Western Indian Ocean J. Mar. Sci. Vol. 11, No. 1, pp. 41-54, 2012

Genetic Identification and Population Structure of Juvenile Mullet (Mugilidae) Collected for Aquaculture in East Africa

Oskar Henriksson[1,2,*], Augustine W. Mwandya[3], Martin Gullström[4], Marika Thorberg[1] and Mats Grahn[1]

[1]School of Life Sciences, Södertörn University, Box 4101, SE-141 04 Huddinge, Sweden; [2]Department of Zoology, Stockholm University, SE-106 91 Stockholm, Sweden; [3]Department of Animal Science and Production, Sokoine University of Agriculture, Box 3004, Morogoro, Tanzania; [4]Department of Ecology, Environment and Plant Sciences, Stockholm University, SE-106 91 Stockholm, Sweden.

Keywords: Mullet, mtDNA, CO1, AFLP, population genetic structure, Western Indian Ocean, aquaculture, *Valamugil buchanani*

Abstract—There is a growing demand for wild caught juvenile fish to supply the market for aquaculture. We investigated the local genetic structure of juvenile mullets collected at five sites around Bagamoyo (Tanzanian mainland) and Zanzibar, East Africa. Fish were caught at low tide using a seine net in the same manner used for aquaculture. Specimens were morphologically identified and then genetically identified using direct sequencing of the CO1 gene with cross-referencing to a recent paper on mullet phylogeny. Molecular variance analyses were used to infer genetic subdivision between the sampling sites and population structure using the Bayesian assignment test. Our results revealed that samples morphologically identified as *Mugil cephalus* were in fact *Valamugil buchanani* and, potentially, an unknown species, and we also found evidence of gene flow from other species that may have affected the gene pool. Bayesian analysis revealed a clear genetic population structure within the sampled fish community with a unique mainland cluster. Our findings may have important implications for management and conservation of mullets in the region and elsewhere.

INTRODUCTION

Knowledge on the genetic structure of harvested fish populations is becoming more important for their management and conservation (Hauser & Carvalho, 2008).In species with a strong genetic structure, intense harvesting of a population may lead to local extinction and loss of genetically distinct and locally-adapted stocks (Hiddink *et al.*, 2008). Several ecological and behavioural factors influence the genetic structure of fish populations, the most important being homing behaviour (Gerlach *et al*., 2007), timing of

Corresponding author: OH
Email: oskar.henriksson@sh.se

reproduction (Selkoe *et al*., 2006) and habitat specialization (Knudsen *et al*., 2006), whereas extrinsic factors such as pelagic larval duration have been shown to be of less importance than previously thought (Weersing & Toonen, 2009). The genetic population structure of marine fishes was earlier considered panmictic based on the assumption that their larvae can survive in the water column for extended periods and therefore disperse far (>1000 km) from spawning grounds in oceanic currents. However, recent studies have shown that this picture is more complicated (see review by Cowen & Sponaugle, 2009). Laikre *et al*. (2005) believe that marine organisms fall into three population categories – open, closed and continuous. An open population is, in essence, a panmictic population in which all spawning individuals have an equal chance of mating with each other. In a continuous population, the likelihood of two individuals mating decreases with distance and, in a closed population, only individuals that belong to a population mate with each other. The literature provides evidence of all three population types (Knutsen *et al*., 2003; Dorenbosch *et al*., 2006), suggesting that spawning mode, as well as the ability to return to the same spawning site are contributing factors. In the Western Indian Ocean, previous studies of genetic structure in marine fish are limited and have mainly focused on broad-scale patterns of genetic connectivity (Ridgeway & Sampayo, 2005; Dorenbosch *et al*., 2006; Visram *et al*., 2010).

Grey mullets are distributed worldwide from approximately 42°S to almost 51°N where they inhabit estuarine, intertidal, freshwater and coastal marine habitats (Odum, 1970; Ross, 2001). Reproductive patterns in grey mullet involve migration from shallow coastal habitats to offshore waters where spawning takes place in large schools. Thereafter, larvae and juveniles migrate to inshore environments where they inhabit shallow intertidal habitats such as mangrove creeks (Odum, 1970; Saleh, 2008). Grey mullet is considered to be isochronal spawners, characterized by synchronous gamete development and spawning of all eggs at once or in batches within successive nights (Render *et al*., 1995). The mullets (Family Mugilidae) are important in commercial and subsistence fisheries in many parts of the world (FAO, 2000; Ross, 2001) and, because of their high tolerance to environmental change, they have a great potential for aquaculture in many countries (Oren, 1981; Lee & Menu, 1981; Pillay & Kutty, 2005). They constitute priority species for marine aquaculture development in East Africa (Mmochi & Mwandya, 2003). Previous local-scale population genetic studies on the grey mullet (*Mugil cephalus*) in Florida, using allozymes, suggested it had little or no genetic structure (Campton & Mahmoudi, 1991; Huang *et al*., 2001). A recent study by Liu *et al*., (2010) using Amplified Fragment Length Polymorphism (AFLP) revealed high levels of genetic structuring in *M. cephalus* on a spatial scale of 2000 km in the China Seas. AFLP has proven to be useful in population genetic studies (Sonstebo *et al*., 2007) and has been applied with great success on a wide range of organisms (Bensch & Akesson, 2005). The strength of AFLP is that no prior genetic information is needed about the study organism; however, due to the none-specificity of AFLP analysis, proper species identification is crucial. In this study, we employed direct sequencing of the CO1 gene located in the mitochondrial DNA (mtDNA) as a means of genetic identification of our samples. CO1 is currently used as a DNA barcode in the BOLD database (Ratnasingham & Herbert, 2007). However, the phylogeny of mullets is under revision and there is a great deal of confusion regarding the correct nomenclature of mullet species. Thus, for this paper, we decided to only use CO1 sequences provided by Durand *et al*. (2012).

