Genetic diversity of Mahisefid (*Rutilus frisii kutum* Kamensky 1901) in different rivers of the south Caspian Sea using PCR-RFLP

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Abstract

Mahisefid is the most popular fish in Iran with the highest economic value. The analysis of mitochondrial DNA has been extensively used as a marker for population genetic studies and is a powerful tool in studies of gene flow and evolutionary biology. Two hundred ninety four samples were collected from Sefid Rud River (100), Lamir River (98), Shir Rud River (48) and Tajan River (48) during spawning season. Out of 24 enzymes tested, four enzymes, namely *Tas*I, *Hae*III, *Hinf*I and *Hinc*II were selected for this study. In the present study, the haplotype and nucleotide diversity of Mahisefid in four important rivers where fingerlings are produced, were carried out by using PCR-RFLP at mtDNA ND5/6 region. A total of 20 haplotypes were studied so that AAAA and BAAA haplotypes had the most frequency. The average haplotype frequency of AAAA and BAAA haplotypes were 29.93% and 27.55%, respectively. The maximum nucleotide diversity was 0.94%, the minimum was 0.80% and the average was 0.88%. Divergence between Lamir and Sefid Rud River and Shir Rud was 0.2% and between Lamir and Tajan, and Shir Rud River it was 0.1%. The average evolutionary distance was 0.015. The maximum evolutionary distance was 0.294 between ADAA and AAAB, ABAB and BDBA, BBAA and AABB. The average number of bases surveyed was 121.2 and the average number of fragments was 30.30. The study suggests that there was a low genetic variability in four populations of Mahisefid in the south of Caspian Sea. Mahisefid population can be divided into two main clusters, the first clade consists of Shir Rud River and Lamir River populations and the second clade consists of Tajan River and Sefid Rud River populations. The clustering of Mahisefid populations was not in accordance with their geographical areas or river systems.

Keywords: *Rutilus frisii kutum*, Genetic characteristics, mtDNA, Iran

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Introduction

Mahisefid is one of the most important fish for fisheries and stocking programs in the south of Caspian Sea. Fingerlings are produced from different rivers and are released to improve fisheries captures. It is important to identify the population in each river for sustainable fisheries and genetic conservation (Abdolhay, 1997; Abdolhay and Baradaran, 2006; Abdolhay et al., 2011). This species is reported as a candidate species to be of least concern in the south Caspian Sea basin according to IUCN criteria. The 2000 IUCN Red List lists this species as the red list (IUCN 2010).

The mtDNA evolves much faster than nuclear DNA and thus contains more sequence diversity compared to ncDNA, so that it facilitates the identification of closely related species (Bavornlak et al., 2009; Brown et al., 1979; Brown et al., 1982; Vawter and Brown, 1986). In addition, maternal inheritance of the mtDNA generally results in a lack of heteroplasmy (Hayashi and Walle, 1985; Lansman, 1983). A high copy number of mitochondrial genome by a factor up to 10,000 (1000 mitochondria per cell, each 10 copies of the genome) is advantageous (Alberts et al., 1990). In PCR restriction fragment length polymorphism (PCR-RFLP), a conserved region of DNA sequence is amplified using PCR, followed by digestion with restriction enzymes (REs), which can reveal genetic variation between species (Partis et al., 2000). A careful selection of REs prevents ambiguous results caused by intraspecies polymorphisms (Wolf et al., 1999).RFLP has been applied on several species, to describe the population structure including on *Silurus glanis*, (Triantafyllidis et al., 1999) Eleven Danish brown trout hatchery strains were studied using PCR-RFLP analysis of the ND-l and ND-5/6 segments of the mitochondrial genome. For comparison, data from the wild trout representing three Danish river systems were also included (Hansen and Loeschcke, 1996), Atlantic Mugilidae species (Trape et al., 2009), and *Rutilus rutilus* (Rezvani Gilkholahi et al., 2006). Reduced variability in terms of nucleon diversity and number of haplotypes were observed in most hatchery strains. However, computer simulations showed that even with relatively large numbers of female spawners considerable loss of haplotypes could take place over time. Therefore, reduced variability in some of the strains did not necessarily indicate a critical loss of allelic variation at nuclear loci. The genetic relationships among the strains were compared with information from hatchery managers on the origin of the strains. In one case, a strain supposed to be recently founded from wild trout appeared to be of a mixed wild and hatchery origin. Genetic differentiation among strains (F_{ST} = 0.23) was of the same order of magnitude as that observed among wild Danish trout populations. However, minimal differentiation (G_{ST} = 0.01) was observed among the four most important strains, supplying 80% of all hatchery trout stocked in Denmark.

