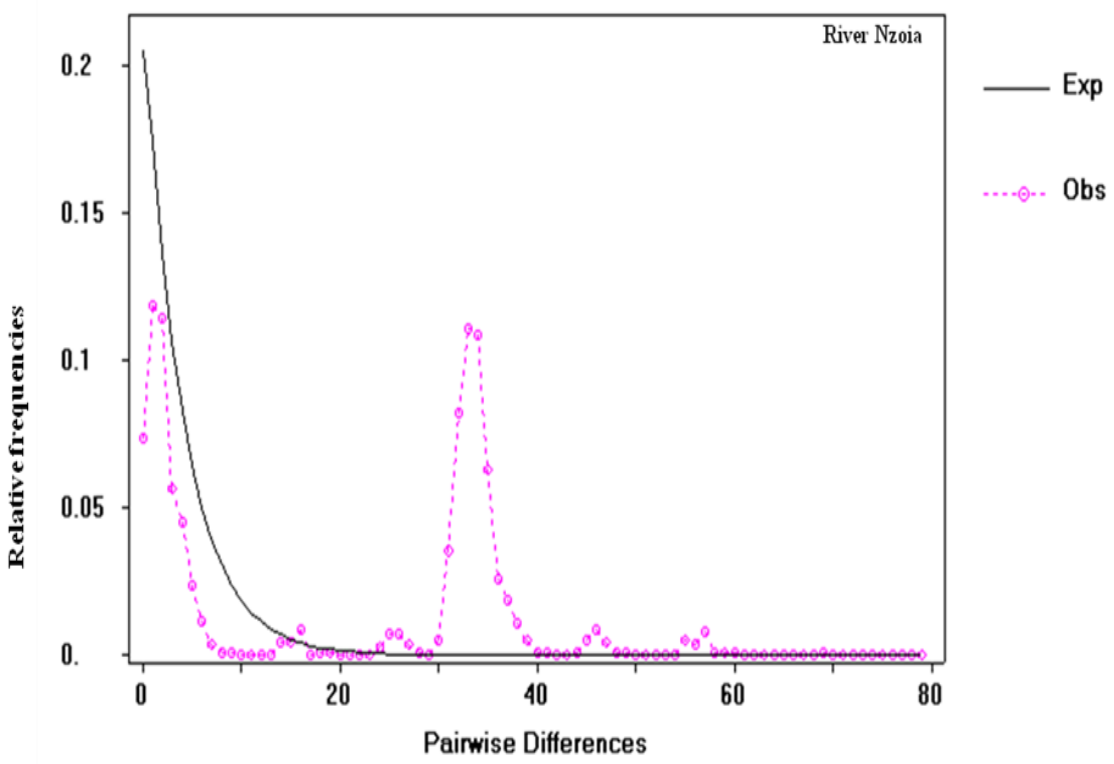

Figure 7: Mismatch distributions based on the control region sequence of the River Nzoia population

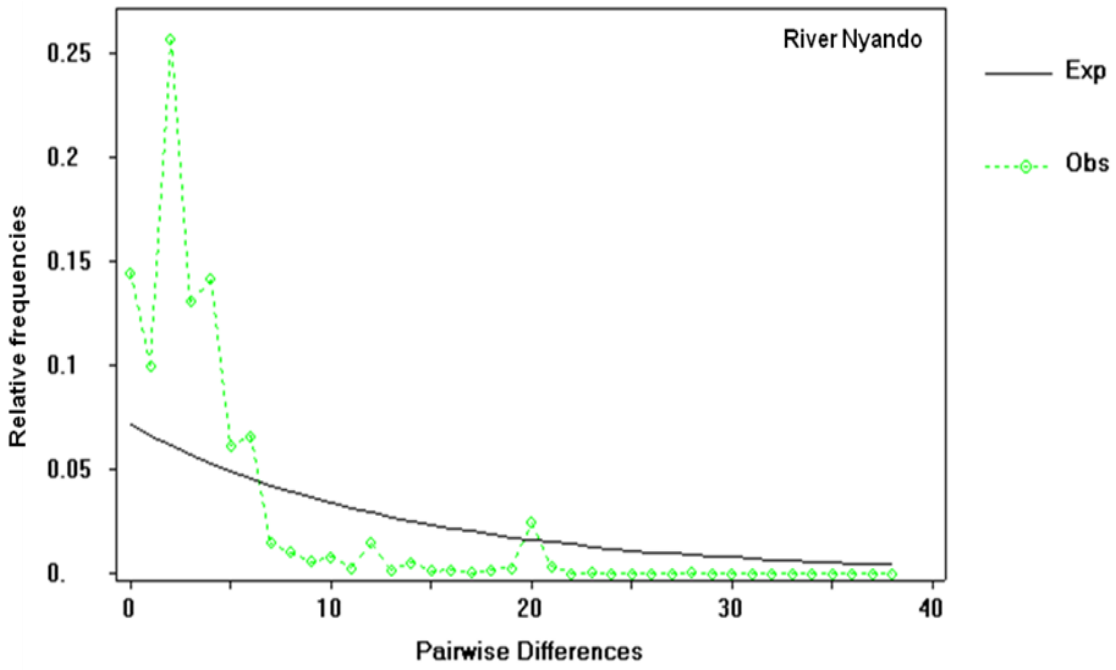

Figure 8: Mismatch distributions based on the control region sequence of the River Nyando population

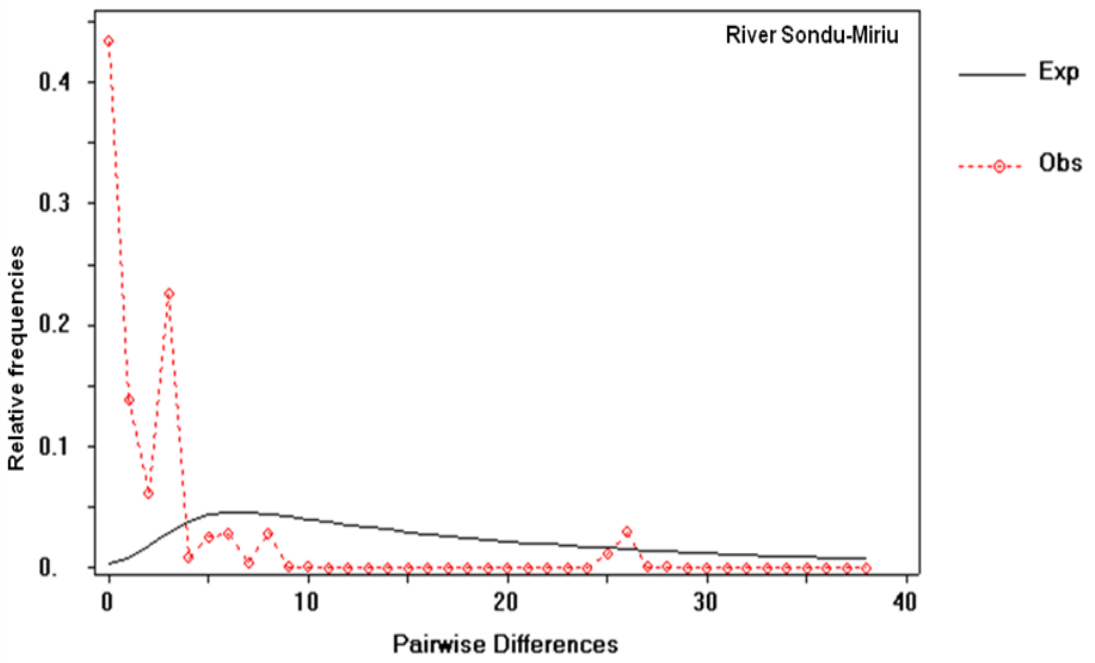

Figure 9: Mismatch distributions based on the control region sequence of the River Sondu-Miriu population

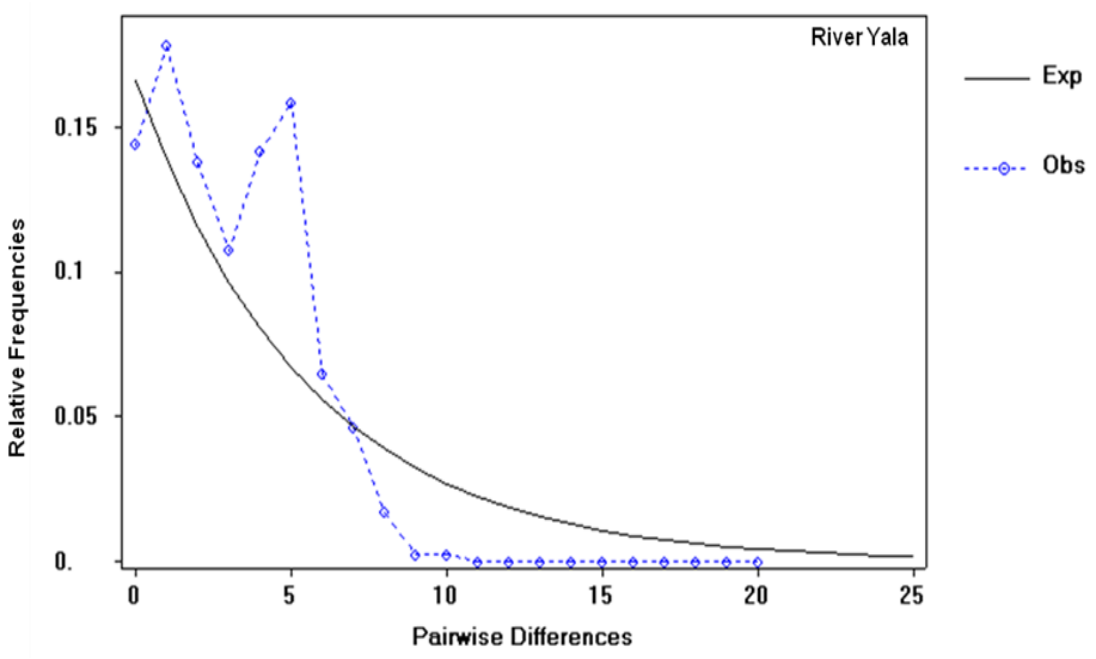

Figure 10: Mismatch distributions based on the control region sequence of the River Yala population

4.2.4 The Haplotype Networks

The haplotype network for the four river-based populations of *Barbus altianalis* samples from the Kenya side of Lake Victoria formed two major haplotype groupings (Clades I and II) and four minor groupings (Figure 11). The 49 haplotypes formed 6 networks when subjected to a 95% parsimony criterion. The major network 1 which was labelled as clade I contained 11 haplotypes; it was separated from network 2 labelled clade II by 29 mutational steps and network 5 (haplotype 41) by 22 mutational steps. Clade II comprised 33 haplotypes; it was separated from network 3 (haplotypes 13 and 26) by 17 mutations; from network 4 (haplotype 18) by 18 mutations; and from network 6 (haplotype 16) by 8 mutations. Clade I consisted of haplotypes from the Nzoia River population only, while Clade II was made up of haplotypes from all four rivers.

