



Genetic diversity and population structure of the endangered ripon barbel, *Barbus altianalis* (Boulenger, 1900) in Lake Victoria catchment, Kenya based on mitochondrial DNA sequences

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Summary

Approximately 850 bp of the mitochondrial control region was used to assess the genetic diversity, population structure and demographic expansion of the endangered cyprinid *Barbus altianalis*, a species known to be potamodromous in the Lake Victoria drainage system. The 196 samples taken from the four main rivers draining the Lake Victoria catchment (Nzoia, Yala, Nyando and Sondu-Miriu) yielded 49 mitochondrial DNA haplotypes; 83.7% thereof were private haplotypes restricted to particular rivers. The overall mean haplotype diversity was high (0.93663 ± 0.008) and ranged between 0.566 (Sondu – Miriu) and 0.944 (Nzoia). The overall mean nucleotide diversity was low (0.01322 ± 0.00141), ranging from 0.0342 (Sondu – Miriu) to 0.0267 (Nzoia). Population differentiation tests revealed strong and highly significant ($P \leq 0.001$) segregation of populations in the four river basins. F_{ST} values among the four river-based populations ranged from 0.05202 to 0.44352. The samples formed two main haplotype networks based on a 95% parsimony criterion, each exhibiting a strong signature of past population expansion. The smaller network was restricted to the River Nzoia, 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. The degree of population differentiation and the number of river-specific haplotypes are too high to be explained by recent anthropogenic impacts alone and suggest 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 populations.

Introduction

The ichthyofauna of the Lake Victoria ecosystem are some of the most globally threatened biodiversities as a result of anthropogenic and other ecological impacts. Lake Victoria, the second largest freshwater lake in the world, is drained on the Kenyan side by four main rivers: the Nyando, Nzoia, Yala and Sondu-Miriu. Of these, the Nzoia and Sondu-Miriu are the largest, with a recognizable fishery (Cadwalladr, 1965a,b; Ochumba and Manyala, 1992).

The River Nzoia is the most extensive and largest Kenyan river emptying into Lake Victoria. It has numerous tributaries arising from the Cherangany Hills and slopes of Mount Elgon

(Cadwalladr, 1965a,b). River Nyando is one of the feeders of Lake Victoria and criss-crosses the sugar belt region in Kenya where it is apparently polluted by sugar factory effluent. The Yala drains the central highlands west of the Rift Valley, as does the Nyando, which has its sources near Mount Tinderet ($0^{\circ}06'9''S$; $35^{\circ}21'9''E$) 2640 metres above sea level (Wright, 1978). 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 fluctuating high rainfall causing regular massive floods in the lower reaches, especially during the long rainy season.

Sondu Miriu is a large river with an extensive floodplain. The lower part of the river supports fishing activities and has fish landing sites.

Following ecological changes in the lake, a number of studies have documented the resultant effects on its indigenous fish species (Witte et al., 1992; Abila et al., 2004; Njiru et al., 2008). 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 and other human activities could be increasing the nutrient loads in the rivers, probably affecting the dissolved oxygen content and pH of the water (Vuai et al., 2012). Direct discharge of municipal and untreated sewage into rivers degrades and alters habitats for both riverine and lacustrine fish species (Ntiba et al., 2001). These impacts on river water quality could pose a challenge to migratory fish species, 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 the River Sondu-Miriu hampers upstream migration for spawning among potamodromous fish. Similarly, such vegetation limits the entry of newly hatched fry into the lake from the river (Ochumba and Manyala, 1992), predisposing the fry to higher predation pressure and lack of suitable diets.

In Kenya, *Barbus altianalis* (Boulenger, 1900), 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 Lake Victoria in the 1950s and 1960s (Corbet, 1961). Overfishing has reduced riverine species from annual catches of 2500 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. Declining fishing potential in rivers of the Lake Victoria basin has affected the livelihoods of local communities (Ochumba, 1984), hence the need for urgent conservation and management measures for riverine fish species.