Krieg et al., (2000) had studied genetic diversity of 13 wild populations and 8 cultured populations while Gross et al., (2002) had studied different

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populations of carp in European and East Asian subspecies. Polymorphisms within the mitochondrial NADH-3,4 dehydrogenase (*ND-3/4*) and NADH-5,6 dehydrogenase (*ND-5/6*) gene regions were studied by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis among common carp populations belonging to the European (two farmed strains and three wild populations) and East Asian (Amur wild carp, Vietnamese wild carp and Japanese Koi carp) subspecies, *Cyprinus carpio carpio* and *Corythoichthys haematopterus*, respectively (Gross et al., 2002). The polymorphism was detected using eight and six restriction enzymes, and a total of seven composite haplotypes were identified. Both distance-based and maximum likelihood phylogenetic inference methods clustered the haplotypes into four distinct groups, the European (two haplotypes), Amur (two haplotypes), Vietnamese (two haplotypes) and Koi (one haplotype), and their distributions strictly follow the geographic origin of the populations. The populations were clustered into two highly divergent groups (average net nucleotide divergence, 2.4%), the European and the East Asian populations, suggesting an ancient separation. Six enzymes (*Hin*fI, *Alu*I, *Hpa*II and *Taq*I at ND-3/4 ; *Eco*47I and *Bsu*RI at ND-5/6) yielded diagnostic restriction sites for discriminating the European and East Asian maternal lineages that can be applied for monitoring genetic purity of the European farmed strains.

The RFLP technique has been used as a molecular tool for evaluating genetic variation and relationships in several aquatic species including Artemia species (Baxevanis et al., 2005; Bossier et al., 2004; Eimanifar et al., 2006; Gajardo et al., 2004), *Cyprinus carpio* L. (Gross et al., 2002; Lehoczky et al., 2005), Nebraska paddlefish (Szalanski et al., 2000), brown trout (Hansen and Loeschcke, 1996), *Salmo trutta* L. (Bardakci et al., 2006), lobster (Stamatis et al., 2004), sturgeon (Rezvani Gilkolaei, 2000; Wolf et al., 1999) , *Penaeus japonicus* (Sugaya et al., 2002), scallop (Pastene et al., 1997) *Penaeus monodon and Penaeus merguiensis* (Daud, 1995) and Populations of *Atherina boyeri* (Spiridoula et al., *2008)* . Some literatures used gene cythochrome b in anchovy *Engraulis encrasicolus* (Rea et al., 2009), *Rutilus rutilus* (Rezvani et al., 2000) and D-loop and ND 5/6 of Acipenserida (Pourkazemi, 1996). The objective of this study was to study the genetic variation among the population of Mahisefid in 4 rivers using RFLP markers.

Materials and methods

The caudal fin tissues (2-3g) of Mahisefid (*Rutilus frisii kutum*) were collected from four rivers along the south of the Caspian Sea during migration in 2005. A total of 294 adult individual specimens comprising 100 from Sefid Rud River, 50 from Lamir River, 48 from Shi Rud River and 48 from Tajan River were sampled. These regions are located at the south-west Caspian Sea. The samples were collected from fin tissue of broodstock when they migrated to the rivers for reproduction. The broodstock

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were caught by shill (which is the method of collection of fish in river) and by case net. All fin samples were preserved in 96% alcohol and transferred to the genetic laboratory. DNA was extracted using phenol chloroform methods (Taggart et al., 1992).Approximately 50mg of alcohol preserved fin tissue was placed in 600 ml STE (sodium Tris EDTA) buffer, 30 ml SDS (10% sodium dodecyl sulphate), and digested overnight with 15 ul l proteinase K (20mg/ml, Roche) at 55°C. The DNA was extracted following the method of (Taggart et al., 1992). Approximately 0.5-1.0 mg of the DNA sample and control lambda DNA were run on a 1% agarose gel and stained with ethidiumbromide (10mg/ml; Sigma1) to check its quality and relative quantity. The actual concentration of DNA was measured more accurately by a spectrophotometer by taking readings at 260 nm. PCR amplification fragments approximately 2470 base pair (bp) in length from the N/D 5 were amplified by PCR from total genomic DNA.

The primer sequences were as follows:

ND5- 5′ AAT AGT TTA TTC AGT TGG TCT TAC 3′
ND6- 5′ TAA CAA CGA TGG TTT TTC ATA TCA 3′

Amplification reaction mixtures consisted of 100 ng DNA template, 0.5 ml of dNTPs, 25 pmol primers each, 3 ml $MgCl_2$, 5ml 10X reaction buffer, µl Taq polymerase, with sterilized double distilled water added to make up the volume to 50 µl (Table 1). The PCR was conducted for 30 cycles using an automatic thermal cycle (Eppendorf Germany) at 94°C for 5min denaturation, 48°C for 1min annealing and 72°C for 1.5min extension (Table 2). All products from the PCR amplification were confirmed as being of equal length by electrophoresis on 1.5% agarose gel buffered with Tris boric EDTA (TBE), stained with ethidium bromide, visualized under ultraviolet (UV) light and were subjected directly to digestion with restriction endonucleases. The PCR products were stored at - 20°C until used.