The objectives of the study were to:

- Confirm the identity of samples morphologically identified as *Mugil cephalus* by direct sequencing of the CO1 gene.
- Examine the population structure of grey mullets around Zanzibar (Unguja Island) and the neighbouring Tanzanian mainland using AFLP.

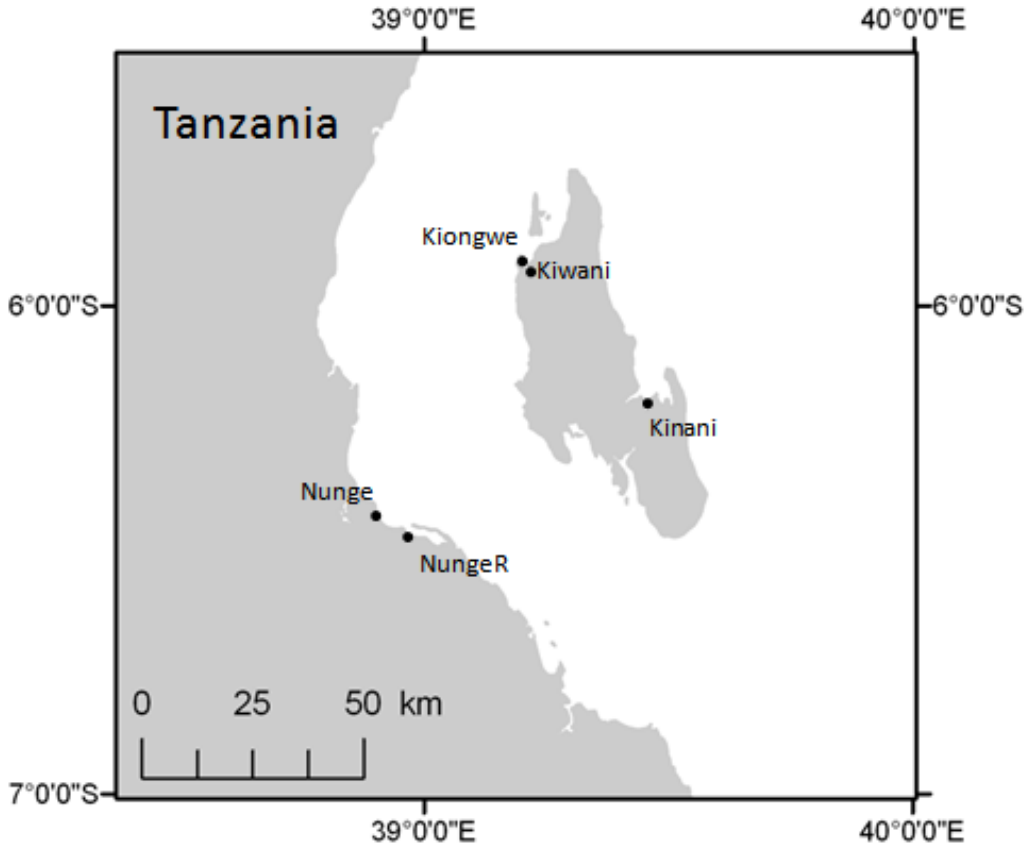

Figure 1. Sampling localities around Zanzibar and on the mainland of Tanzania.

MATERIALS and METHODS

Study sites

Fish were collected during January 2008 at five sites on the coasts of Zanzibar (Unguja Island) and the Tanzanian mainland, East Africa (Fig. 1). The sampling sites at Zanzibar were situated in the forested mangrove creeks of Makoba Bay (Kiongwe and Kiwani) and Chwaka Bay (Kinani). On the Tanzanian mainland, sampling was conducted in a forested mangrove creek (Nunge) of Bagamoyo as well as in a nearby deforested area (Nunge Reserve, hereafter abbreviated to Nunge R), which is used as a reservoir for solar salt works (see Mwandya *et al*. 2009). Mangrove creeks were chosen for sample collection because mullets are commonly distributed within this type of environment (Mwandya *et al*., 2010a, b). All the sampling sites are influenced by monsoon winds, with two pronounced rainy seasons from March to May (the south-east monsoon) and October to December (the north-east monsoon; McClanahan, 1988). Tides in the region are strong and semi-diurnal with a tidal range of approximately 3.5 m. The mangroves are intertidal but retain water even during low spring tide. None of the sampling sites have permanent freshwater input and were characterized by sand or mud bottom with no macrophyte cover. The distance between the sampling sites ranged from 500 m to 100 km in a hierarchical design (see Table 3 for pair-wise distances) to assess at which spatial scales genetic structuring becomes evident (analysing variability within creeks, between creeks and between Zanzibar and the Tanzanian mainland).

Sample collection

A total of 118 schooling fish were caught during low tide using a 17 x 2 m seine net with a stretched mesh size of 1.9 cm. Each haul swept an area of approximately 170 m^2. All fish collected were juveniles between 6.9 and 13.8 cm in total length, the mean length being 9.9±1.8 cm. After each haul, the tail tissue of freshly killed specimens was immediately preserved in 70% ethanol solution and stored at 4°C. Each specimen was morphologically identified to species level according to Smith & Heemstra (1991).

DNA extraction

DNA was extracted from each individual as described by Laird *et al*. (1991).

DNA-barcoding using cytochrome oxidase subunit 1 (CO1) sequencing

Thirty individuals were used for the mtDNA analysis, but only 18 were successfully amplified. Primers used in a previous study by Ward *et al*. (2005), proven to be effective for a variety of fish species, were chosen for amplification of the CO1 mitochondrial region. PCR reactions were also performed according to Ward *et al*. (2005).