Recent studies indicate that *Barbus altianalis*, which formed a major component of riverine fisheries, no longer migrates upstream to breed but comprises stationary populations concentrated at the mouths of the Nzoia, Sondu-Miriu, Yala and Nyando rivers in the Lake Victoria watershed (Ojwang et al., 2007). Whether such philopatric behaviour is a response to recent environmental changes, or whether these river-specific groupings represent previously unidentified populations is not clear. Furthermore, it is unclear how fishing pressure impacts genetic diversity and hence the long term evolutionary survival of the species. Genetic diversity studies are critical in the management of inland water fisheries (Mwanja and Fuerst, 2003), and elucidating genetic structure and gene flow patterns in populations of *B. altianalis* may contribute towards defining management and conservation units for the species. The European and North American cyprinids have been genetically characterized (Gilles et al., 2001; Turner et al., 2004). More recently, Muwanika et al. (2012) used mitochondrial control region and cytochrome *b* sequences to study genetic diversity in *Labeobarbus* species in the Lake Victoria and Albertine basins in Uganda, and reported the presence of two divergent *Barbus* lineages in the Lake Victoria region. Due to high levels of sequence polymorphism, the control region is useful in population diversity studies (Chen et al., 1998). These studies demonstrate the importance of molecular data in the management of cyprinid fish species.

The aim of this study is to use mitochondrial control region DNA sequence variation to assess levels of genetic diversity and structure in populations of *B. altianalis* from the four major rivers draining Lake Victoria, Kenya.

Materials and methods

The study area

Four main rivers flowing into Lake Victoria from the Kenyan catchment were sampled between May and August 2011. Sampling sites identified by Ojwang et al. (2007) were adopted for the study (Fig. 1). Samples were collected from the following stations: Nzoia-Ugunja Bridge and Nzoia-Webuye before discharge on Nzoia River; Yala water works and Yala Kakamega Bridge on Yala River; Nyando-Ahero and Nyando-Koru on Nyando River and Sondu-Nyakwere and Sondu-Sondu Bridge on Sondu-Miriu River.

Sample collection

Samples of *Barbus altianalis* were collected by electrofishing and the total weight (in grams) and total length (in cm) of the samples determined. Fish samples were sexed according to the classification of Witte and van Densen (1995). Approximately 0.5 cm² muscle tissue was clipped from each fish and preserved in 95% ethanol for DNA analysis.

Samples were also fixed in 4% formalin and preserved in 70% ethanol and stored at the University of Eldoret laboratory.

Genomic DNA extraction

Genomic DNA was extracted from approximately 25 mg of tissue per individual using the QIAmpTM DNeasy[®] DNA isolation kit (Qiagen Inc.) following the manufacturer's procedures. The quality of the extracted DNA was checked by electrophoresing 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 loaded along with the samples to confirm the presence of high molecular weight genomic DNA. The DNA concentration was quantified spectrophotometrically.

Mitochondrial DNA sequencing

Approximately 850 bp of the mitochondrial control region was amplified using common carp primers *Cyprinus carpio*, Carp-Pro (5'AACCTCTACCCCTGGCTACCAAAG-3') and Carp-Phe (5'CTAGGACTCATCTTAGCATCTTCAGTG-3') (Chang et al., 1994). PCR amplifications were performed in 25-ml volumes. Each reaction contained 9 ml of DNA (3 ng ml⁻¹), 0.8 ml of sterile water, 2.5 ml of 10× reaction buffer (Super-Therm), 4 ml of 25 mM MgCl₂ (Super-Therm), 0.5 ml of 10 mM deoxynucleoside-triphosphate mixture (dNTPs) (Roche), 0.2 ml of *Taq* polymerase (5 U ml⁻¹) (Super-Therm), and 4 ml of each primer (6 mM) (forward and reverse) per reaction. Target fragments were purified from excised gel bands using a Zymoclean Gel DNA recovery kit according to manufacturers instructions and sequenced at InqabaBiotec (Hatfield, Pretoria, South Africa). All fragments were sequenced in both directions to allow for the reconciliation of ambiguous positions. Sequences were aligned using the CLUSTAL W option (Thompson et al., 1997) of BioEdit 7.0.9 (Hall, 2005) and by visual inspection. Sequences are publicly available under GenBank accession numbers KC860272-KC860467.

Sequence analysis

To determine levels of genetic diversity within and between the populations in the four rivers, 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 TCS 1.21. Control region sequence data were used, with gaps coded as missing and run at a 95% confidence interval (Clement et al., 2000). The network was created with a fixed connection limit of 100 steps.

