Population genetic structure studies of *Liza aurata* based on mtDNA control region sequences analyses in the southern coasts of the Caspian Sea

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Introduction

Nowadays many species are endangered as a result of habitat loss. Decreases in population lead to reduced genetic diversity, which can cause survival crisis in a population (Cecconi *et al*., 1995). Nowadays optimal management of fish stocks needs information on population structure of species that is provided to researchers through genetic science. Bereavement of science about stock composition will lead to the fracture of fisheries management and unsuitable harvest of stocks (Papasotiropoulos *et al*., 2007). One of the beneficial methods to demonstrate genetic diversity is haplotype analysis of the D-loop region, an index which is very important and determinant for the preservation of species. Significant genetic variation is found in the D-loop region, even among individuals within a given species. Grey mullets are not endemic species of the Caspian Sea. Juveniles of *L. aurata*, *L. saliens* and *Mugil cephallus* were introduced from the Black Sea into the Caspian Sea during the years 1930-1934. But only the introduction of *L. aurata* and *L. saliens* was successful and they adapted well to the ecological conditions of the Caspian Sea (Fazli *et al*., 2008). Papasotiropoulos *et al*. (2007) used mtDNA sequences analysis to explore phylogenetic relationship among five species of the Mugilidae family. Furthermore Erguden *et al*., 2010 examined eight Mugilid species from the Mediterranean Sea and the Black Sea on the basis of the 16S rDNA

gene of mitochondrial DNA. To date there is still not enough genetic analysis about *L. aurata* stock except that Ghodsi *et al*. (2011) studied genetic diversity of *L. aurata* in the coasts of the Golestan province by microsatellite method and Nematzadeh *et al*. (2012) determined genetic differences and phylogenic relationships among six Mugilidae species (*Mugil cephalus*, *M. capito, Liza subviridis, L. saliens, L, aurata, Valmugil buchanani*) using PCR-sequencing. In this study, we used partial sequences of the mtDNA control region to estimate the genetic diversity and differentiation of *L. aurata*. Accordingly, the main objectives of this study were to examine the population genetic structure and evolutionary history of golden grey mullet stocks in Iranian waters of the Caspian Sea.

Materials and methods

In total, 33 fin samples were taken from *L. aurata* collected by beach seine from Gilan (Anzali) , Mazandaran (Sari) , and Golestan (Gomishan) Provinces in January and April of 2012. The specimens were taken to Molecular Genetics Laboratory located at the Caspian Sea Ecology Research Center and stored under -20ºC. DNA was extracted from 50 mg of fin sample using the method of ammonium acetate (McQuown *et al*., 2000). PCR was carried out using the primers D-loop F1 Forward-GGCATTTGGTTCCTACTTCAGG and 12S1-H Reverse-TGCGGAGACTTGCATGTGTAAGT for the D-loop fragment, which were

designed based on Atabeyoglu (2007). PCR reactions were prepared in 50 µL total volume as follows: PCR Buffer (1X), $MgCl_2$ (1.8 mM), $dNTP_S$ (0.1 mM), Primer F (1.5 Pmol), Primer R (1.5 Pmol), *Taq* DNA polymerase CinnaGen company (unit 1) and DNA template (100-200 ng). Reactions of PCR amplification were conducted in a thermal cycler (Auto-Q, Quanta biotech, England) using the following conditions: denaturation step at 95ºC for 30 sec, annealing at 49ºC for 30 sec and extension at 72ºC for 30 sec for 30 cycles. PCR products were purified by electrophoresis in a 1.5 % agarose gel. Lastly, the positive PCR products were used as templates for mtDNA sequencing by dNTP method (Pherson *et al*., 2000). The purified DNAs of each sample with primer were transferred to the BIONEER Company in South Korea for sequencing. The mtDNA control region sequences of all samples were aligned using Clustal X (Thomson *et al*., 1997) in BioEdit. Genetic diversity indices, number of haplotypes (*N*), haplotype diversity (*h*), nucleotide diversity (*P*), polymorphic sites (s) and fixation index (F_{ST}) were estimated using the software DnaSP (Rozas *et al*., 2003). The mean difference of paired nucleotide (Tamura *et al*., 2007) within and among samples of regions and the Neighbor-Joining (NJ) tree was constructed using MEGA (ver.5.05). Genetic distance within samples was estimated using (Nei, 1978) model. Estimation of gene flow (Nm) was derived using the equation:

$Nm=[(1/F_{sT})-1]/2$ (Weir and Cockerham, 1984).