AFLP

Prior to AFLP analyses, all samples were randomized relative to their sampling site to minimize possible effects of between-batch variation in the PCR reactions (Bensch & Åkesson, 2005). The concentration of their DNA was determined using a Nanodrop © ND-1000 (Thermo Scientific) spectrophotometer and then diluted to the working concentration of 25ng/µl. The AFLP analyses were performed according to Vos *et al.* (1995) with the modification described by Bensch & Åkesson (2005). Pre-amplification was carried out using two selective nucleotides, an EC-forward primer and an MG-reverse primer. The primer combination for the selective amplification step was a FAM-labelled E-primer with CGT as selective nucleotides and the M-primer with GTA as selective nucleotides. The labelled DNA fragments were separated by capillary electrophoresis, ABI3730XL (Applied Biosystems), at Uppsala Genome Centre, using a 500bp DNA ladder as size standard. The data were subsequently scored using Gene Mapper software 3.0 (Applied Biosystems) at default settings with no normalization. AFLP score (Whitlock *et al*., 2008) was used to normalize data based on peak height and to determine the optimum scoring conditions by genotype calling. The heights of the loci were exported to AFLP score and used to generate a 1/0 data matrix for further analysis. The analysis range was 50-500bp, the locus selection threshold was 200 RFU and the relative phenotype calling threshold was set to 100%, yielding a total of 133 variable bands. We manually checked that duplicate samples yielded the same genotypes.

Data analysis

Differences in total length of the individual fish were analysed using one-way ANOVA. Prior to analysis, Levene's test was used to establish whether the various data fulfilled the assumption of homogeneity of variances. When assumptions were not met even after transformation, the non-parametric Kruskal-Wallis test was used. A *posteriori* pairwise comparison of means was performed using the Games-Howell approach. Due to the large number of tests, the significant level was adjusted using the Bonferroni correction method.

DNA-barcoding

Analysis of the mitochondrial sequences was made using MEGA software 4.0 (Tamura *et al*., 2007). Forward and reverse sequences were aligned and modified by hand to create consensus sequences. The consensus sequences were compared to sequences in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) by BLAST search and the closest hits in GenBank presented by Durand *et al*., (2012) were aligned to the sampled sequences. All sequences were aligned using ClustalW and a neighbour-joining tree was constructed with the Kimura-2 parameter (K2P) distance model and tested with 500 bootstrap replicates using MEGA software (Tamura *et al*.,

2007). All individuals were arranged in groups based on the consensus neighbour-joining tree and genetic distances with standard errors were calculated within and between groups using the K2P model. Finally, the results from CO1 were compared with the value considered by Hebert *et al*. (2004) to be the threshold for species or congeneric species differentiation.

AFLP

The genetic stock structure was investigated with the Bayesian approach in STRUCTURE 2.3 (Pritchard *et al*., 2000, Falush *et al*., 2007). All individuals were assigned to genetic clusters with no prior information and the assumption of admixture and correlated allele frequencies.

This model assumes that the mullet belong to K putative parental populations that may or may not be present today. The admixture proportions represent the proportion of an individual's genome that originates from a K parental population (Francois & Durand, 2010). The most likely number of clusters was calculated according to Evanno *et al*. (2005). A constrained analysis of principal coordinates (CAP) was performed in the R environment (R Development Core Team 2009) using the VEGAN package (Oksanen *et al*., 2009) with Jaccard distance measures to visualize the AFLP data. CAP entails multidimensional scaling that accommodates non-Euclidian dissimilarity indices.

The hierarchical sampling design enabled us to compare variation at different spatial scales and therefore detect the smallest spatial scale of genetic structuring using AFLP data. Five sites at which samples of 13 to 35 individuals were collected were involved in this analysis. The population structure of the mullet was investigated using analysis of molecular variance (AMOVA) software and F-statistics in ARLEQUIN 3.11 (Excoffier *et al*., 2005). The in-file for ARLEQUIN was prepared using the R software package, AFLPdat (Ehrich, 2006). The AFLP genotypes were analyzed using AFLP-SURV 1.0 (Vekemans *et al*., 2002) to obtain values of partitioning of the genetic diversity (F_{ST}). Both the global F_{ST} and pairwise values were analysed between sites. In the F_{ST} analyses, the allele frequencies were analysed assuming Hardy-Weinberg equilibrium, using the Bayesian method assuming non-uniform prior distribution (Zhivotovsky, 1999). A total of 1000 permutations of individuals were tested between sites to calculate the p-values for F_{ST}.

RESULTS

DNA-barcoding

None of the individuals morphologically identified as *Mugil cephalus* using Smith & Hemstra (1991) proved genetically to be this species. According to the phylogenetic analysis of the mtDNA marker CO1, the samples seemed to belong to more than one species, possibly as many as three. The majority (13 of 18) of the analysed samples clustered with *Valamugil buchanani* (Fig. 2a). All the bootstrap values at the level of groups were high, supporting the robustness of the tree in Fig. 2a. Identifications of the samples based on a BLAST search of data provided by Durand *et al*. (2012) yielded *V. buchanani, Moolgarda seheli*, and *M. cunnesius*. The *M. cunnesius* consensus rate in GeneBank was low (91% similarity), indicating that this might be an undescribed species (pers. comm. J.D. Durand). The genetic distances of the CO1 sequences within clades determined from the Kimura 2-parameter model were generally much smaller than the genetic distances between clades (Table 1a). The highest genetic variation (0.6% within clade) was found in the *M. cunnesius* clade, followed by *M. seheli* (0.3%), *V. buchanani* (0.07%), *M. cephalus* (0.03%) and *Chelon labrosus* (0%; Table 1). Applying the tenfold threshold recommended by Hebert *et al*. (2004), the average genetic variation between *M. cunnesius* and the other taxa should be at least 6% to qualify as a separate species (Table 1b). The genetic distances between groups were more than tenfold compared to the genetic distance within groups for *V. buchanani, C. labrosus* and *M. cephalus,* with nucleotide distances ranging between 0.161±0.016