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). 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.

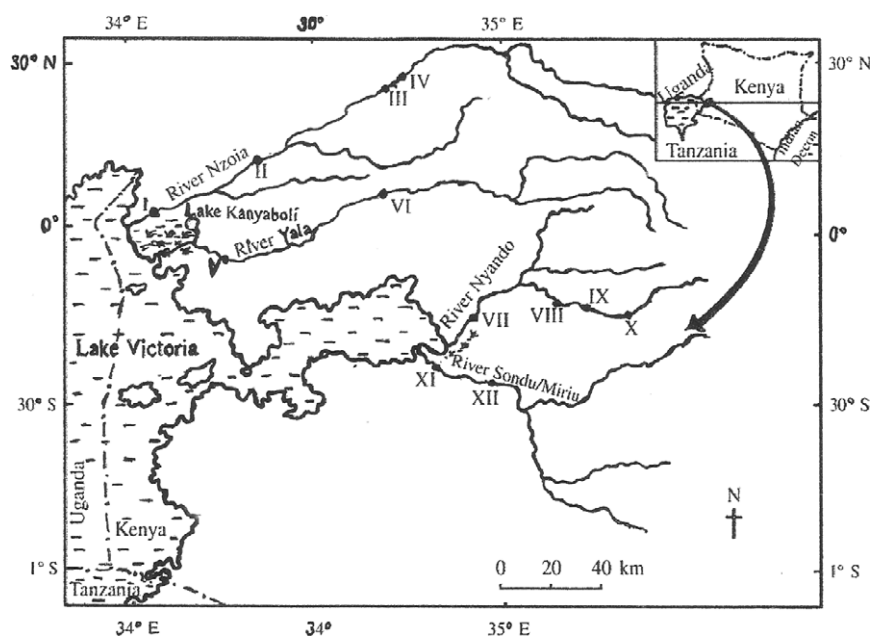


Fig. 1. Positions of sampling stations on four rivers draining the Kenyan side of Lake Victoria: (I, Nzoia-Ugunja Bridge; IV, Nzoia-Webuye before discharge; V, Yala water works; VI, Yala Kakamega Bridge; VII, Nyando-Ahero; X, Nyando-Koru; XI, Sondu-Nyakwere; XII, Sondu-Sondu Bridge). Map modified from Ojwang et al. (2007)

Mismatch distribution analyses (Harpending, 1994) describing pairwise differences between individuals within the population were carried out on the control region sequences to investigate evidence for demographic changes among the populations of *B. altianalis* using the program DNASP 4.9 (Librado and Rozas, 2009). The shape of the distributions can be used to deduce whether a population has undergone a historical population expansion (Rogers and Harpending, 1992; Rogers, 1995).

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). The raggedness index distinguishes a unimodal distribution from a ragged distribution. Secondly, departures from mutation-drift equilibrium were determined using the coalescent-based neutrality estimators: Fu's F_s (Fu, 1997) and Tajima's D (Tajima, 1989) estimated using Arlequin 3.11 (Excoffier et al., 2005).

Results

Genetic diversity inferred from haplotype diversity, nucleotide diversity and the haplotype network

The 196 individuals sampled from the four rivers yielded 49 haplotypes (Table 1, Fig. 2). The overall mean haplotype diversity (h) was high (0.93663 ± 0.008), ranging from 0.566 in the Sondu-Miriu to 0.944 in the Nzoia. The overall mean nucleotide diversity (π) was low (0.01322 ± 0.00141), ranging from 0.0342 in the Sondu-Miriu to 0.02670 in the Nzoia (Table 2).

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). 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, and 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 the respective rivers. This suggests that 'private alleles' could be used as indicators for stock identification.

The samples formed two major haplotype groupings (networks) and four minor groupings (Fig. 2). The 49 haplotypes formed six networks when subjected to a 95% parsimony criterion. Network 1 contained 11 haplotypes; it was separated from network 2 by 29 mutational steps and network 5 (hap 41) by 22 mutational steps. Network 2 comprised 33 haplotypes; it was separated from network 3 (haps 13 and 26) by 17 mutations; from network 4 (hap 18) by 18 mutations; and from network 6 (hap 16) by 8 mutations. Major network 1 consisted of haplotypes from the Nzoia River population only, while major network 2 was made up of haplotypes from all four rivers.