Figure 12 is a version of the haplotype network which includes haplotypes of *Barbus altianalis* from the four Kenyan rivers sampled in this study and 36 samples from the Ugandan side of Lake Victoria (Muwanika *et al*., 2012). The 63 haplotypes formed two major groupings (clades) and four minor groupings (Figure 12). The 63 haplotypes formed 6 networks when subjected to a 95% parsimony criterion. The major network 1 which was labelled as clade I contained 32 haplotypes; it was separated from network 2 (labelled clade II) by 13 mutational steps and network 3 (haplotype 47) by 16 mutational steps. Clade II comprised 26 haplotypes; it was separated from network 4 (haplotype 52) by 2 mutational steps, network 5 (haplotypes 11 and 20) by 5 mutational steps and network 6 (haplotype 14) by 12 mutations. Clade I consisted of haplotypes from the Nzoia river and all samples from the Ugandan side of Lake Victoria, which is equivalent to the clade I in the haplotype

network generated from the experimental samples only (Figure 11), while clade II comprised haplotypes from all four rivers draining the Kenyan side of the lake, namely the Nzoia, Yala, Nyando and Sondu-Miriu, equivalent to the clade I in the haplotype network generated from the experimental samples only (Figure 11).

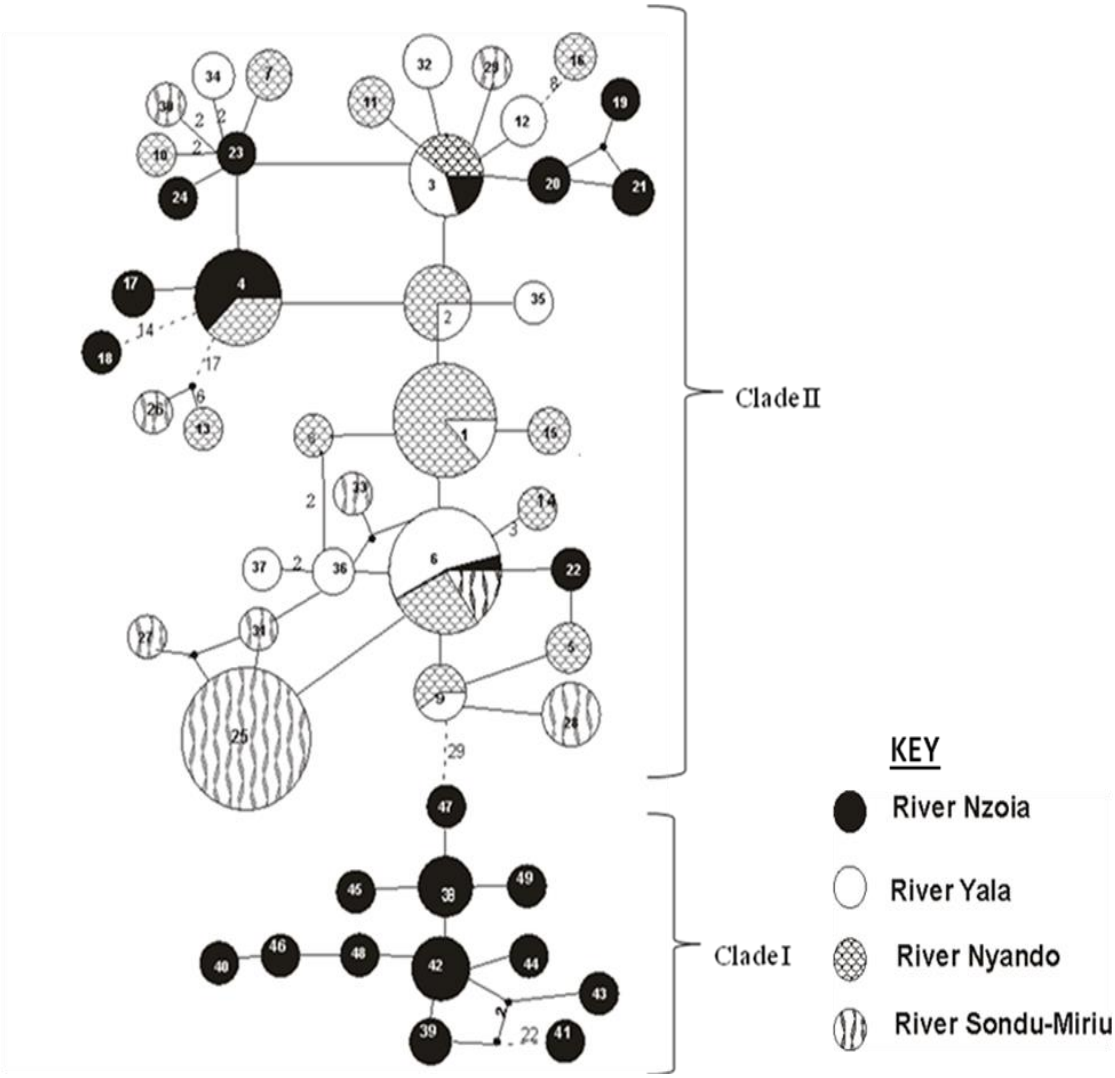

Figure 11: Haplotype network showing mutational relationships between 49 observed haplotypes of *Barbus altianalis.*

(Based on 850bp nucleotides of the mitochondrial (control region))

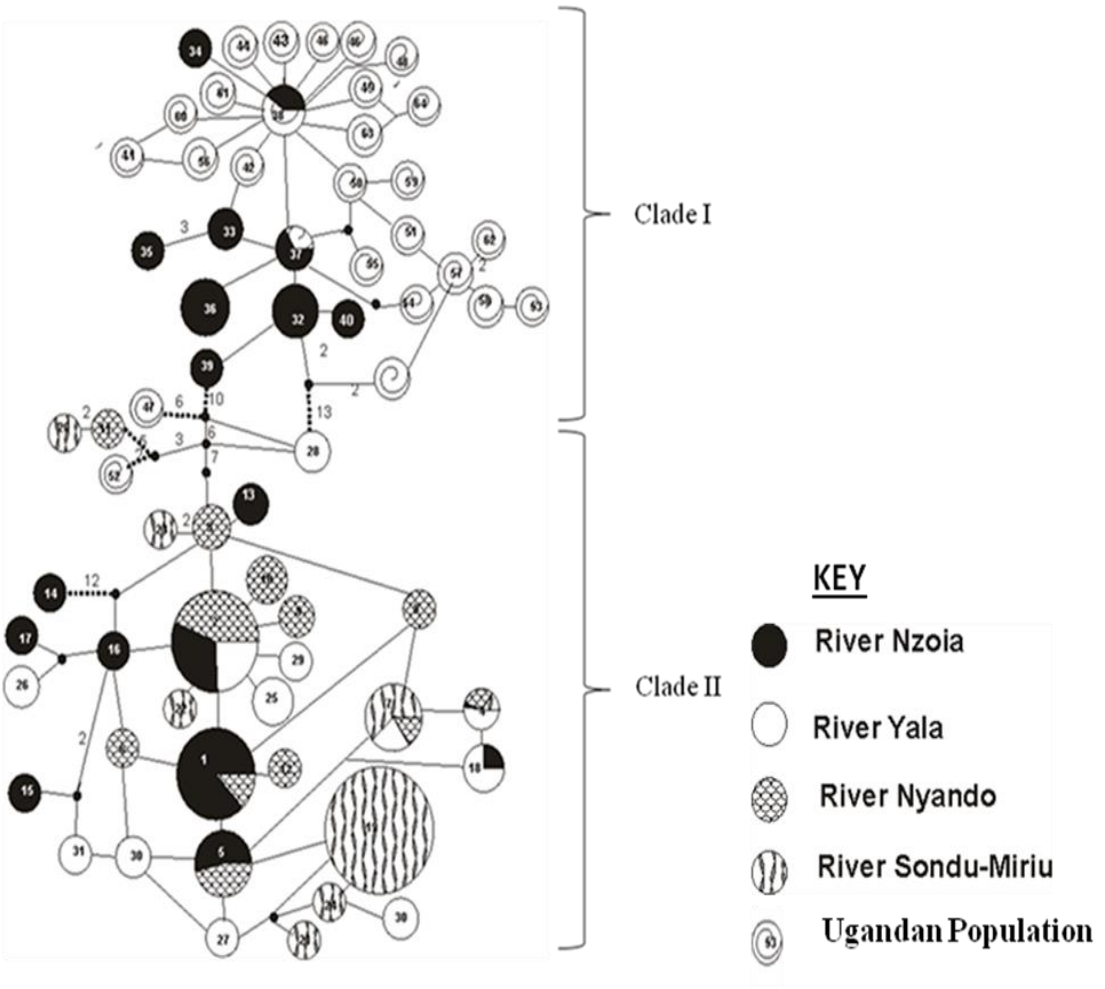

Figure 12: Haplotype network based on 361bp nucleotides of the mitochondrial (control region).

(Relationships between 63 observed haplotypes of *Barbus altianalis* from both the Kenya and Uganda sides of Lake Victoria)

4.2.5 Phylogenetics of *Barbus altianalis* based on mtDNA

Figures 13 and 14 represent phylogenetic trees based on 850bp and 361bp nucleotides respectively of the mitochondrial control region. Figure 13 represents phylogenetic relationships between study samples from this study only, whereas samples from Uganda (Muwanika *et al*., 2012), downloaded from the NCBI genbank, are included in the tree presented in Figure 14. As both trees have a similar overall structure, only relationships revealed in Figure 14 have been discussed. All Kenyan and Ugandan samples form a very strongly supported monophyletic clade with respect to the outgroups, *Cyprinus carpio* and *Procypris rabaudi*. Support levels for this clade are (100%/100%/1.00: neighbour joining bootstrap percent/ maximum parsimony bootstrap percent/ Bayesian posterior probability). This is further subdivided into two sister clades, clade I and clade II. Clade I is very strongly supported (100%/100%/1.00) and comprises all haplotypes from Uganda and some haplotypes from the River Nzoia. Moderately supported clade II (82%/82 %/-) comprises haplotypes derived from all four rivers sampled in this study. Clade II was further divided into two sister clades: very strongly supported clade IIA comprised three haplotypes from Nyando and Sondu-Miriu populations, whereas the larger and moderately supported clade II B contained haplotypes derived from all four rivers.