Table 1. a) Estimates of average evolutionary divergence between sequence pairs (K2P distances within groups) derived from the juvenile mullet CO1 data. D = the number of base substitutions per site derived from averages of all sequence pairs within each group, SE = standard error, N = number of samples, and GenBank = the number of sequences derived from this source. All distance estimates are based on the pairwise analysis of 26 sequences. b) Estimates of evolutionary divergence over sequence pairs between groups (K2P distances between groups).

a

	D	S.E.	samples	GenBank
V. buchanani (A)	0.0070	0.0014	13	1
M. seheli (B)	0.0353	0.0058	3	2
M. cunnesius (C)	0.0626	0.0084	2	1
C. labrosus(D)	0.0034	0.0024	0	2
M. cephalus (E)	0.0000	0.0000	0	2

b

	M. seheli (B)	*M. cunnenius* (C)	*C. labrosus*(D)	*M. cephalus* (E)
V. buchanani (A)	0.096 ±0.012	0.161 ±0.016	0.183 ±0.020	0.237 ±0.024
M. seheli (B)		0.166 ±0.017	0.185 ±0.019	0.227 ±0.022
M. cunnesius (C)			0.157 ±0.017	0.222 ±0.020
C. labrosus(D)				0.231 ±0.023

(mean K2P distance ± standard error) and 0.237±0.024, results which imply that these are distinct species. The smallest genetic distances were found between *V. buchanani* and *M. seheli*, where the mean K2P distance of within-group divergence was 0.096±0.012. The pair-wise difference between the *V. buchanani* clade and the *M. seheli* clade did not rise above the tenfold threshold level and the genetic variation was too low to assign them to different species (Hebert *et al*., 2004).

AFLP

Bayesian analysis of the number of genetic clusters (based on the AFLP analyses) showed that the log-likelihood posterior probabilities were lacking modal distribution, so the optimum number of clusters had to be calculated according to the method of Evanno *et al*. (2005). We found support for a distinct ΔK peak at K=2 (Fig. 3a). Of the two genetic clusters, cluster 2 was only found at the Tanzanian mainland (Nunge R and Nunge) and not at Zanzibar (Fig. 3b). The CAP plot supported the Bayesian analysis; the two groups on the plot corresponded to the two genetic clusters revealed by Bayesian analysis (Fig. 2b). However, the different clades identified by the neighbour-joining tree based on the mitochondrial CO1 DNA sequences did not correspond with the two genetic clusters identified by the Bayesian analysis of the AFLP data. The DNA barcoded individuals were overlaid on the CAP plot of the AFLP data to visualize the distribution of the barcoded samples. AMOVA analysis revealed that 69% of the genetic variation was within sites, 10% between sites on either the mainland or Zanzibar, and 21% were partitioned between the mainland and Zanzibar (Table 2). The global FST values between sites was 0.0308 ($p<0.05$), indicating