Population genetic structure

The population differentiation test was highly significant ($P < 0.001$) and revealed strong segregation among the four river-based groups. F_{ST} values derived from comparison among these groups ranged from 0.05202 to 0.44352 (Table 3). The group from the River Nzoia was most genetically distinct, showing the highest F_{ST} values when compared with the population groupings from the other three rivers.

Demographic expansion

The overall mismatch distribution for all *Barbus altianalis* samples was bimodal, and did not correspond to the distribution expected for a stationary population (Fig. 3a). In contrast, the overall mean values of Fu's F_s and Tajima's D (-0.40116 and -1.18535 , respectively) were not significantly different from zero, suggesting a relatively stationary population. The negative values obtained, however, do indicate past population growth (Fu, 1997). Mismatch

Table 1
Aligned mitochondrial control region haplotypes (variable positions) from 4 riverine *Barbus altianalis* populations from Lake Victoria basin, Kenya. Dots = identity with *Barbus altianalis* haplotype I sequence and dashes = gaps

Haplotypes	Hap_1
Hap_1	ATTCTCTTTAGATATATACTGATGTACAAAATCCCGGCGAGTTAGTTTACAAAAGCTATGCACCTCTTATTCTGTTTATCTATTCGCAACCAACCCATCACAGA GGATTGGATT
Hap_2	C.....
Hap_3	C.....G.....
Hap_4	C.....C.....
Hap_5	C...G...T...T.....
Hap_6	T...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.....
Hap_12	G...C...G.....
Hap_13	C...C...CG...C...A...CCG...T...T...C...C...AC...C...T...T.....
Hap_14	T...T...T.....TGG.....
Hap_15	C.....
Hap_16	G...C...C...G...G...TGG...C...G...CCC...
Hap_17	C...C...CG...GCC...CG...CT...C...C...G...TC.....
Hap_18	T...GA...C...CG...GCC...CG...CT...C...C...G...TC.....
Hap_19	T...CT.....C...C...C...G...C.....
Hap_20	T.....C...C...C...G...C.....
Hap_21	T...A.....T...T.....
Hap_22	G...T...T.....
Hap_23	C...C...C...G...C.....
Hap_24	C...C...C...G...C.....
Hap_25	TT...T...T.....
Hap_26	C...T...C...C...CG...C...T...A...CCG...T...T...G...C...C...AC...C...T...T.....C.....
Hap_27	CA.....TT...T...T.....
Hap_28	C...T...T...G.....
Hap_29	G...C...C...G...C.....
Hap_30	C...A...T...C...G.....
Hap_31	T.....TT...T...T.....
Hap_32	C...C...C...G.....
Hap_33	T...T...T...C.....
Hap_34	C...T...C...T...G.....
Hap_35	A...C...C...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...T...CA...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

Haplotypes

Hap_44	C.....G..CAGCACTT..CGGCT..TA..T..A..C...T..T..A...A...T...G...A..CC...T..GC.....GGC.....
Hap_45	C..G.CAGC...CTT..CGGCT..TA..T..A..C...T..T..A...A...T...G...A..CC...T..GC.....AG..C.....
Hap_46	C.....G..CAGCACTT..CGGCT..TA..T..A..C..A...T..T...GA...A...T...G...A..CC...T..GC.....G..C.....
Hap_47	C.....G.CAGC...CT...CGGCT..TA..T..A..C...T..T..A...A...T...G...A..CC...T..GC.....G..C.....
Hap_48	C.....G..CAGCACTT..CGGCT..TA..T..A..C..A...T..T..A...A...T...G...A..CC...T..GC.....G..C.....
Hap_49	C.....G..TCAGC...CTT..CGGCT..TA..T..A..C...T..T..A...A...T...G...A..CC...T..GC.....G..C.....

The goodness of fit between the observed and expected distributions under a sudden expansion model was tested. The SSD was significant for groups from the Nzoia and Sondu-Miriu rivers, and the observed distribution curves did not significantly differ from the simulated distribution curves for demographic expansion.