Phylogenetic trees

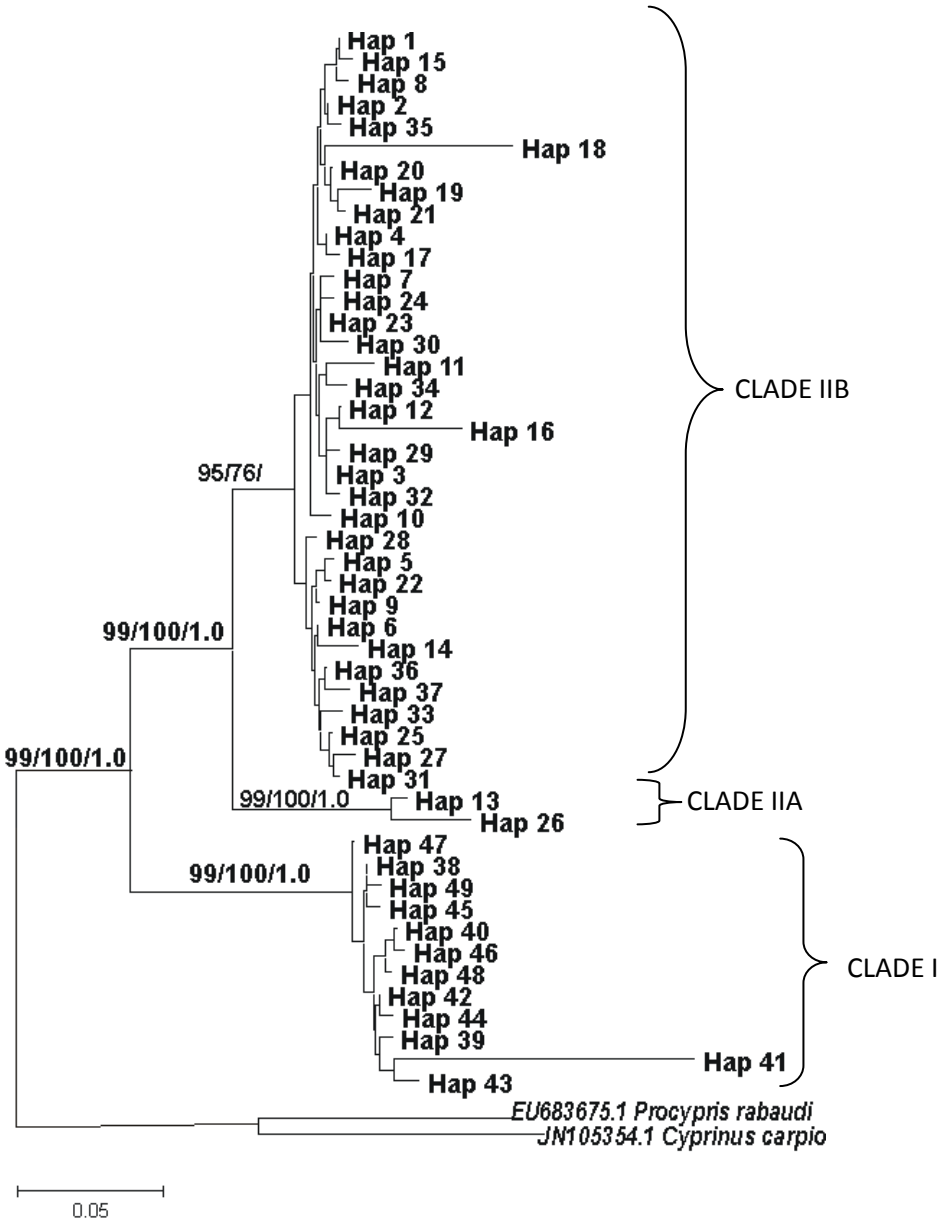

Figure 13: A neighbour joining haplotype tree based on 850bp of the mitochrondrial (control region) for 196 experimental samples

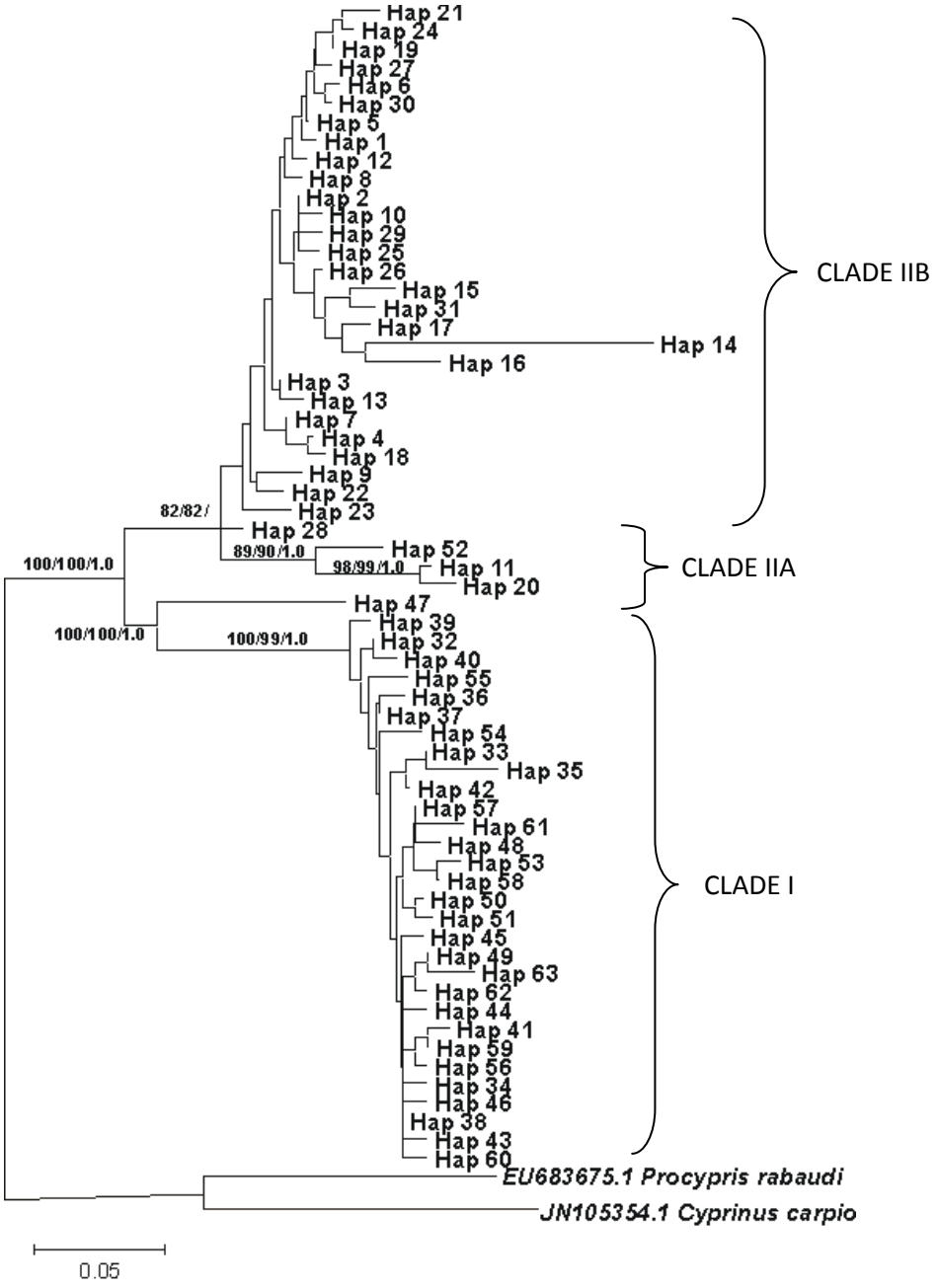

Figure 14: A neighbour joining haplotype tree based on 361bp of the mitochondrial (control region).

(Sequence of *Procypris rabaudi*, EU683675.1 and *Cyprinus carpio* JN105356.1) were used as an out-groups. The sequences were for 196 experimental samples and 35 samples from Uganda, downloaded from the NCBI Genbank (Haplotypes 38-63))

4.2.6 mtDNA Cytochrome c Oxidase I (CO1)

The 21 individuals sampled from the four rivers yielded 5 different haplotypes (Figure 15). The most widely distributed haplotypes were haplotype 2, found in three rivers Nyando, Nzoia and Sondu-Miriu and haplotype 3 found in rivers Nyando, Nzoia and Yala. Haplotype 1 had samples from Nzoia, and Haplotypes 4 and 5 each had samples from Nyando. The Haplotype networks reflected the 2 major Clades as observed in the d-loop networks. The pairwise population F_{ST} comparisons indicated highest genetic structuring among the Nzoia and Nyando populations (F_{ST} = 0.690). Nzoia and Sondu-Miriu populations had a high genetic structuring (F_{ST} = 0.711), but not significantly differentiated. Clade I samples were significantly differentiated from the Sondu-Miriu (F_{ST} = 0.836), and Clade II samples (F_{ST} = 0.685).

Table 12: Population differentiation (F_{ST} values) between four populations of *Barbus altianalis* based on 658bp nucleotides of the mitochondrial CO1 gene

* = significant, P< 0.001.

	Nyando	**Nzoia**	**Sondu-Miriu**	**Yala**	**Clade I**	**Clade II**
Nyando						
Nzoia	0.691*					
Sondu-Miriu	0.041	0.711				
Yala	-0.157	0.666*	1.000			
Clade I	0.796*	0.076	0.836*	0.807		
Clade II	-0.054	0.577*	-0.197	-0.101	0.685*	

The haplotype network based on 658bp of the mitochondrial CO1 gene (Figure 15) yielded 5 haplotypes and a structure which corresponded to that observed in the haplotype network based on the mitochondrial control region (Figure 11). There were two groups of haplotypes, as Haplotype 1, equivalent to Clade I (Figure 15) and derived from the river Nzoia only, was separated from Clade II, comprising haplotypes 2, 3, 3 and 5 by 6 mutational steps. Haplotypes within Clade II were separated from neighbouring haplotypes by one mutational step only, and were derived from all four rivers, namely the Nzoia, Yala, Nyando and Sondu-Miriu.

The structure of the tree based on the CO1 marker sequences of the study samples only, (Figure 16) was similar based on the mitochondrial control region (figure 13 and 14). The two major clades were clearly defined with the probability values of (99%/99%/100%: neighbour joining bootstrap percent/ maximum parsimony bootstrap percent/ maximum likelihood bootstrap percent) (Figure 16). When CO1 and control region sequences were concatenated, the resulting neighbour-joining tree displayed a similar topology to those based on the markers separately (Figure 17).