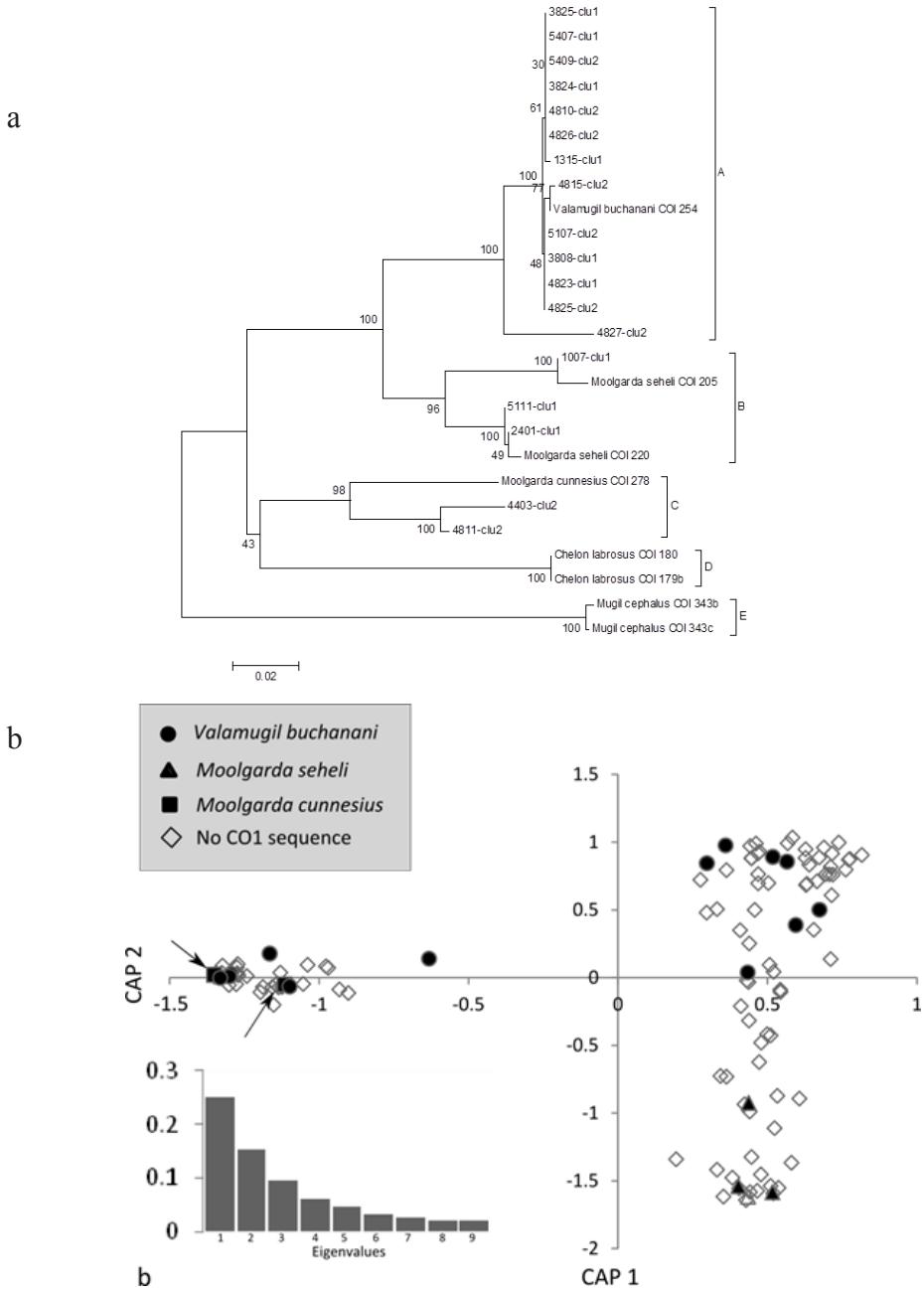

Figure 2. a) Evolutionary relationships of juvenile mullet collected around Zanzibar and on the Tanzanian coast. The evolutionary history was inferred using the neighbour-joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Clu 1 or Clu 2 represents the assigned clusters based on the Bayesian assignment test of the AFLP data. Letters (A-E) depict the major clades present. Species names are from Genebank based on the sequences published by Durand *et al*. (2012). b) CAP analysis based on dissimilarities of the AFLP phenotypes of juvenile mullet collected around Zanzibar and on the Tanzanian coast. The CO1 sequenced individuals are overlaid for visual reference. Arrows show the position of the two samples identified as *Moolgarada cunnesius*. Eigenvalues are represented by a bar graph.

Table 2.Three-level locus by locus AMOVA analysis of grey mullet AFLP phenotypes sampled around Zanzibar and Bagamoyo on the Tanzanian mainland. Global FST values of 0.30 and all levels of differentiation in the AMOVA were significant ($p<0.05$).

Source of variation	Sum of squares	Variance components	Percent variation
Mainland vs Zanzibar	269.35	3.60	20.59
Among sites within groups	151.65	1.79	10.23
Within sites	1367.89	12.11	69.17
Total	1788.88	17.50	

strong overall genetic structuring. Pairwise tests of genetic differentiation between sites were all significant except for those at Nunge vs Nunge R and Kinani vs Kiongwe (Table 2). A comparison of genetic and geographical distances yielded no consistent patterns (Table 3). Likewise, variability in fish size between sites was not related to genetic differentiation (Table 3). Although pairwise tests showed that the mean fish size differed significantly between Kinani, Kiwani and Kiongwe respectively, genetic differentiation was only found between Kiwani and the other two sites, and not between Kinani and Kiongwe (Table 3). Furthermore, the genetic structure also differed between Kinani and both sites in Bagamoyo (Nunge and Nunge R), while the mean fish size differed only between Kinani and Nunge and not between Kinani and Nunge R. Fish from the two neighbouring mainland sites, Nunge and Nunge R, differed neither genetically nor in size (Table 3).

DISCUSSION

In this study, we sampled what was thought to be *Mugil cephalus* harvested in the manner practiced by local fishermen and fish farmers to collect wild fingerlings for aquaculture (Mmochi & Mwandya, 2003). All samples were morphologically identified as *M. cephalus* according to Smith & Heemstra (1991). However, two distinct genetic clusters emerged when analysing the AFLP data using the Bayesian assignment test in STRUCTURE 2.2. Field identification of juvenile mullet can be difficult and we thus employed genetic barcoding of the CO1 gene to ensure that we had sampled a single species and not two species as indicated by the Bayesian assignment test. Since the phylogeny of mullets is under revision (Papasotiropoulos *et al*., 2002; 2007, Durand *et al*., 2012), we only used DNA sequences provided by Durand *et al*. (2012) in the analyses. We found that the samples

Table 3. Pairwise F_{ST} values representing genetic distances of juvenile mullet collected around Zanzibar and on the Tanzanian coast (above diagonal) and geographical distances in km between the collecting sites (below diagonal). Values denoted (*) were significant ($p<0.005$) and a plus sign indicates that the mean fish size was significantly different between sites ($p<0.05$).

	Nunge	NungeR	Kinani	Kiwani	Kiongwe
Nunge		0.0427	0.0764*	0.1470*	0.0459*
Nunge R	3		0.1944*	0.1767*	0.1767*
Kinani	70	67+		0.1918*	0.0100
Kiwani	45+	42+	53+		0.1700*
Kiongwe	45+	42	53+	0.5+	