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

Population differentiation tests indicate that the Nzoia group is the most genetically distinct. Both the haplotype network analysis (Fig. 2) and the mismatch distribution analysis (Fig. 3d) show that the Nzoia River comprises two distinct haplotype groups; one of these forms part of network 2, whereas the other is strongly differentiated and solely comprises network 1 (Fig. 2). Some samples of *Barbus*

Table 2

Indices of diversity and neutrality based on 850 nucleotides of the mitochondrial control region of four *Barbus altianalis* populations (Nyan-do, Nzoia, Sondu-Miriu and Yala rivers) and network 1 and network 2 (see Fig. 2) derived from Lake Victoria catchment, Kenya

Index	Nyando	Nzoia	Sondu	Yala	Network 1	Network 2
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)	0.00571	0.02670	0.00342	0.00491	—	—
No. segregating sites	44	90	34	20	—	—
Tajima's D	-1.69107*	0.18165	-2.15443**	0.07888	-2.25506**	-2.06606**
Fu's F_S	-3.88520	-0.18262	0.69906	-4.29284	-3.03775	-17.54685***
SSD	0.00653	0.94563**	0.39764**	0.00426	0.00656**	0.00151**
Raggedness index	0.02249	0.00759	0.17070	0.00792	0.06324	0.01134
No. in Network 1	0	25	0	0	—	—
No. in Network 2	60	23	47	41	—	—

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, standardized squire difference, goodness of fit test statistic between observed and expected distributions under a sudden expansion model. Bold values are significant.

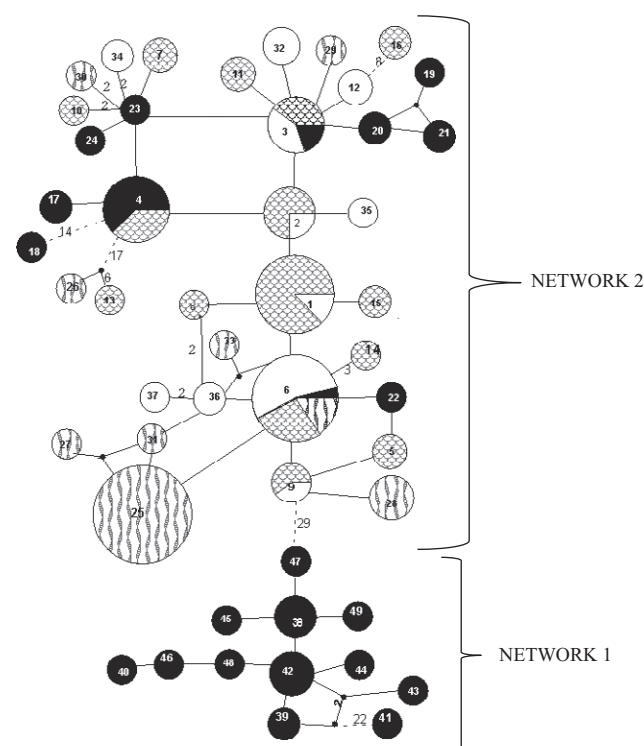


Fig. 2. Haplotype networks showing mutational relationships between 49 haplotypes derived from 196 samples of *Barbus altianalis*. The overall network contains six separate networks, each defined by a 95% parsimony criterion. Solid lines = joining haplotypes within a network, indicating 95% parsimony connections. Dotted lines = different networks, indicating a <95% parsimony connection. Haplotypes are shaded as: wavy lines = Sondu-Miriu River, fish scales = Nyando River, Black = Nzoia River, White = Yala River. Circle size is proportional to the prevalence of the haplotype. Haplotypes are identified by the numbers inside the circles. Branch labels represent numbers of mutation steps that exist between haplotypes; where no label is given, a single mutational step is assumed

altianalis from the River Nzoia have been shown to manifest strong phylogenetic affinity to specimens found in Uganda's water bodies, while others are genetically distinct (Muwanika et al., 2012; E. J. Chemoiwa, unpubl. data). These findings suggest that the Nzoia–Uganda *Barbus altianalis* might harbour cryptic *B. altianalis* species. The high genetic diversity could therefore be due to admixture of phylogenetically different individuals (E. J. Chemoiwa, unpubl. data).