Results and discussion

After alignment, DNA sequences of the control region (D-loop) in golden grey mullet were 900 bp. One sequence data from each region were submitted to GenBank (accession numbers. KF418242 , KF465679 , KJ769204). Individual nucleotides were compared to identify conserved and mutated nucleotides. From a total of 923 study areas, a total of 489 protected sites and 420 polymorphic sites were observed in the gene sequence. The haplotype diversity (h) values of golden grey mullet were 0.989±0.002, 1.000±0.044, and 1.000±0.052 in the Gilan, Mazandaran, and Golestan regions, respectively. The nucleotide diversity (P) values were 0.085, 0.023, and 0.024 in the Gilan, Mazandaran, and Golestan regions, respectively. The highest nucleotide diversity was observed in the Gilan region (0.085) and lowest in Mazandaran (0.023) (Table 1).

The genetic diversity was calculated respectively(0.989±0.002, 1.000±0.044, 1.000±0.052) in the Gilan, Mazandaran and Golestan Provinces, according to Nie (1987) model. Based on the AMOVA (Analysis of Molecular Variance), the highest genetic variations (0.71) were observed within populations and lowest (0.09) was among populations within regions (Table 2).

The high rates of F_{sT} value were observed between Gilan and Mazandaran (0.695), also between Gilan and Golestan (0.692), and the lowest was among Golestan and Mazandaran (0.067) Provinces. Based on the Nei (1987) model, the highest rate of gene flow (3.470) was observed between Mazandaran and Golestan regions, and in addition the lowest rate (0.958) was between Mazandaran and Gilan (Table 3).

Table 1: Haplotype diversity (h), nucleotide diversity (P), observed heterozygosity (H_o), expected heterozygosity (H_e) and Hardy-Weinberg equilibrium (HWE) of golden grey mullet samples in the Caspian Sea.

Location	N	h	P	H_o	H_e	HWE	Tajima′D
Gilan	14	0.989	0.085	0.374	0.353	0.024	-1.389
Mazandaran	10	1.000	0.023	0.301	0.298	0.021	-0.869
Golestan	9	1.000	0.024	0.281	0.203	0.033	-0.443

Table 2: Analysis of molecular variance (AMOVA) of golden grey mullet in the Caspian Sea. Sum square (SS), p value (Pro).

Source of variation	SS	Est.var.	%	Pro
Among regions	23.47	0.024	0.20	0.030
Among populations within regions	13.41	0.092	0.09	0.021
Within populations	96.58	0.132	0.71	0.026

Table 3: Gene flow (Nm) of golden mullet samples in the Caspian Sea.

Nm	Gilan	Mazandaran	Golestan
Gilan	-	0.958	1.020
Mazandaran	0.958	-	3.470
Golestan	1.020	3.470	-