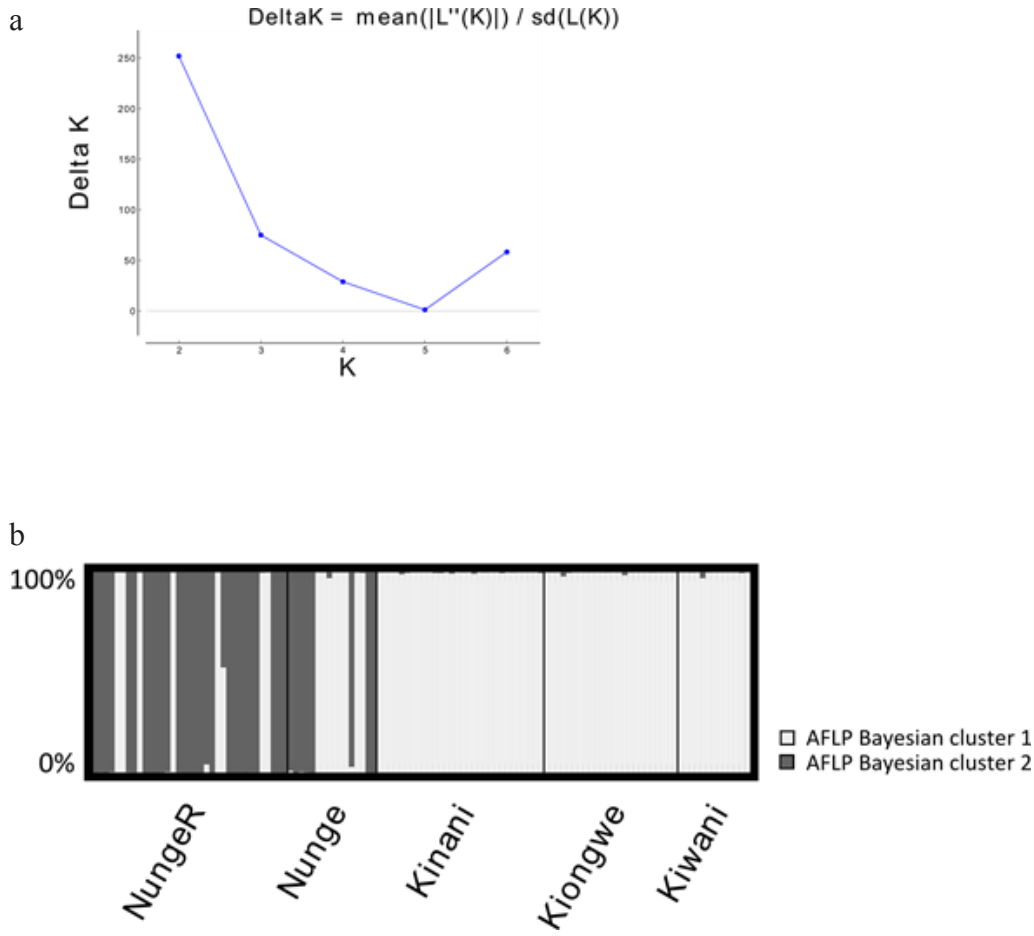

Figure 3. a) Optimum number of genetic clusters of juvenile mullet collected around Zanzibar and on the Tanzanian coast calculated according to Evanno *et al*. (2005). b) Genetic composition of juvenile mullet at sample sites around Zanzibar and on the Tanzanian coast. Each bar represents an AFLP phenotype.

morphologically identified as *M. cephalus* were, in fact, not genetically identified as such. They were identified as *Valamugil buchanani* (13 samples), *Moolgara seheli* (3 samples) and *Moolgarda cunnesius* (2 samples). It is unclear whether *V. buchanani* and *M. seheli* actually constitute two distinct species based on the tenfold within vs between difference in species recommended by Herbert *et al*. (2004), but the DNA sequences scored 91% hits on *M. cunnesius*, the highest in the Durand *et al*. (2012) dataset. Two species had very high within-clade genetic diversity, *M. cunnesius* (0.063 ±0.008) and *M. seheli* (0.035 ±0.006), and could not be distinguished as unique species when compared to the other clades.

Individuals identified by DNA-barcoding as *V. buchanani* were found in both the genetic clusters identified by the Bayesian analysis of the AFLP markers. AFLP has been used to detect differences between species and also for the construction of phylogenetic trees, not just at sub-species level but also for clearly separable species (Graves, 2009). In some instances, AFLP has outperformed mtDNA and genetic barcoding in species phylogenies (Dasmahapatra *et al*., 2009, Mendelson & Wong, 2010).

Our two markers, the CO1 gene (mtDNA) and AFLP (nuclear DNA), revealed two different scenarios with no correspondence between them. The two AFLP clusters each contained several species identified by

the CO1 neighbour-joining tree (cluster 1 contained *V. buchanani* and *M. seheli* and cluster 2 contained *V. buchanani* and *M. cunnesius*). The AFLP variation in cluster 1 was greater within *V. buchanani* than between *V. buchanani* and *M. seheli*. In addition, *V. buchanani* mtDNA haplotypes were found in both the AFLP-based genetic clusters, with no clear delineation in the AFLP phenotypes in the continuum between *V. buchanani* and *M. seheli*. It is also worth noting that Durand *et al*. (2012) questioned the validity of the two genera, *Valamugil* and *Moolgarda*.

A weak correspondence between nuclear and mtDNA markers in phylogeographic analyses is not uncommon (Toews & Brelsford, 2012) and our combined information on AFLP markers and CO1 sequences did not clearly separate out different species. The pairwise comparisons between sampling sites nevertheless revealed that there were some fine-scale genetic differences. Our hierarchical sampling design, ranging from 500 m to 70 km, enabled us to establish the spatial scale of the genetic partitioning we encountered. The pair-wise differences in AFLP markers between Kiwani and Kiongwe could be explained by the sampling of different species at different ends of the genetic continuum between *V. buchanani* and *M. seheli*, thus exaggerating the genetic differences by the geographical proximity of the sites. The clear distinction between the two Bayesian clusters cannot be explained alone in terms of their different species; cluster 1 included fish from all the sites while cluster 2 only incorporated fish from mainland sites.