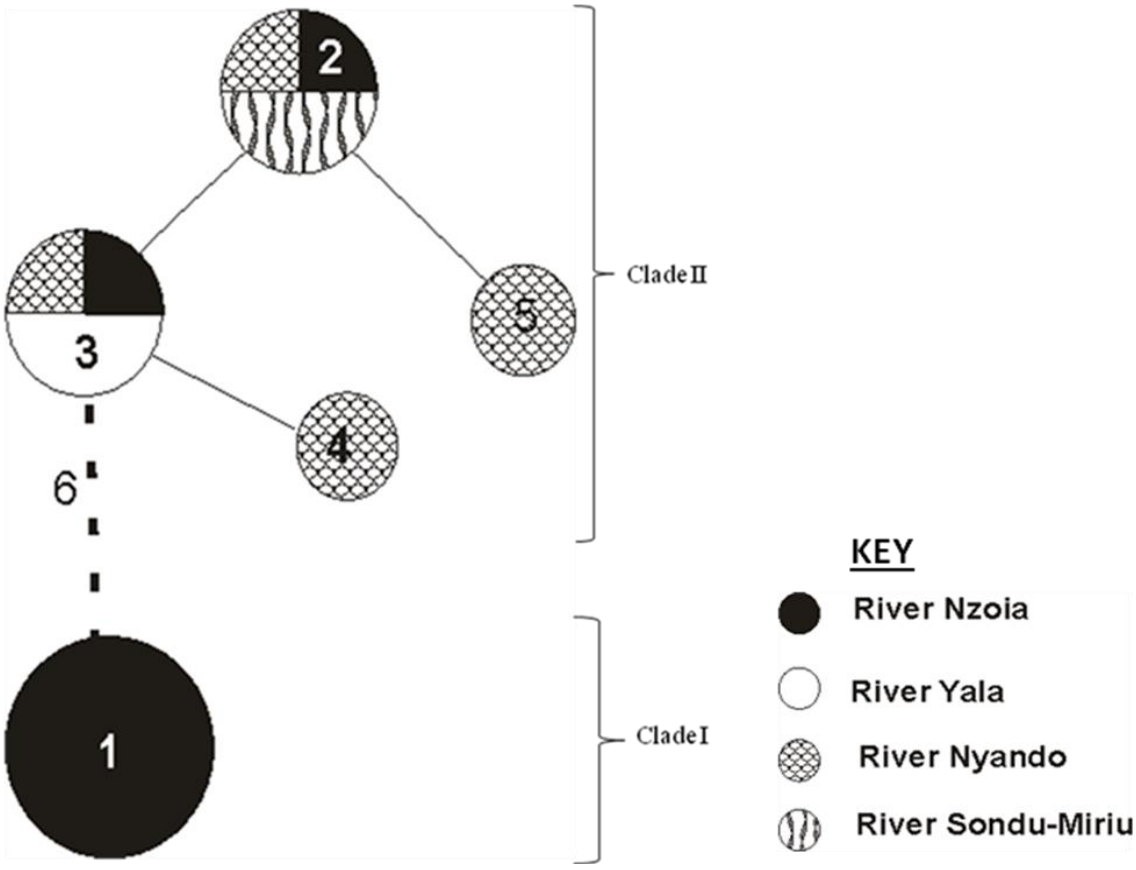

Figure 15: Haplotype network based on 658bp nucleotides of the mitochondrial CO1 gene showing mutational relationships between the observed 5 haplotypes

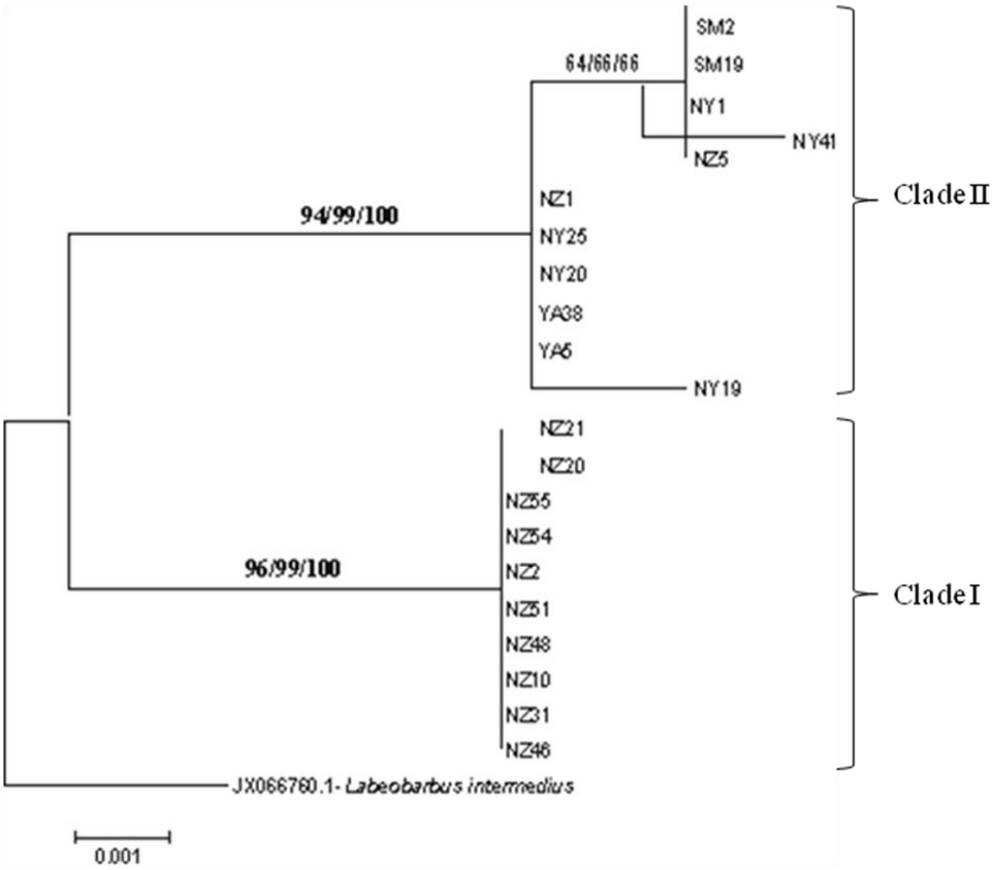

Figure 16: Neighbour joining haplotype tree based on analysis of 658bp of the mitochondrial CO1 gene of *Barbus altianalis.*

(Samples taken from four rivers draining the Kenyan side of Lake Victoria**)**

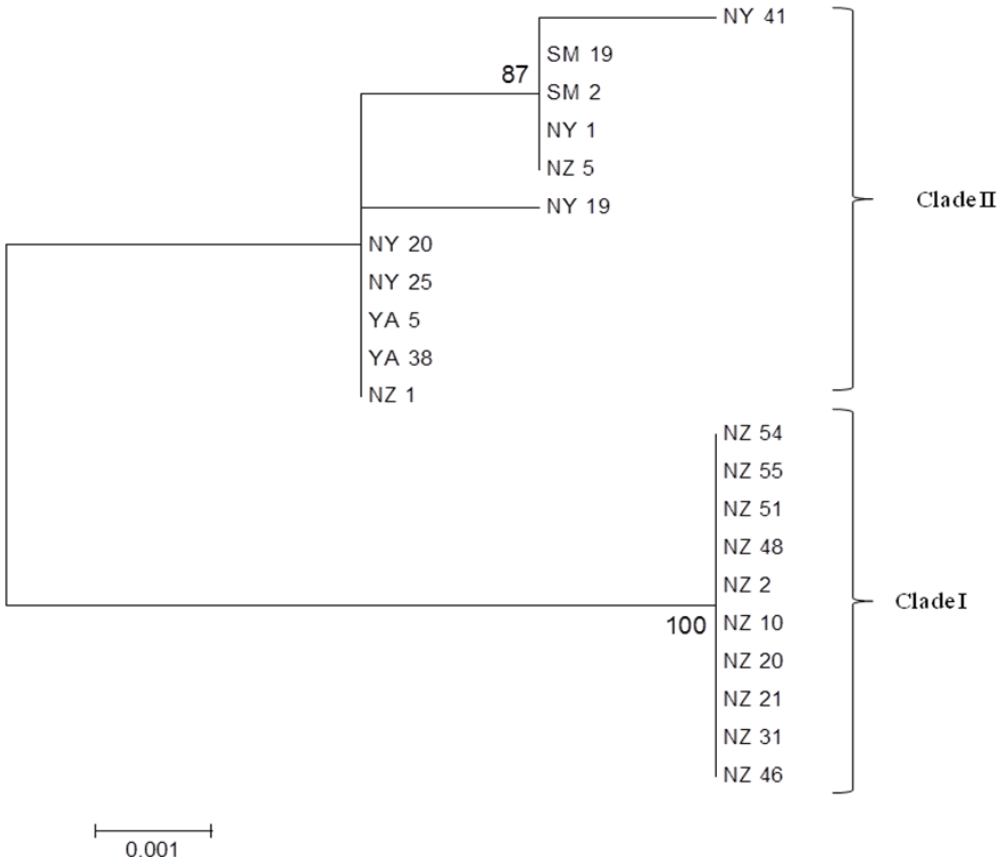

Figure 17: Unrooted neighbor-joining tree based on analysis of a concatenation of the mitochondrial control region and CO1 markers.

(1316 nucleotides) illustrating evolutionary relationships among *B. altianalis* samples derived from four rivers draining the Kenyan side of Lake Victoria

For the CO1 gene, neighbour joining, maximum parsimony and Bayesian inference analyses yielded congruent trees, which are presented as one tree (Fig 18), with support levels from all three methods indicated at the nodes. *Tor* and *Neolissochilus* formed strongly supported monophyletic sister groups, and were sister to members of the genera *Labeobarbus*, *Barbus* and *Varicorhinus*. Neither *Barbus* nor *Varicorhinus* species formed monophyletic groups, as the mainly-*Varicorhinus* clade also contained the species *Labeobarbus caudovittatus*, whereas *Varicorhinus jubae* formed a clade (C) with *Barbus gananensis*.

Moderately supported clade A (83/85/0.91) comprised *Barbus* species generally in a sister relationship to *Labeobarbus* and the experimental sequences. Well-supported Clade D (90/87/0.99) comprises experimental Clade E, derived from the rivers Nzoia, Yala, Nyando and Sondu-Miriu sister to Clade F. Within F, Clade G is sister to Clade H. Clade H is a mixed unsupported clade comprising 5 *Labeobarbus* species (*intermedius, gorgorensis, platydorsus, surkis* and *acutirostris*). Within moderate to weakly supported clade G (67/72/0.81), moderately supported *Labeobarbus intermedius* Clade I (71/74/0.86) is sister to well-supported Clade J (90/94/0.98) comprising experimental samples from the Nzoia River.