Table 3

Population differentiation (i.e. F_{ST} values) between four populations of *Barbus altianalis* based on 850 nucleotides of the mitochondrial control region

	Nyando	Nzoia	Sondu-Miriu	Yala
Nyando				
Nzoia	0.41344*			
Sondu-Miriu	0.30879*	0.44352*		
Yala	0.05202*	0.38690*	0.2061*	

*Significant, $P < 0.001$.

Due to their migratory behaviour, genetic homogeneity would be expected among the *Barbus altianalis* populations in the Lake Victoria catchment. Our data, however, show differentiation into four river-based populations, as evidenced by the strong river-specific haplotype distribution and the recovery of significant population differentiation among river-based groups. This suggests low dispersal of *B. altianalis* in the Lake Victoria catchment, contrary to the species' known potamodromous nature, which could potentially lead to interactions of the different riverine populations. However, two major and four minor networks are defined by 95% parsimony criteria. While major network 1 (Fig. 2) is confined to the Nzoia River and appears to be non-migratory, major network 2 comprises samples harvested from all four rivers draining the Kenyan side of Lake Victoria. Although the majority of more peripheral haplotypes are confined to specific rivers, the major, more central haplotypes within network 2 (haplotypes 1, 2, 3, 4 and 6) are found in two or more rivers, consistent with present or past migratory behaviour as might be found in a potamodromous species. Since anthropogenic effects on the populations are a relatively recent phenomenon, we hypothesize that the strong population differentiation observed in this study is unlikely to be related to human-induced environmental changes. Rather, the river-based groupings of *Barbus altianalis* are likely to have existed as separate populations for long periods of time, and network 2 might correspond to a migratory form of this species.

High haplotype diversity and low nucleotide diversity was found in the four Lake Victoria catchment rivers. Such haplotype and nucleotide diversity combinations are characteristic of populations that have all experienced rapid population