The Nei (1978) model was used to estimate the genetic distance. Hence the genetic distance estimated between Gilan and Mazandaran, Gilan and Golestan, as well as between Golestan and Mazandaran Provinces were 0.458, 0.452, and 0.081, respectively. Phylogenic relationships among golden grey mullet were calculated by MEGA software and divergence time was estimated using Tahjima's test (Tajima, 1993). The results obtained from genetic differences showed that there were significant differences among Gilan with Mazandaran and Golestan Provinces (Fig. 1).
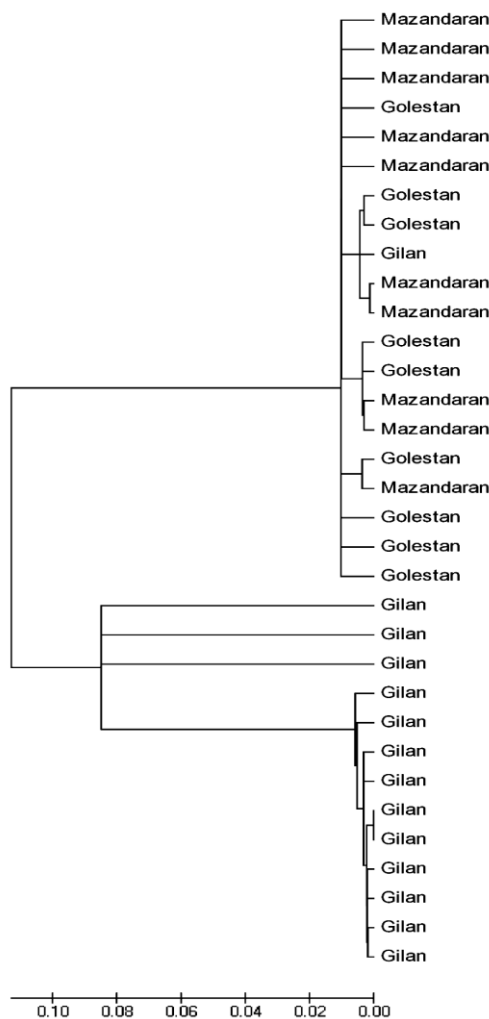
Figure 1: Neighbor-Joining (NJ) tree drawn for golden grey mullet in the Gilan, Golestan and Mazandaran Provinces.

Grey mullets are distributed worldwide, inhabiting coastal and brackish waters of all temperate and tropical regions of the globe (Papasotirpoulos *et al*., 2007), so they can be compared with marine species. Zhongduo *et al*., 2014 investigated high sequence variation and low population differentiation of mitochondrial control regions of wild large yellow croaker as a marine species in South China Sea. they found that the haplotype diversity values of different populations were all higher than 0.980. Furthermore Jean *et al*. (2014) surveyed population genetic structure in the endemic cyprinid fish *Microphysogobio alticorpus* in Taiwan by the cyt *b* and d-loop region analyses and found the average haplotype diversity value was 0.896. Our study indicated high levels of haplotype and nucleotide diversities of *L. aurata* in the southern coasts of the Caspian Sea. Our results obtained from this study on halplotype diversity are in agreement with the results on mullet fish of the Mediterranean Sea (Erguden *et al.*, 2010) and golden grey mullet (Rossi *et al*., 1998; Caldara *et al*., 2002; Papasotirpoulos *et al*., 2007). A similar conclusion was reached by Naderi (2011) who observed high levels of halplotype diversity and more than one population of *L. saliens* in the southern coasts of the Caspian Sea using microsatellite method. Two different populations of *L.aurata* in the southern coasts of the Caspian Sea is a sign of our data analysis in our study. The genetic distance between Gilan

Province and Mazandaran and Golestan Provinces indicates the high difference between the Gilan population and other populations in Mazandaran and Golestan regions. In this study, the gene flow rates between Gilan and Mazandaran and that between Gilan and Golestan Provinces were low which indicated the infrequent rates of immigration between Gilan region and the two other regions. Hereupon the reproductive isolation occurred in the Gilan population which is a factor for the constitution of different populations. Inbreeding of populations within a species produced a unique gene pool of that population, and reproductive characteristics are the main elements for population differentiation (Turan *et al*., 2005). High levels of gene flow were observed among grey mullets in Ghaneh (2011) (5.05) and Ghodsi *et al*. (2011) (5.153-39.264) investigations. In this study the observed genetic diversity for golden grey mullet was 1.000 in the Golestan and Mazandaran Province, which is in agreement with marine species such as *Sciaenops ocellatus* (Gold and Richardson, 1991), *Clupea harengus* and *Brevoorita tyrannus* (Kornfield and Boydanowicz, 1987; Avise *et al*, 1989) but in contrast with *Anguilla rostrata*, *Arius felis* and *Cynoscion nebulosus* (Avise *et al*., 1989). The F_{ST} was calculated at 0.692 and 0.695 between Gilan province and Golestan and between Gilan and Mazandaran Provinces ($p \leq 0.05$), respectively. The overall standardized F_{ST} value among all samples in Jean *et*

al. (2014) was 0.876. In contrast to the present study, Ghodsi *et al*. (2011) calculated the F_{ST} value at 0.016. As a conclusion two populations of *L.aurata* exist in the Iranian waters of the Caspian Sea based on our study as a result of different ecological status in these regions.

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