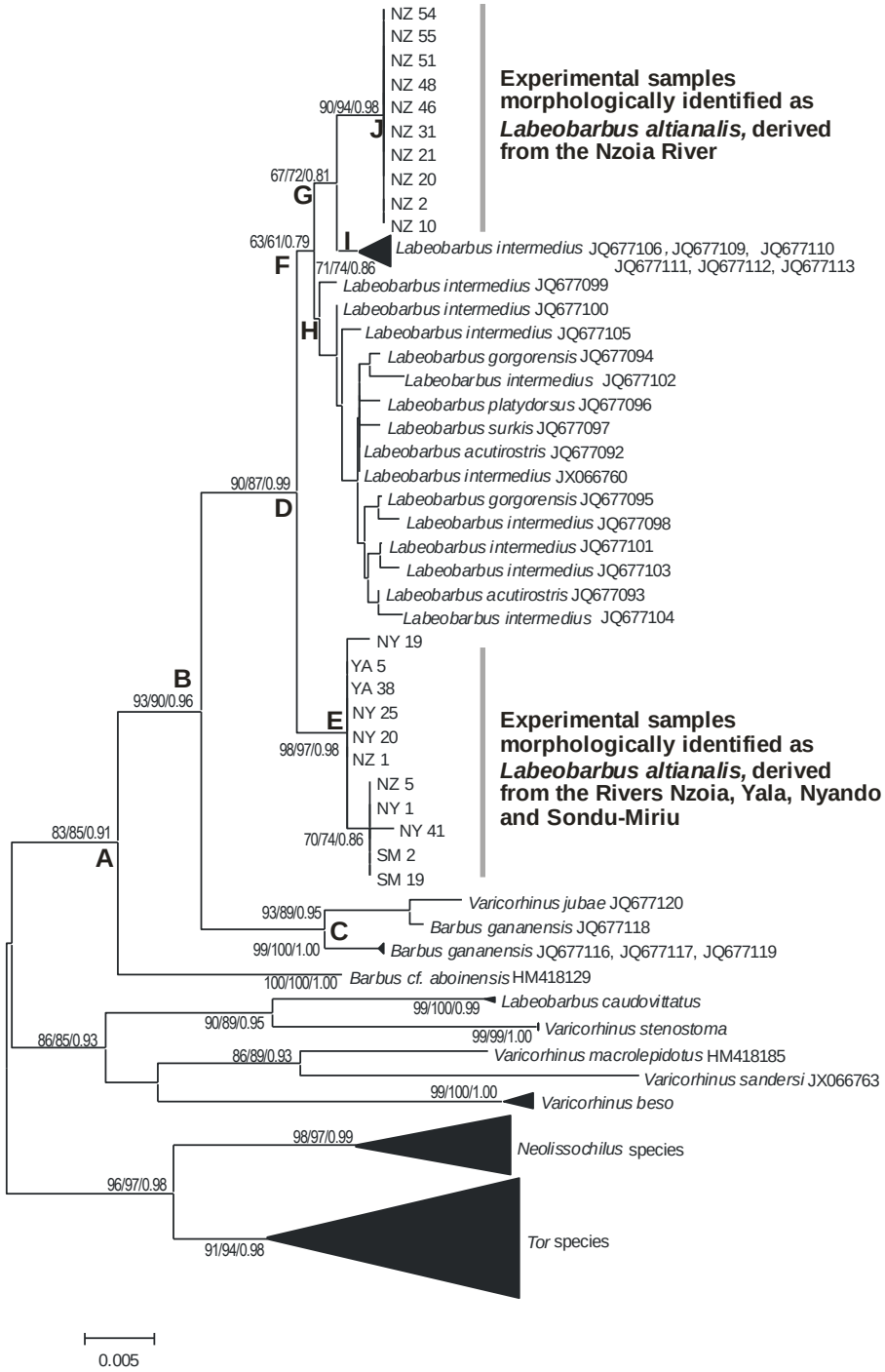

Figure 18: Neighbour joining tree based on 619bp of the mitochondrial cytochrome c Oxidase 1 gene.

4.2.7 Genetic distances

The CO1 genetic distance between the experimental samples in clades I and II is 1.10% (0.00% - 1.32%) (Table 13 and 14). The mean genetic distance between 5 *Labeobarbus* species (*L. intermedius, L. acutirostris, L. surkis, L. platydorsus, and L. gorgorensis*) is 0.47% (0.16% - 1.09%). Members of clades I and II differ from the above species of *Labeobarbus* by between 0.66% and 1.09%, within the range by which they differ from each other. *L. Caudovittatus* differs from the other *Labeobarbus* species by distances of 5.88% – 7.11%. This is clearly distinct from the other *Labeobarbus* species, and forms a clade with all of the *Varicorhinus* species, suggesting that it may have been misidentified and is really a *Varicorhinus* species. Species level differences within the genera *Varicorhinus* (mean 6.7%) and *Neolissochilus* (mean 3.9%) are much greater than those within *Labeobarbus* (Table 14).

Table 13: Genetic distance (%) between study samples (1-21) based on 615 nucleotides of the mitochondrial Cytochrome c Oxidase 1 gene

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	NZ_1																				
2	NZ_2	0.99																			
3	NZ_5	0.16	1.16																		
4	NZ_10	0.99	0.00	1.16																	
5	NZ_20	0.99	0.00	1.16	0.00																
6	NZ_21	0.99	0.00	1.16	0.00	0.00															
7	NZ_31	0.99	0.00	1.16	0.00	0.00	0.00														
8	NZ_46	0.99	0.00	1.16	0.00	0.00	0.00	0.00													
9	NZ_48	0.99	0.00	1.16	0.00	0.00	0.00	0.00	0.00												
10	NZ_51	0.99	0.00	1.16	0.00	0.00	0.00	0.00	0.00	0.00											
11	NZ_54	0.99	0.00	1.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00										
12	NZ_55	0.99	0.00	1.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00									
13	NY_1	0.16	1.16	0.00	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16								
14	NY_19	0.16	1.15	0.33	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15	0.33							
15	NY_20	0.00	0.99	0.16	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.16	0.16						
16	NY_25	0.00	0.99	0.16	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.16	0.16	0.00					
17	NY_41	0.33	1.32	0.16	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	0.16	0.49	0.33	0.33				
18	SM_2	0.16	1.16	0.00	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	0.00	0.33	0.16	0.16	0.16			
19	SM_19	0.16	1.16	0.00	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	0.00	0.33	0.16	0.16	0.16	0.00		
20	YA_5	0.00	0.99	0.16	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.16	0.16	0.00	0.00	0.33	0.16	0.16	
21	YA_38	0.00	0.99	0.16	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.16	0.16	0.00	0.00	0.33	0.16	0.16	0.00

Table 14: Genetic distance (%) among Sample from clade I and clade II and selected outgroups based on 615 nucleotides of the mitochondrial CO1 gene (below diagonal).

Mean genetic distances among species within selected genera are presented below the diagonal

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Clade II		1.10														
2	Clade I	1.10															
3	*L. intermedius*	0.93	0.66			0.47											
4	*L. acutirostris*	0.93	0.82	0.49													
5	*L. surkis*	1.09	0.99	0.66	0.16												
6	*L. playtdorsus*	1.09	0.99	0.66	0.16	0.33							6.7				
7	*L. gorgorensis*	1.09	0.99	0.66	0.16	0.33	0.33										
8	*L. caudovittatus*	7.11	6.25	6.24	6.06	6.24	6.23	5.88									3.9
9	*Barbus Sp.*	2.96	3.19	2.85	2.85	3.02	3.02	3.02	5.70								
10	*V. jubae*	2.78	3.37	3.02	3.19	3.37	3.36	3.37	7.01	2.87							
11	*V. beso*	6.90	6.78	6.40	6.22	6.41	6.40	6.41	6.41	6.15	7.53						
12	*V. macroleipdotus*	6.54	6.42	5.86	6.23	6.41	6.40	6.41	5.68	5.69	6.80	5.48					
13	*V. stenostoma*	7.11	6.25	6.43	6.80	6.98	6.97	6.98	3.72	6.07	7.38	7.20	6.05				
14	*Tor sp.*	6.95	6.31	6.31	5.94	6.13	6.12	6.13	7.07	6.18	7.25	7.13	6.86	8.01			
15	*N. hexastichus*	6.30	5.85	5.85	5.30	5.49	5.48	5.49	7.34	6.60	6.59	7.53	6.78	8.87	4.88		
16	*N. hexagonolepis*	7.42	6.95	7.14	6.77	6.96	6.95	6.59	7.73	7.35	7.72	7.71	7.14	8.88	5.79	2.35	
17	*N. stracheyi*	6.68	6.60	6.60	6.04	6.22	6.22	5.86	7.76	6.99	6.98	8.32	7.57	9.29	4.34	4.08	5.20

CHAPTER FIVE

DISCUSSION

5.1 Morphometrics

The morphometric characteristics of *B. altianalis* showed partial differentiations. Althoug

5.1.1 Morphometric Characteristics

The present study strongly suggests the existence of intra-specific morphometric variation of *Barbus altianalis* populations on the Kenyan region the Lake Victoria catchment. Studies have shown that morphometric characters are suitable in describing intra-specific variation since they are often influenced by several factors such as the genotypic composition, water quality, feeding habits, feed types, organism interrelationship (predation), weather conditions, and habitat type (Murta, 2000; Costa *et al*., 2003; Turan *et al*., 2005). Any significant differences in the above factors would consequently lead to variation in fish morphology. Morphometric characters in *Barbus* have been shown to exhibit plasticity (Verep *et al*., 2006; Osuka and Mlewa, 2011), and this could be related to changes in species' habitat throughout its life (Hinder and Jonsson, 1993; Peres-Neto and Magnan, 2004) (Plates 1-4).

The morphometric characteristics of *Barbus altianalis* from River Sondu-Miriu and River Nyando did not vary significantly. This could partly be attributed to similarity in food types as a result of common source and a more similar catchment in terms of activities in the riparian zone and weather conditions (Mungai *et al*., 2011). These populations occupy similar habitats and are generally subjected to similar environmental and selective pressures. Both rivers are located within the same catchment area with agriculture as the dominant activity (Mungai *et al*., 2011). Differences in length of the barbels are mostly associated with feeding and feeding

habit of fish (Leveque, 1997). Osuka and Mlewa (2011) found significant differences in morphometric traits between three species of *Barbus* within the Chepkoilel reservoir in the Kenyan highlands and attributed these to different feeding habits. The observed morphometric congruence between Rivers Sondu-Miriu and Nyando in the current study could thus partly be attributed to similarities in diet and the physico-chemical parameters in these rivers (Mungai *et al*., 2011).

Pairwise comparisons between Rivers Yala, and Nyando, Yala and Sondu-Miriu, Yala and Nzoia, Nzoia and Nyando, Nzoia and Sondu-Miriu, based on Mann-Whitney U Test (Table 3) indicated significant differences in morphometric traits. This could probably be due to difference in food types within the river systems. Diet has been shown to cause variation in the morphology not only in fish but also in most organisms (Meyer, 1988; Fermon and Cibert, 1998). Food types within a system determine the feeding habit of an organism. A fish may change from herbivory to carnivory which will in turn alter its morphology (Fermon and Cibert, 1998) as an adaptation to enhance feeding efficiency.

The observed differences in morphometric traits may also be related to different environmental conditions which varied significantly since the rivers run through areas of relatively different altitude. De Silva and Liyanage, (2009) observed that altitude greatly influences water quality parameters like temperature and turbidity, factors responsible for morphometric changes in fish. Similarly food availability and water depth could also be contributing to different morphological features of *B. altianalis* in the four rivers studied.

Rivers Yala and Nzoia flow through areas of high temperatures while Nyando and Sondu-Miriu both run through a catchment experiencing lower temperatures. River Sondu-Miriu has been largely viewed to have significant differences in turbidity as compared to rivers Yala and Nzoia (Vuai *et al*., 2012). Variations in turbidity have been recorded to influence differences in fish morphology (Pierce *et al*., 1994; Vega *et al*., 2002; Cheng-Hsin *et al*., 2010).

All the four rivers have different water depths which could also be partly responsible for variation in morphology of *B. altianalis*. Water depth determines eye diameter (Vuai *et al*., 2012). The depth further has a connection with water volume thus affecting the velocity and discharge of the river water (Cheng-Hsin *et al*., 2010). Fishes in high volume waters such as River Nzoia in this study are generally big as compared to those in low volume waters (Turan *et al.*, 2005). This is probably an adaptive mechanism of fish to aid in movement, feeding, and even defence from predation since larger water bodies have higher diversity of organisms that are interrelated (Turan *et al.*, 2005).

5.1.2 Growth Variability of Morphometric Traits

Types of growth in fish morphometric characters have been proposed to be included in most taxonomic studies more so when checking for differences between species and families (Musa *et al*., 2012). There are generally two types of growth in organisms. Isometric growth, which is where growth of all parts of an organism grows at the same rate as the various body parts, hence the shape is consistent throughout development and allometric growth where different parts of the organism grows at different rates (Verep *et al*., 2006).