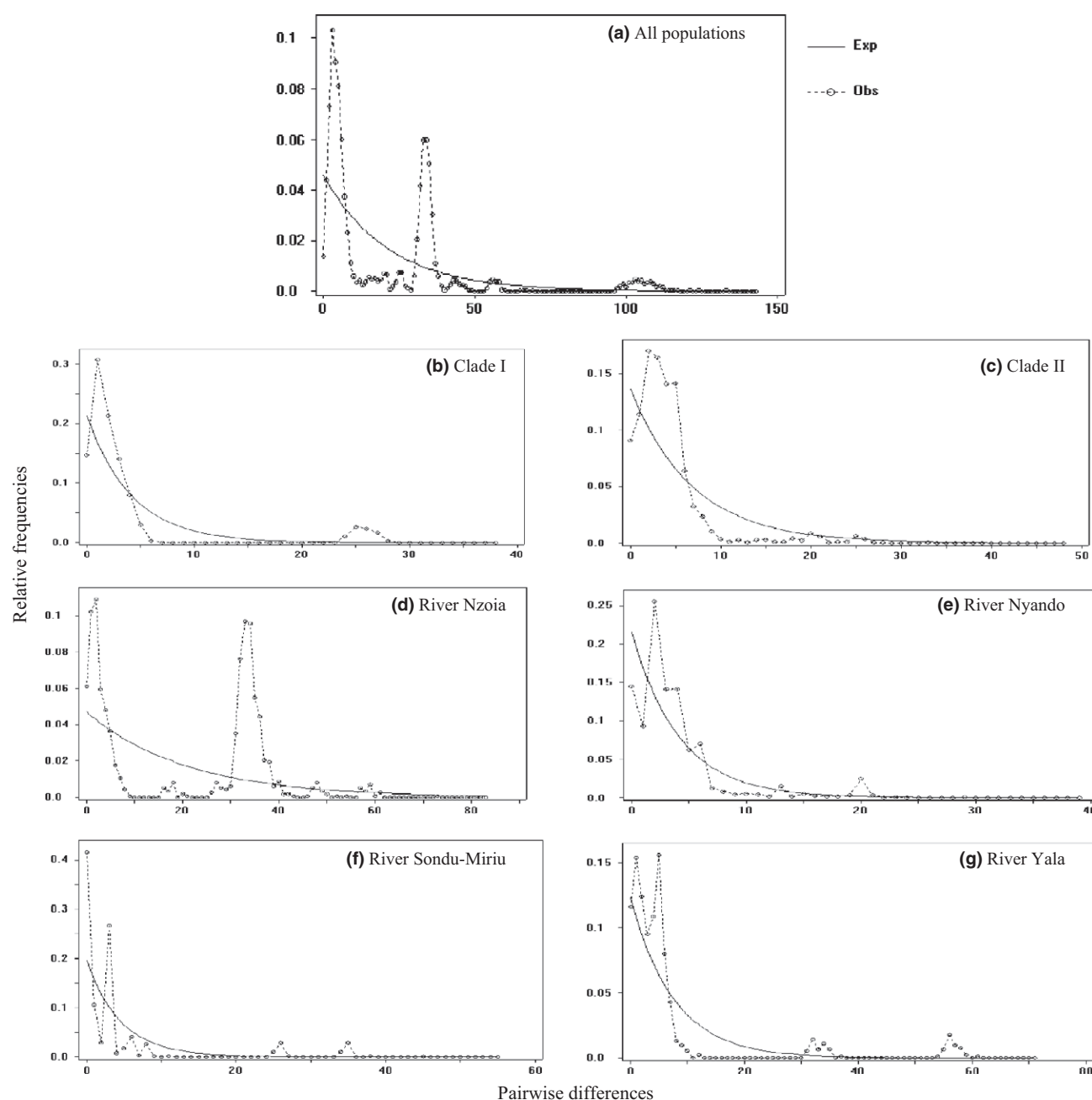


Fig. 3. Mismatch distributions based on 850 nucleotides of the mitochondrial control region of various population groupings of *Barbus altianalis* in rivers draining the Kenyan side of Lake Victoria. Solid line = expected population distribution under a model of constant population size; dotted line = observed distribution

expansion after a period of low effective population size (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. Although mismatch distribution analysis did not clearly show the bell-shaped signature of population expansion in any of the four river based groups (Fig. 3d–g), analyses of Fu's F_s and Tajimas D statistics, as well as goodness of fit tests provide some evidence for population expansion in the Nzoia, Sondu-Mirio and Nyando rivers, but not in the Yala River.

The four Lake Victoria catchment populations separated into two principal 95% parsimony networks. Both of these showed mismatch distributions with a single peak

characteristic of a past population expansion. This demographic scenario is further supported by significantly negative Tajima's D test statistics for both networks, and a highly significant Fu's F_s for network 2, comprising samples from all four rivers. The Fu's F_s test statistic is a powerful test for historical demographic expansions; significantly negative values are indicative of an excess of new mutations resulting from either selective pressure or a past sudden population expansion after a bottleneck.

Although it has been suggested that *Barbus altianalis* no longer exists in Lake Victoria, Ojwang et al. (2007) have reported that large stationary populations do still occur. Our analyses of the mitochondrial control region revealed the persistence of strongly genetically differentiated populations in the four rivers draining the Kenyan side of the Lake

Victoria catchment. These populations may represent hitherto unrecognized non-migratory populations that have survived despite anthropogenic pressures. Other studies have since identified river-restricted populations of potamodromous Lake Victoria cyprinids. Studies based on mitochondrial DNA (Nakamya, 2010; Muwanika et al., 2012) and nuclear microsatellites (Lysell, 2009) have revealed strong population differentiation between two populations of *Labeo victorianus* in the Sio and Mara rivers, suggesting the existence of both migratory and non-migratory populations. Further, our identification is of a 95% parsimony network in which central haplotypes are found in more than one river, whereas peripheral haplotypes are river-specific points to past migratory behaviour in *Barbus altianalis*.

Labeo and *Barbus* populations in Lake Victoria are subject to high fishing pressure mainly during the seasonal migration, where gravid females and juveniles in flood pools are targeted (Ogutu-Ohwayo, 1990). The existence of robust localized populations of *Barbus altianalis* should provide new impetus for conservation of these species and perhaps other potamodromous 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. Morphological and morphometric characterization of the *Barbus altianalis* along its known range in East Africa is important to ascertain its taxonomic status.

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