Aquaculture activities in the Western Indian Ocean region depend on the collection of wild juvenile mullet (Mmochi & Mwandya, 2003). This practice is used in many countries due to the positive results gained from wild seed collection and the high cost of development of commercial hatchery facilities (Suloma & Ogata, 2006). Although the effect of harvesting wild juveniles on the mullet stock has not been well studied, an increasing demand for juvenile mullet and fry with the expansion of aquaculture may have negative effects on the capture fisheries. Harvesting wild fry for aquaculture poses dangers both in terms of the introduction of disease into aquaculture facilities and a reduction in the wild harvested stock. Furthermore, harvesting a single wild stock for aquaculture can lead to a loss in genetic diversity as well as reduced genetic fitness and resistance to disease within an aquaculture system (Spielman *et al*., 2004). This does not seem pertinent in the case of *V. buchanani*, considering the high within-species genetic variability described by its AFLP data. The main concern for aquaculture development in East Africa should thus be not to overharvest the juveniles, leading to collapse of the wild stocks. This study has further shown that the species currently regarded as *Mugil cephalusis*, in fact, is *Valamugil buchanani* and, as there are differences in growth rate and maximum size between the two species (Froese & Pauly, 2010), it is possible that aquaculture systems may be deemed a failure due to the fact that the fish under culture are slower-growing than *M. cephalus*. These are important concerns that need to be addressed as soon as possible.

Acknowledgments–We would like to thank A. Angerbjörn and B. Borg for comments on earlier versions of the manuscript. This study was funded by the Swedish International Development Cooperation Agency (Sida) through the Sida/SAREC Bilateral Marine Science Programme between Sweden and Tanzania.

References

Bensch S, Åkesson M (2005) Ten years of AFLP in ecology and evolution: why so few animals? Molecular Ecology 14: 2899-2914

Campton DE, MahmoudiB (1991) Allozyme variation and population structure of striped Mullet (*Mugil cephalus*) in Florida. Copeia 2: 485-492

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. Annual Review of Marine Science 1: 443-466

Dasmahapatra KK, Hoffman JI, Amos W (2009) Pinniped phylogenetic relationships inferred using AFLP markers. Heredity 103: 168-177

Dorenbosch M, Pollux BJA, Pustjens AZ, Rajagopal S, Nagelkerken I, van der Velde G, Moon-van der Staay SY (2006) Population structure of the *Dory snapper, Lutjanus fulviflamma*, in the western Indian Ocean revealed by means of AFLP fingerprinting. Hydrobiologia 568: 43-53

Durand J-D, Shen K-N, Chen W-J (2012) Systematics of the grey mullets (Teleostei: Mugiliformes: Mugilidae): molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy. Molecular Phylogenetics and Evolution 64: 73-92

Ehrich D (2006) AFLPdat: a collection of R functions for convenient handling of AFLP data. Molecular Ecology Notes 6: 603-604

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14: 2611-2620

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7: 574-578

FAO (2000) The state of the world fisheries and aquaculture. FAO, Rome, 158 pp

François O, Durand E (2010) Spatially explicit Bayesian clustering models in population genetics. Molecular Ecology Resources 10: 773-784

Froese R, Pauly D (eds) (2010) Fish Base. http//www.fishbase.org

Gerlach G, Atema J, Kingsford MJ, Black KP, Miller-Sims V (2007) "Smelling home can prevent dispersal of reef fish larvae." Proceedings of the National Academy of Sciences 104: 858-863

Graves J (2009) Deeper AFLPs. Heredity 103: 99

Hauser L, Carvalho GR (2008) Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish and Fisheries 9: 333-362

Hebert P, Stoeckle M, Zemlak T,Francis C(2004) Identification of birds through DNA barcodes. PLoS Biology 2: e312, 1657-1663

Hiddink JG, MacKenzie BR, Rijnsdorp A, DulvyNK, Nielsen EE, Bekkevold D, Heino M, Lorance P, Ojaveer H (2008) Importance of fish biodiversity for the management of fisheries and ecosystems. Fisheries Research 90: 6-8

Huang CS, Weng CF, Lee SC (2001) Distinguishing two types of grey mullet, *Mugil cephalus* L. (Mugiliformes: Mugilidae), by using glucose-6-phosphate isomerase (GPI) allozymes with special reference to enzyme activities. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 171: 387-394

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. Bmc Genetics 6: 1471-2156

Knudsen R, Klemetsen A, Amundsen P, Hermansen B (2006) Incipient speciation through niche expansion: an example from the Arctic charr in a subarctic lake. Proceedings of the Royal Society B: Biological Sciences 273: 2291-2298

Knutsen H, Jorde PE, André C, Stenseth C (2003) Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. Molecular Ecology 12: 385-394

Laikre L, Palm S, Ryman N (2005) Genetic population structure of fishes: implications for coastal zone management. Ambio 34: 111-119

Laird P.W, Zijderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A (1991) Simplified mammalian DNA isolation procedure. Nucleic Acids Research 19: 4293

Lee CS, Menu B (1981) Effects of salinity on egg development and hatching in grey mullet (*Mugil cephalus*). Journal of Fish Biology 19:179-188

Liu J, Brown CL, Yang T (2010) Phylogenetic relationships of mullets (Mugilidae) in China Seas based on partial sequences of two mitochondrial genes. Biochemical Systematics and Ecology 38: 647-655

McClanahan TR (1988) Seasonality in East Africa's coastal waters. Marine Ecology Progress Series 44: 191-199

Mendelson TC, Wong MK (2010) AFLP phylogeny of the snubnose darters and allies (Percidae: Etheostoma) provides resolution across multiple levels of divergence. Molecular Phylogenetics and Evolution 57: 1253-1259