All populations exhibited negative allometric growth thus the variation in *Barbus altianalis* found in the four different rivers was also brought about by the type of growth. The gradual allometric growth could be best described by a quadratic regression concave downwards. In negative allometric growth, growth of all parts of an organism is proportionally slower than the standard length (Verep *et al*., 2006; Musa *et al*., 2012).

5.2 Genetic diversity and genetic structure of *Barbus altianalis*

The Lake Victoria ecosystem is undergoing unprecedented ecological changes due to anthropogenic and other impacts that threaten the long term existence of its biodiversity. Besides cichlids, the cyprinids are the other indigenous group of fishes whose populations have severely been affected by the ecological changes in the Lake Victoria basin. Because of their dependence on two aquatic environments, potamodramous species are especially sensitive to environmental disturbance (Saura and Faria, 2011). The present study reports a detailed assessment of genetic diversity, population structuring and demographic expansion within the populations of *B. altianalis* in light of the ecological changes in the Lake Victoria basin.

The genetic structure of fish populations is of considerable interest because of its importance for the management and conservation of fishery resources (Hauser *et al*., 2002). Highly exploited fishery resources like the Lake Victoria *B. altianalis* are expected to suffer loss of genetic diversity (Hauser *et al*., 2002) and the finding of high genetic diversity in the studied populations suggest that these populations have persisted and have not been adversely affected by overfishing and other human induced environmental perturbations. Both the haplotype networks and the population

differentiation tests based on CO1 data indicate that the Nzoia population is the most genetically distinct. The Nzoia population also exhibited the highest genetic diversity and, unlike the Yala, Sondu–Miriu and Nyando populations which appear to be stable. The Nzoia population is the only one that contains both 'species' groups; one group is found in all four Kenyan rivers, and the other is found in the Nzoia river and on the Ugandan side of the lake. Phylogeographically, it is interesting that the Nzoia river which is closest to Uganda, shares a species with Ugandan population studied by Muwanika *et al.* (2012). The high genetic diversity observed in morphologically-identified *B. altianalis* from the Nzoia river could therefore be due to admixture of phylogenetically different individuals.

Due to their migratory behaviour, genetic homogeneity would be expected among the *B. altianalis* populations in Lake Victoria. The results of this study however shows that the four *Barbus* populations are genetically differentiated, as evidenced by the strong river specific haplotype distribution with high numbers of river-specific private haplotypes, and the results of the population differentiation test. This strongly suggests low dispersal of *B. altianalis* in the Lake Victoria catchment, contrary to the species' known potamodramous nature (Mugo and Tweddle, 1999), which would lead to interactions of the different riverine populations in Lake Victoria. However, this finding contrasts with the morphological findings that indicate that Sondu and Nyando fish are morphologically similar. As *Barbus* species have high morphological plasticity, it appears that the morphological plasticity is environmentally induced and not genetic. It is likely that the river-specific populations of *B. altianalis* studied have existed as separate populations for long periods of time. It is therefore highly probable that the Lake Victoria catchment harboured two populations of *B.*

altianalis populations, the now 'extinct' migratory populations and the 'extant' non-migratory river dwelling populations. Such loss of the migratory populations then created favourable conditions for the observed expansion of the riverine populations.

High haplotype diversity and low nucleotide diversity was found in *B. altianalis* in the four Lake Victoria catchment rivers. Such haplotype and nucleotide diversity combinations are characteristic of populations that have experienced rapid population expansion after a period of low effective population size (Grant and Bowen, 1998). Recently expanded populations tend to show low nucleotide diversity but high haplotype diversity values (Wang *et al*., 2004; Lin *et al*., 2008). The results were consistent with mismatch distribution analysis in two of the four populations: Rivers Nzoia and Nyando populations and the neutrality test. However other studies have also demonstrated the tendency of fish populations to display high haplotype but low or medium nucleotide diversities (Grant and Bowen, 1998; Chen *et al*., 2004; Ball *et al*., 2007; Liu *et al*., 2009). The haplotype and nucleotide diversity reported in this study are comparable to those reported by Muwanika *et al* (2012) in *B. altianalis* from Lake Edward and Lake Kazinga in Uganda.

5.3 Demographic history

The four populations of Lake Victoria catchment separated into two distinct clades. The two major clades both exhibited a skewed unimodal distribution of pairwise differences, consistent with a sudden population expansion after a bottleneck event or population growth in recently founded populations. This demographic scenario is further supported by the negative values for Tajima's D and Fu's Fs. Fu's Fs is a powerful test of historical demographic expansions (Rogers and Harpending, 1992; Ramos-Onsins and Rozas, 2002). Significantly negative values of these test statistics

are interpreted as indicative of the presence of an excess of new mutations as a result of either selective pressure or a past sudden population expansion after a bottleneck. However, a positive value of this test may indicate presence of an excess of old mutations in the population (Tajima, 1989; Alonso and Armour, 2001; Russell *et al*., 2008).

A significant p-value may be usually obtained when there is deviation from the normal situation caused by the occurrence of; a neutral population, constant population size, or lack of recombination among the individuals of a given population. Significantly negative values of this test, as obtained for Clade II, are interpreted as indicative of presence of an excess of new mutations as a result of either selective pressure or past sudden population expansions after a bottleneck (Rogers and Harpending, 1992; Alonso and Armour, 2001).

5.4 Phylogenetic relationships of *Barbus altianalis* based on mtDNA

All analyses of both genetic marker systems, CO1 and the control region of the mitochondrial DNA, revealed two well-supported main groups, suggesting the presence of two cryptic species and two sub-species within the study sample of *B. altianalis* from the Kenyan side of Lake Victoria. The phylogenetic analyses of mtDNA control region of a study sample morphologically identified as *B. altianalis* suggest that there are two cryptic species (clades I and II) within this group and possibly two subspecies within clade II (clades IIa and IIb). This would account for some of the high genetic variation within currently recognized *B. altianalis* species in Kenyan Lake Victoria catchment rivers.

These clades are defined on the basis of the phylogenetic species concept, as they are strongly supported monophyletic clades (species = clade I and clade II; subspecies = clades IIa and IIb). It is likely that clades I and II are valid species under the genetic species concept, as they are separated by a mean CO1 genetic distance of 1.10% (Table 13 and Table 14), which is greater than the mean genetic distance of 0.47% (0.16% - 1.09%) for 5 recognised *Labeobarbus* species (*L. intermedius, L. acutirostris, L. surkis, L. platydorsus, L. gorgorensis*). As *B. altianalis* Clades 1 and II differ from the above species of *Labeo barbus* by between 0.66% and 1.09%, is consistent with the designation of these clades as *Labeobarbus* species. These two clades are also reflected in the haplotype analysis as groups separated by a relative large number of mutational steps. Clade I occurred only in the Nzoia River population and in the Ugandan samples of Muwanika *et al.*, (2012), whereas Clade II was represented in all four rivers, the Nzoia, Nyando, Yala and Sondu-Miriu. However, species level differences within the genera *Varicorhinus* (mean 6.7%) and *Neolissochilus* (mean 3.9%) are much greater than those within *Labeo barbus* (Table 14). Based on genetic distances alone, this suggests that the *Labeo barbus* species analysed in the present study may be a cryptic species complex, and that their taxonomic status warrants some revision.

Other workers have reported cryptic species in cyprinids. Using mitochondrial DNA, Dimmick *et al* (2001) identified a previously undescribed genetically distinct species, *Barbus intermedius,* in Ethiopia. Kohlmann *et al.*, (2003), using mtDNA and a nuclear marker, reported that a high degree of differentiation supports the subspecies status of *Cyprinus carpio carpio* assigned to European carp and *Cyprinus carpio haematopterus* assigned to Asian carp.

However, the results from the phylogenetic analysis in the present study did not concur with study by Muwanika *et al.,* (2012) who reported that the two lineages of *B. altianalis* in Uganda population were not genetically distinct. The results both from phylogenetic and haplotype network analyses support a component of the Nzoia population as a species that is also found in the Ugandan samples of Muwankia *et al* (2012), but not in the other Kenyan rivers, the Nyando, Yala and Sondu-Miriu.

The two experimental clades identified in this study could represent *B. altianalis* and either a new unknown species or a species that occurs within the Lake Victoria system but has never been sequenced before. Clade H (Figure 18) contains 5 *Labeobarbus* species separated by a relatively low mean genetic distance of 0.47% (0.16% - 1.09%). Each species is also not monophyletic within this clade. This could imply that *Labeobarbus* species can show very low levels of genetic differentiation, and show incomplete lineage sorting, or that alleles were recently exchanged among these putative species (Kebede, 2012). The other interpretation is that clade H represents a species complex, and that its members are not distinct species, and warrant taxonomic revision.

Clade G consists of *Labeobarbus intermedius* (I) sister to the experimental samples from the River Nzoia (J). The genetic distance separating I and J is about 0.47%, which, in DNA barcoding terms, is too low to be a species level difference, although it is consistent with the mean genetic distance separating 5 *Labeobarbus* species, previously mentioned. As *Labeobarbus* appears to show generally low genetic differentiation, Clades I and J could represent sister species, or they could be sub-species or Evolutionary Significant Units (ESUs). Clade E, comprising experimental

haplotypes from all four Kenyan rivers, is distinct from clade F, with a genetic distance of 1.10% (Table 14). Clade E could be a distinct species (new or previously unsequenced for CO1). However information from other DNA regions, including nuclear sequences, would help shed light on this.

Thus, based on genetics alone, this study suggests that the *Labeobarbus* and *B. altianalis* species analysed here may be part of a species complex. However if the *L. intermedius*, *L. acutirostris*, *L. surkis*, *L. platydorsus*, *L. gorgorensis* are regarded as distinct species (Table 14, Figure18), the two distinct genetically diverse experimental clades of *B. altianalis* are likely to be two distinct cryptic species, precedent for which is found in many fish taxa (Amazaki *et al*., 2003; Trevor and Marti, 2003; Bostock *et al*., 2006; Kon *et al*., 2007; Milhomem *et al*., 2008).

5.5 Implications for fishery management

Changes in ecosystems and biotic communities in Lake Victoria involved the extinction of about two hundred species, and other species have a great risk of extinction (Witte *et al*., 1992a; Witte *et al*., 1992b; Witte *et al*., 2000). These changes should provide a motive for scientists to investigate evolutionary information from remaining native species which could disappear in the future, in order to implement conservation plans to avoid an ecological disaster (Nakamya, 2010). Indeed, knowledge and understanding of genetic structure, evolutionary processes and ecosystems are precious to plan conservation systems of endangered species like *Barbus* species (Nakamya, 2010).