Table 1: Amplification reaction solution for PCR amplification

Materials	Concentration	Quantity
DNA	100ng	1 >µl
Taq DNA polymerase	5u/ µl	0.5-0.2µl
dNTPs	10 mMol	1µl
$MgCl_2$	50 mMol	3µl
PCR buffer	10X	5µl
Primer F	20pmol	1µl
Primer R	20pmol	1µl
Double Distilled water	20	37.6µl
Total volume	……..	50 µl

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Table 2: PCR condition for amphylication of mtDNA ND5/6

Stages	Steps	Temperature(^{o}C)	Time(min)	cycle(s)
1	Denaturation	94	5	1
		94	0.5	30
		48	1	
2	Annealing	72	1.5	
3	Extension	72	10	1

The PCR products were digested by 20 restriction enzymes according to the manufacture recommendations as follows: *TasI, HaeIII, HinfI, HincII, SalI, DraI, AccI, AvaII, XhaI, BshNI, AvaI, BclI, BshII, MspI, PstI, RsaI, SdnI, TaqI, TruI, VspI*. 3 mg of PCR products incubated at 37^{o}C for 4-6 h with appropriate amounts of the enzymes under conditions described digested the mtDNA. Polyacrylamide gel (6%) electrophoresis was carried out in the standard gel matrix including 7.5ml of 30% polyacrylamide, 3.5ml of 10 TBE, 27.5ml of distilled water, 300 µl of 10% APS and 32.5 µl of TEMED. Electrophoresis was performed at a constant voltage (60V for about 4 h) and silver stained. The DNA fragments were exposed to UV light to visualize the mtDNA fragments. A 100 bp DNA ladder molecular weight marker (MBI, FermentasTM, Iran) was included on each gel for bp comparisons. The size of mtDNA fragments were measured by using software (photo documentation system, model: Doc-008. XD, v. 10) A, B, C, etc., in the order of detection designated the cleavage patterns by each enzyme. Data was analyzed with Reap software. The Computer package DFRAG (Schaeffer & Sederoff, 1981, version 3.03) and BIOPROFILE (Vilber-Lourmat Ltd.) were used to analyze the restriction digest profiles. Restriction fragment length sizes (bp) were assigned using the standard marker VI and HindIII fragment ladder and a correlation between distance migrated and band size was calculated for each gel. Different genotypes are described by capital letters. The composite haplotype of an individual is the collection of each of the single enzyme genotypes. Nucleotide diversity (π) is defined as the average number of either nucleotide differences or substitutions per site for a group of DNA sequences (alleles) sampled (Nei and Tajima 1981; Nei 1987). The extent of DNA polymorphism in a population can be measured by nucleotide diversity (π). This can be assigned both within and between populations. When there is polymorphism within populations, the extent of nucleotide divergence between populations for a group of sampled DNA sequences is measured by the average number of net nucleotide substitutions per site (*dA*), where the effect of within-population polymorphism has been subtracted. Both π and *dA* are estimated based on the proportion of shared restriction fragments between haplotypes

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(Nei and Miller, 1990). The nucleotide diversity in a population (π) was calculated by:

$$\pi = 2\sum_{Kj}\frac{d_{ij}}{[n(n-1)]}$$ (Nei and Tajima, 1981)

d_{ij} is an estimate of the number of nucleotide substitutions per site between DNA sequences *i* and *j*, and *n* is the number of DNA sequences examined.

The number of nucleotide substitutions between haplotypes (d_{ij}) is based on the total number of restriction sites (m_i *and* m_{ji}) and the number of shared restriction sites (m_{ij}) between haplotypes *i* and *j*. The d_{ij} was calculated for all enzyme classes, classified according to their nucleotide recognition sequence number (*r*) (Nei, 1987) which in the present study was 4, 5, 5.3 and 6 for different enzymes.

The calculation of shared restriction sites for each enzyme class

The proportion of shared restriction sites between haplotypes i and j for each enzyme class (S_{ij}) was estimated by:

$$S_{ij} = \frac{2m_{ij}}{(m_i + m_j)}$$

The calculation of $d_{ij(K)}$ for each enzyme class as:

This is computed as:

$$\hat{d}_{ij(k)} = -\lceil \log_e S_{ij} \rceil / r$$

(Nei and Li, 1979)

where *r* is defined as above and *(k)* refers to the k^{th} class of restriction enzymes.