Mmochi AJ, Mwandya AW (2003) Water quality in the integrated mariculture pond system (IMPS) at Makoba Bay, Zanzibar, Tanzania. Western Indian Ocean Journal of Marine Science 2: 15-23

Mwandya AW, Gullström M, Öhman MC, Andersson MH, Mgaya YD (2009) Fish assemblages in Tanzanian mangrove creek systems influenced by solar salt farm constructions. Estuarine, Coastal and Shelf Science 82: 193-200

Mwandya AW, Mgaya YD, Öhman M.C, Bryceson I, Gullström M(2010a) Distribution patterns of the striped mullet (Mugil cephalus) in mangrove creeks of Zanzibar, Tanzania. African Journal of Marine Science 32: 85-93

Mwandya AW, Gullström M, Andersson MH, Öhman M.C, Mgaya YD, Bryceson I (2010b) Spatial and seasonal variations in fish assemblages of mangrove creek systems in Zanzibar (Tanzania). Estuarine, Coastal and Shelf Science 89: 277-286

Odum WE (1970) Utilization of the direct grazing and plant detritus food chain by striped mullet *Mugil cephalus*. In: Steele JH (ed) Marine food chains. Oliver and Boyd, Edinburgh, pp 222-240

Oksanen J,Kindt R, Legendre P, O'Hara B, SimpsonGL, Solymos P, Henry M, Stevens H, Wagner H (2009) vegan: Community Ecology Package. R package version 1.15-4. http://CRAN.R-project.org/package=vegan

Oren OH (ed) (1981) Aquaculture of grey mullets. Cambridge University Press, Cambridge, United Kingdom, 507 pp

Papasotiropoulos V, Klossa-Kilia E, Alahiotis SN, Kilias G(2007) Molecular phylogeny of grey mullets (Teleostei: Mugilidae) in Greece: Evidence from sequence analysis of mtDNA segments. Biochemical Genetics 45: 623-636

Papasotiropoulos V, Klossa-Kilia E, Kilias G Alahiotis S(2002) Genetic divergence and phylogenetic relationships in grey mullets (Teleostei: Mugilidae) based on PCR-RFLP analysis of mtDNA segments. Biochemical Genetics 40: 71-86

Pillay TVR, Kutty MN (2005) A*quaculture – principles and practices*. Blackwell Scientific Publications Limited, Oxford, pp43-55

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959

R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Ratnasingham S, HebertPDN(2007) BARCODING: bold: The Barcode of Life Data System (http://www.barcodinglife.org). Blackwell Publishing Ltd 7: 355-364

Render JH,Thompson BA, Allen RL (1995) Reproductive development of striped mulletin Louisiana estuarine waters with notes on the applicability of reproductive assessment methods for isochronal species. Transactions of the American Fisheries Society 124: 26-36

Ridgway T, Sampayo EM (2005) Population genetic status of the Western Indian Ocean: what do we know? Western Indian Ocean Journal of Marine Science 4: 1-9

Ross ST (2001) *The inland fishes of Mississippi*. University Press of Mississippi, Jackson, 624 pp

Saleh M (2008) *Capture-based aquaculture of mullets in Egypt*. In: Lovatelli A, Holthus PF (editors) Capture-based aquaculture: global overview. FAO Fisheries Technical Paper No 508, Rome, FAO,pp 109-126

Selkoe KA, Gaines SD, Caselle JE, Warner RR (2006) Currents shifts and kin aggregations explain genetic patchiness in fish recruitments. Ecology 87:3082-3094

Smith M, Heemstra PC (1991) *Smith's sea fishes*. Southern Book Publishers, Johannesburg

Suloma A, Ogata HY (2006) Future of rice-fish culture, desert aquaculture and feed development in Africa: the case of Egypt as the leading country in Africa. JARQ 40: 351-360

Spielman D, Brook BW, Briscoel DA, Frankham R (2004) Does inbreeding and loss of genetic diversity decrease disease resistance? Conservation Genetics 5: 439-448

Sontestbo J, Borgstrom R, Heun M (2007) A comparison of AFLPs and microsatellites to identify the population structure of brown trout (*Salmotrutta* L.) populations from Hardangervidda, Norway. Molecular Ecology 16: 1427-1438

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596-1599

Toews DPL, Brelsford A (2012) The biogeography of mitochondrial and nuclear discordance in animals. Molecular Ecology 21: 3907–3930

Visram S, Yang M-C, Pillay RM, Said S, Henriksson O, GrahnM,Chen CA (2010) Genetic connectivity and historical demography of the blue barred parrotfish (*Scarus ghobban*) in the western Indian Ocean. Marine Biology 157: 1475-1487

Vekemans X, Beauwens T, Lemaire M, Roldan-Ruiz I (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. Molecular Ecology 11: 139-151

Vos P, Hogers, R, Bleeker M, Reijans M, van de Lee T, Hornes M, Friters A, Pot J, Paleman J, Kuiper M, Zabeau M (1995)AFLP –a new technique for DNA-fingerprinting. Nucleic Acids Research 23: 4407-4414

Ward R, Zemlak T, Innes B, Last P, Hebert P (2005) DNA barcoding Australia´s fish species. Philosophical Transactions of the Royal Society of London, Series B, Biological sciences 360: 1847-1857

Weersing K, Toonen R (2009) Population genetics, larval dispersal, and connectivity in marine systems. Marine Ecology Progress Series 393: 1-12

Whitlock R, Hipperson H, Mannarelli M, Butlin RK, Burke T (2008)An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. Molecular Ecology Resources 8: 725-735

Zhivotovsky LA (1999) Estimating population structure in diploids with multilocus dominant DNA markers. Molecular Ecology 8: 907-913