Aquaculture has employed this knowledge for a long time to help in brood stock selection, in monitoring genetic drift and hybridization, and in improving vigor of farmed fish. In addition to that, aquaculture can also be used to restock areas where species are endangered and thus restore their wild stocks (Nakamya, 2010). Assessing genetic variations is very important to try to protect populations, conserving or improving their genetic diversity (Daniels *et al.*, 1997; James and Ashburner, 1997). The protection of aquatic resources against anthropogenic changes is mainly based on stock assessment models (Walters and Martell, 2004; Haddon, 2010) and genetics data are still poorly used to guide fishery managers and stakeholders. These methods raise disagreement amongst the scientist community but some of them argued about the utility of this data to manage fisheries (Waples, 2008). In particular, this study has revealed the possibility of two species within *B. altianalis*, and of two subspecies within clade II and this suggests that it requires separate management strategies.

This study in therefore provide information that would then be used to guide policy makers to set up more meaningfull regional management and conservation strategies, which will help revive the fish's reduced numbers. *Barbus altianalis* should be considered as a candidate for aquaculture to restock areas where species are endangered and thus restore their wild stocks of *B. altianalis.* It is important to collect the brood stocks from all the rivers especially from the River Nzoia which had the highest genetic diversity, in order to maintain the diversity of the *Barbus*.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has provided baseline information on morphology, genetic diversity, population structuring, demographic expansion and phylogenetics within the populations of *Barbus altianalis* in Kenyan part of the Lake Victoria basin. In this study mtDNA analysis has revealed the persistence of strongly genetically and morphologically differentiated populations between the four rivers draining Lake Victoria catchment. These populations could represent hitherto unrecognized non-migratory populations that have not been negatively impacted. Morphometric differentiation between the rivers is likely to be mainly due to the differences in the anthropogenic activities within the catchments. As far as the external morphology is concerned, this study confirms a high phenotypic plasticity in *Barbus* spp. which could lead to a misclassification of specimens if not supported by molecular tools.

The existence of robust localized populations of *B. altianalis* should provide new impetus for conservation of these species and perhaps other potamodramous species in the Lake Victoria basin. The sedentary populations represent unique genetic resources that should be protected from over-fishing and habitat degradation by instituting river basin specific management and conservation measures. Specific refuges should be identified, recognized and protected as conservation units. Further studies should investigate the ecological factors contributing to the persistence of these populations.

The strong genetic distinctiveness and differentiation of the four populations suggests that the population division is not a recent phenomenon induced by anthropogenic changes in Lake Victoria catchment.

This study provides the first phylogenetic comparisons of *B. altianalis* populations from the Kenyan and Ugandan sides of Lake Victoria. The Nzoia population is different from the other three in that it appears to contain two cryptic species of *B. altianalis*, one with affinity to Lake Victoria and Lake Albert from Uganda, and the other with affinity to the species of *B. altianalis* found in the Kenyan rivers Yala, Nyando and Sondu-Miriu. Further, the Kenyan rivers are likely to contain two cryptic sub-species of *B. altianalis*.

6.2 Recommendations

- Further studies should investigate the ecological factors contributing to the persistence of these populations as well as morphological and morphometric characterization of the *B. altianalis* along its known range in East Africa to ascertain its taxonomic status.
- Further taxonomic analysis of *B. altianalis* population to verify their taxonomic status: are they pure *B. altianalis* or do the population represent two or more sub-species.
- Undertake phylogenetic analysis of the genus *Barbus* and related genera in Lake Victoria and the East Africa Rift Valley system.
- The four Lake Victoria catchment populations should be considered as Evolutionary Significant Units (ESU) and catchment specific conservation measures instituted to protect these populations. This should be instituted together with river basin management to control the pollution of the rivers.

- Microsatellite markers should be employed to further investigate the genetic structure of *B. altianalis.*
- Consider artificial breeding/aquaculture of *B. altianalis*, i.e. Consider *B. altianalis* as a candidate aquaculture species to restock areas where species are endangered and thus restore their wild stocks.

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APPENDICES

Appendix I: Morphometric measurements taken on 19 characters of *B. altianalis*

Measurement	Abbreviation	Description
Total length	TL	Ameasurement from the most anterior point to the most posterior point.
Standard length	SL	A measurement from the rostral tip of the upper jaw to the origin of the caudal fin, caudal measuring point is the middle of the borderline between the caudal peduncle and caudal fin.
Body depth	BD	The greatest vertical depth of the body mostly between points near rostral origins of dorsal fin and of the pelvic fin.
Head length	HL	A measurement from the rostral tip of the premaxillae to the caudal end of the gill cover.
Snout length	SnL	A measurement from the rostral tip of the premaxillae to the rostral point of the bony orbit.
Eye diameter	ED	Maximum eye length from the most anterior point to the most posterior point of the orbit.
Interorbital width	IOW	Distance between the nearest edges of the eyes, measured across the top of the head of a fish.
Dorsal fin base length	DFB	A measurement between the most anterior and the most posterior point of the dorsal fin base.
Anal fin base length	AFB	A measurement between the most anterior and the most posterior point of the anal fin base.
Predorsal length	PDL	Length measurement from the rostral tip of the upper jaw to the most anterior point of the dorsal fin base.
Preanal length	PAL	Length measurement from the rostral tip of the upper jaw to the most anterior point of the anal fin base.
Prepectoral length	PPL	A measurement from the rostral tip of the upper jaw to the most anterior point of the pectoral fin base.
Preventral length	PVL	Measured from the rostral tip of the upper jaw to the most anterior point of the pelvic fin base.
Caudal peduncle length	CPL	The distance between the vertical line through the caudal point of the anal fin and that of through the caudal border.
Caudal peduncle depth	CPD	The distance between the vertical line through the caudal point of the anal fin and that of through the caudal border.
Pelvic fin base length	PvFB	A measurement between the most anterior and the most posterior point of the pelvic finbase.
Pectoral fin base length	PFB	A measurement between the most anterior and the most posterior point of the pectoral fin base.
Length of the anterior barbel	LAB	A measurement of frontal barbel.
Length of the posterior barbel	LPB	A measurement of hind barbel.
Occipital length	OcL	Length from rostral tip of the upper jaw to the point where predorsal scales originates.

Appendix II: Identification of populations

CODE	POPULATION
NZ -	Nzoia
YA -	Yala
NY -	Nyando
SM –	Sondu-Miriu
NYK-	Nyando Koru
NYM-	Nyando Ahero
NZB-	Nzoia Webuye before discharge
NZU-	Nzoia Ugunja Bridge
SMB-	Sondu-Sundu Bridge
SMW-	Sondu Nyakwere
YAB-	Yala waterworks
YAC-	Yala Kakamega Bridge

Appendix III: Preparation of 10X TBE

To make 500µl 10X TBE,

Tris: 53.89g

Boric acid: 24.96g

EDTA: 1.861G

Mix Tris, Boric Acid and EDTA in 450µl of deionised water, adjust the pH to 8.3 thereafter make up 500µl and autoclave.

Appendix IV: DNA Amplification:

PCR master mix for one mtDNA reaction**.**

Reagent	Quantity (µl)
Double distilled water	8.8
Buffer (10X)	2.5
$MgCl_2$ (25um)	4.0
DNTP's (10mM)	0.5
Primer 1	4.0
Primer 2	4.0
Taq – Polymerase (5u/microlitre)	0.2
DNA	1.0
TOTAL	**25.0**

All these kept in a rack placed on ice

PCR thermocycle amplification

D-LOOP

Place in the PCR machine. Adjust thermocycling conditions as follows:
1st Cycle: 2 minutes 95^0C
2nd Cycle: 30 secs 95^0C ..... Denaturation
3rd Cycle: 30 secs 54^0C ..... Annealing
4th Cycle: 1 minute 72^0C ..... Elongation
5th Cycle: 10 minutes 72^0C ..... Final extension
6th Cycle: store at 4^0C
Cycles 2, 3 and 4 are repeated 35 times.

CO1

Place in the PCR machine. Adjust thermocycling conditions as follows:
1st Cycle: 2 minute 95^0C
2nd Cycle: 1 minute 95^0C ..... Denaturation
3rd Cycle: 1 minute 30 secs 59^0C ..... Annealing
4th Cycle: 2 minutes 72^0C ..... Elongation
5th Cycle: 10 minutes 72^0C ..... Final extension
6th Cycle: store at 15^0C
Cycles 2, 3 and 4 are repeated 38 times.

Appendix V: Sample table

Sample	Sample Collection Number	Locality	coordinates of collection locality	CO1 haplotype	Control region haplotype	Genbank no. COI	Genbank no. control region
B. altianalis	NYK_10	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		10		KC860272
B. altianalis	NYK_11	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		5		KC860273
B. altianalis	NYK_12	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		2		KC860274
B. altianalis	NYK_13	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		10		KC860275
B. altianalis	NYK_14	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		10		KC860276
B. altianalis	NYK_15	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		1		KC860277
B. altianalis	NYK_16	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		1		KC860278
B. altianalis	NYK_17	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		1		KC860279
B. altianalis	NYK_18	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		3		KC860280
B. altianalis	NYK_19	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″	4	12		KC860281
B. altianalis	NYK_1	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″	2	5		KC860282
B. altianalis	NYK_2	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		5		KC860283
B. altianalis	NYK_20	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″	3	5		KC860284
B. altianalis	NYK_21	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		3		KC860285
B. altianalis	NYK_22	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		1		KC860286
B. altianalis	NYK_23	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		1		KC860287
B. altianalis	NYK_24	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		5		KC860288
B. altianalis	NYK_25	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″	3	2		KC860289
B. altianalis	NYK_26	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		11		KC860290
B. altianalis	NYK_27	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		9		KC860291
B. altianalis	NYK_28	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		1		KC860292
B. altianalis	NYK_29	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		5		KC860293

B. altianalis	NYK_3	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		1	KC860294
B. altianalis	NYK_30	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		1	KC860295
B. altianalis	NYK_4	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		1	KC860296
B. altianalis	NYK_5	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		10	KC860297
B. altianalis	NYK_6	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		2	KC860298
B. altianalis	NYK_7	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		3	KC860299
B. altianalis	NYK_8	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		1	KC860300
B. altianalis	NYK_9	Nyando, Kenya	S 00°09′57.5″ E 035°11′01.0″		9	KC860301
B. altianalis	NYM_31	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		1	KC860302
B. altianalis	NYM_32	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		6	KC860303
B. altianalis	NYM_33	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		1	KC860304
B. altianalis	NYM_34	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		1	KC860305
B. altianalis	NYM_35	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		2	KC860306
B. altianalis	NYM_36	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		4	KC860307
B. altianalis	NYM_37	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		2	KC860308
B. altianalis	NYM_38	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		2	KC860309
B. altianalis	NYM_39	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		8	KC860310
B. altianalis	NYM_40	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		2	KC860311
B. altianalis	NYM_41	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″	5	6	KC860312
B. altianalis	NYM_42	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		1	KC860313
B. altianalis	NYM_43	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		4	KC860314
B. altianalis	NYM_44	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		1	KC860315
B. altianalis	NYM_45	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		2	KC860316
B. altianalis	NYM_46	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		4	KC860317
B. altianalis	NYM_47	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		3	KC860318
B. altianalis	NYM_48	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		1	KC860319
B. altianalis	NYM_49	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		2	KC860320
B. altianalis	NYM_50	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		1	KC860321

B. altianalis	NYM_51	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		1	KC860322
B. altianalis	NYM_52	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		1	KC860323
B. altianalis	NYM_53	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		2	KC860324
B. altianalis	NYM_54	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		5	KC860325
B. altianalis	NYM_55	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		1	KC860326
B. altianalis	NYM_56	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		3	KC860327
B. altianalis	NYM_57	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		5	KC860328
B. altianalis	NYM_58	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		6	KC860329
B. altianalis	NYM_59	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		2	KC860330
B. altianalis	NYM_60	Nyando, Kenya	S 00°10′23.5″ E 034°55′15.2″		3	KC860331
B. altianalis	NZM_1	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″	3	16	KC860332
B. altianalis	NZM_10	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″	1	33	KC860333
B. altianalis	NZM_11	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		37	KC860334
B. altianalis	NZM_12	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		14	KC860335
B. altianalis	NZM_15	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		36	KC860336
B. altianalis	NZM_19	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		36	KC860337
B. altianalis	NZM_2	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″	1	17	KC860338
B. altianalis	NZM_20	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″	1	36	KC860339
B. altianalis	NZM_21	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″	1	32	KC860340
B. altianalis	NZM_22	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		13	KC860341
B. altianalis	NZM_23	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		13	KC860342
B. altianalis	NZM_24	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		2	KC860343
B. altianalis	NZM_26	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		36	KC860344
B. altianalis	NZM_28	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		3	KC860345
B. altianalis	NZM_29	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		2	KC860346
B. altianalis	NZM_3	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		34	KC860347
B. altianalis	NZM_30	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		15	KC860348
B. altianalis	NZM_5	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″	2	36	KC860349

B. altianalis	NZM_6	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		36	KC860350
B. altianalis	NZM_7	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		2	KC860351
B. altianalis	NZM_8	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		33	KC860352
B. altianalis	NZM_9	Nzoia, Kenya	N 00'05′8.0″ E 034°48′47.4″		35	KC860353
B. altianalis	NZU_31	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″	1	37	KC860354
B. altianalis	NZU_32	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		3	KC860355
B. altianalis	NZU_33	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		3	KC860356
B. altianalis	NZU_34	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		40	KC860357
B. altianalis	NZU_35	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		3	KC860358
B. altianalis	NZU_38	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		5	KC860359
B. altianalis	NZU_41	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		3	KC860360
B. altianalis	NZU_42	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		2	KC860361
B. altianalis	NZU_43	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		32	KC860362
B. altianalis	NZU_44	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		2	KC860363
B. altianalis	NZU_45	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		2	KC860364
B. altianalis	NZU_46	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″	1	32	KC860365
B. altianalis	NZU_47	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		2	KC860366
B. altianalis	NZU_48	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″	1	32	KC860367
B. altianalis	NZU_49	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		32	KC860368
B. altianalis	NZU_50	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		38	KC860369
B. altianalis	NZU_51	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″	1	36	KC860370
B. altianalis	NZU_52	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		18	KC860371
B. altianalis	NZU_53	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		2	KC860372
B. altianalis	NZU_54	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″	1	36	KC860373
B. altianalis	NZU_55	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″	1	32	KC860374
B. altianalis	NZU_56	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		38	KC860375
B. altianalis	NZU_57	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		39	KC860376
B. altianalis	NZU_58	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		3	KC860377

B. altianalis	NZU_59	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		32	KC860378
B. altianalis	NZU_60	Nzoia, Kenya	N 00°07′06.9″ E 034°05′37.2″		3	KC860379
B. altianalis	SMB_1	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		19	KC860380
B. altianalis	SMB_10	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		19	KC860381
B. altianalis	SMB_16	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		19	KC860382
B. altianalis	SMB_19	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″	2	7	KC860383
B. altianalis	SMB_2	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″	2	7	KC860384
B. altianalis	SMB_20	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		7	KC860385
B. altianalis	SMB_21	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		21	KC860386
B. altianalis	SMB_22	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		22	KC860387
B. altianalis	SMB_23	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		5	KC860388
B. altianalis	SMB_24	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		20	KC860389
B. altianalis	SMB_25	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		5	KC860390
B. altianalis	SMB_3	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		5	KC860391
B. altianalis	SMB_4	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		5	KC860392
B. altianalis	SMB_5	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		19	KC860393
B. altianalis	SMB_6	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		7	KC860394
B. altianalis	SMB_7	Sondu-Miriu, Kenya	S 00°21′04.3″ E 034°48′37.7″		23	KC860395
B. altianalis	SMW_26	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″		19	KC860396
B. altianalis	SMW_27	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″		7	KC860397
B. altianalis	SMW_28	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″		19	KC860398
B. altianalis	SMW_29	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″		19	KC860399
B. altianalis	SMW_30	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″		19	KC860400
B. altianalis	SMW_31	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″		19	KC860401
B. altianalis	SMW_32	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″		24	KC860402
B. altianalis	SMW_33	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″		19	KC860403
B. altianalis	SMW_34	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″		19	KC860404
B. altianalis	SMW_35	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″		19	KC860405

B. altianalis	SMW_36	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860406
B. altianalis	SMW_37	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860407
B. altianalis	SMW_38	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	7	KC860408
B. altianalis	SMW_39	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860409
B. altianalis	SMW_40	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860410
B. altianalis	SMW_41	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860411
B. altianalis	SMW_42	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860412
B. altianalis	SMW_43	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	7	KC860413
B. altianalis	SMW_44	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860414
B. altianalis	SMW_45	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860415
B. altianalis	SMW_46	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860416
B. altianalis	SMW_47	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860417
B. altianalis	SMW_48	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860418
B. altianalis	SMW_49	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860419
B. altianalis	SMW_50	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860420
B. altianalis	SMW_51	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860421
B. altianalis	SMW_52	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860422
B. altianalis	SMW_53	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860423
B. altianalis	SMW_54	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860424
B. altianalis	SMW_55	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	7	KC860425
B. altianalis	SMW_56	Sondu-Miriu, Kenya	S 00°20′51.5″ E 034°47′29.3″	19	KC860426
B. altianalis	YAB_1	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″	6	KC860427
B. altianalis	YAB_10	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″	18	KC860428
B. altianalis	YAB_11	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″	5	KC860429
B. altianalis	YAB_12	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″	2	KC860430
B. altianalis	YAB_13	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″	28	KC860431
B. altianalis	YAB_14	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″	25	KC860432
B. altianalis	YAB_16	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″	5	KC860433

B. altianalis	YAB_17	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		1	KC860434
B. altianalis	YAB_18	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		5	KC860435
B. altianalis	YAB_19	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		27	KC860436
B. altianalis	YAB_2	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		26	KC860437
B. altianalis	YAB_23	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		5	KC860438
B. altianalis	YAB_25	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		5	KC860439
B. altianalis	YAB_3	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		18	KC860440
B. altianalis	YAB_30	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		6	KC860441
B. altianalis	YAB_4	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		5	KC860442
B. altianalis	YAB_5	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″	3	1	KC860443
B. altianalis	YAB_6	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		5	KC860444
B. altianalis	YAB_7	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		5	KC860445
B. altianalis	YAB_8	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		25	KC860446
B. altianalis	YAB_9	Yala, Kenya	N 00°05′8.0″ E 034°32′32.2″		5	KC860447
B. altianalis	YAC_32	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		5	KC860448
B. altianalis	YAC_33	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		50	KC860449
B. altianalis	YAC_34	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		1	KC860450
B. altianalis	YAC_35	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		2	KC860451
B. altianalis	YAC_36	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		2	KC860452
B. altianalis	YAC_37	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		25	KC860453
B. altianalis	YAC_38	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″	3	2	KC860454
B. altianalis	YAC_39	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		5	KC860455
B. altianalis	YAC_41	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		5	KC860456
B. altianalis	YAC_42	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		5	KC860457
B. altianalis	YAC_44	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		2	KC860458
B. altianalis	YAC_47	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		29	KC860459
B. altianalis	YAC_48	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		18	KC860460
B. altianalis	YAC_49	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″		6	KC860461

B. altianalis	YAC_50	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″	30	KC860462
B. altianalis	YAC_51	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″	2	KC860463
B. altianalis	YAC_52	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″	5	KC860464
B. altianalis	YAC_55	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″	31	KC860465
B. altianalis	YAC_56	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″	25	KC860466
B. altianalis	YAC_57	Yala, Kenya	N 00°09′34.3″E 34°44′39.2″	28	KC860467
B. altianalis		Uganda		38	JN120443.1
B. altianalis		Uganda		57	JN120445.1
B. altianalis		Uganda		58	JN120447.1
B. altianalis		Uganda		50	JN120449.1
B. altianalis		Uganda		46	JN120451.1
B. altianalis		Uganda		37	JN120453.1
B. altianalis		Uganda		59	JN120455.1
B. altianalis		Uganda		58	JN120457.1
B. altianalis		Uganda		57	JN120459.1
B. altianalis		Uganda		60	JN120461.1
B. altianalis		Uganda		61	JN120463.1
B. altianalis		Uganda		49	JN120465.1
B. altianalis		Uganda		62	JN120467.1
B. altianalis		Uganda		38	JN120469.1
B. altianalis		Uganda		63	JN120471.1
B. altianalis		Uganda		44	JN120474.1
B. altianalis		Uganda		56	JN120473.1
B. altianalis		Uganda		62	JN120468.1
B. altianalis		Uganda		41	JN120476.1
B. altianalis		Uganda		42	JN120472.1
B. altianalis		Uganda		43	JN120470.1
B. altianalis		Uganda		44	JN120466.1

B. altianalis	Uganda	45	JN120462.1
B. altianalis	Uganda	46	JN120460.1
B. altianalis	Uganda	47	JN120458.1
B. altianalis	Uganda	48	JN120456.1
B. altianalis	Uganda	49	JN120454.1
B. altianalis	Uganda	50	JN120452.1
B. altianalis	Uganda	51	JN120450.1
B. altianalis	Uganda	52	JN120448.1
B. altianalis	Uganda	53	JN120446.1
B. altianalis	Uganda	54	JN120444.1
B. altianalis	Uganda	55	JN120477.1
B. altianalis	Uganda	56	JN120475.1
B. altianalis	Uganda		
B. altianalis	Uganda		
Outgroups			
Procypris rabaudi			EU683675.1
Cyprinus carpio			JN105354.1
Labeobarbus intermedius		JX066760.1	

Appendix VI: Publications from the thesis

Chemoiwa E. J., Abila, R., Macdonald, A., Lamb, J., Njenga, E and Barasa, J. E. (2013). Genetic diversity and population structure of the endangered Ripon barbel, *Barbus altanialis* (Boulenger, 1900) in Lake Victoria catchment, Kenya based on mitochondrial DNA sequences. *Journal of Applied Ichthyology*. **29**, 1225-1233

Chemoiwa E. J., Abila R., Lamb J., Njenga E, W., Barasa E. J. and Chirchir E. J. (2014). High genetic differentiation among populations of the endangered Ripon Barbel, *Barbus altianalis* (Boulenger, 1900) in Lake Victoria catchment: Insight from Mitochondrial control region. *Journal of Technology and Socio-Economic Development.* 2014, **3**, 177-184.