The estimation of d_{ij} for two or more enzymes class as

This is computed as:

$$\hat{d}i_j = \sum_k \overline{m}_k r_k d_{ij(k)} \Big/ \sum_k \overline{m}_k r_k$$

where $\overline{m}_k$ is $\frac{(m_{i(k)} + m_{j(k)})}{2}$ (Nei and Tajima, 1981)

Summation was taken over all different enzyme classes. Standard errors for site data based on the proportion of fragments generated by each enzyme class were computed according to Nei and Tajima (1983); and Nei (1987).

Nucleotide divergence is the average number of net nucleotide substitutions per site between different populations, where the effect of intra-population variation is subtracted.

Nucleotide divergence was calculated based on Nei (1987) eq. 10.21 as follows:

$$d_A = d_{xy} - \frac{(d_x + d_y)}{2}$$

d_x and d_y are the π values for populations X and *Y*, and d_{xy} is the average number of nucleotide substitution per site between X

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and *Y*. The d_{xy} value was calculated based on Nei (1987) eq. 10.20.

$$d_{xy} = \sum_{ij} x_i y_j d_{ij}$$

Where d_{ij} is the number of substitutions between the *i*th and *j*th haplotypes and x_i and y_j are the frequencies of the haplotypes in populations X and Y.

Haplotype phylogeny

In order to determine the phylogenetic relationship between haplotypes, the set of d_{ij} values was bootstrapped 100 times (Seqboot, Phylip 3.2; Felsenstein, 1994). This technique generates new multiple data sets by sampling N characters (restriction sites) randomly with replacement, so that the resulting data set has the same size as the original. These hundred data sets were then used to construct a tree using UPGMA and Neighbor Joining methods (Nei, 1987) to create a consensus tree. The numbers on the nodes of the tree represent the number of times that the branch to the right of the node was found in the 100 replicates analysed. The Neighbor-Joining (NJ) method used here is a phylogenetic estimation procedure proposed by Nei (1987) using a distance matrix of nucleotide divergence values among OUTs (haplotypes). The unweighted Pair Group Method with Arithmetic averages (UPGMA) (Nei 1972) used here is another method that estimates the phylogenetic relationship between OTUs based on a distance matrix. An X^2 test based on Monte-Carlo simulation (Roff and Bentzen, 1989) was used to determine whether there were significant differences in the distribution of mtDNA haplotypes between samples. The technique is recommended for mtDNA analyses where restriction-enzyme surveys often reveal many relatively rare haplotypes. One thousand simulations were performed using the Restriction Enzyme Analysis Package (REAP, version 4.0) (McElory et al., 1992). The estimated probability, p, that sets the significance level is given by $\hat{p} = \frac{n}{N}$, where *n* is the number of randomizations that generate an X^2 value greater than that observed and where *N* is the total number of randomized sets.

Results

The primers amplified a fragment about 2470 bp in all samples. The PCR product was digested by 20 restriction enzymes as follows: *TasI, HaeIII, HinfI, HincII, SalI, DraI, AccI, AvaII, XhaI, BshNI, AvaI, BclI, BshII, MspI, PstI, RsaI, SdnI, TaqI, TruI, VspI*. The four restriction enzymes including: *Tas*I, *HaeIII*, *HinfI*, *HincII* showed a polymorphic pattern, six enzymes did not have any restriction site and 14 enzymes showed a monomorphic pattern. A total of 20 haplotypes were studied in which AAAA and BAAA haplotypes had the most frequency. The average haplotype frequency of AAAA was 29.93% and the average haplotype frequency of BAAA was 27.55% (Table 3).

The maximum nucleotide diversity was 0.94%, the minimum was 0.80% and the average was 0.88%. Table 4 shows restriction fragment patterns of four enzymes. Coding regions by restriction enzymes is in table 5.

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Table 3: Number of haplotypes observed in four different rivers following PCR-RFLP analysis and percentage

Row	Haplotype	Lamir (N=98)	%	Sefid Rud (N=100)	%	Shir Rud (N=48)	%	Tajan (N=48)	%
1	AAAA	36	36.73	20	20.00	13	27.08	19	39.58
2	BAAA	28	28.57	29	29.00	13	27.08	11	22.92
3	ABAA	6	6.12	8	8.00	4	8.33	2	4.17
4	ACAA	4	4.08	4	4.00	3	6.25	3	6.25
5	AAAC	1	1.02	6	6.00	3	6.25	-	-
6	AAAB	4	4.08	3	3.00	2	4.17	3	6.25
7	ABAB	5	5.10	2	2.00	2	4.17	-	-
8	BBAA	2	2.04	4	4.00	-	-	2	4.17
9	ABBA	2	2.04	5	5.00	2	4.17	3	6.25
10	BDAA	3	3.06	3	3.00	-	-	-	-
11	AABA	3	3.06	3	3.00	4	8.33	3	6.25
12	BCBA	1	1.02	3	3.00	-	-	-	-
13	AABB	1	1.02	1	1.00	-	-	1	2.08
14	ADAA	1	1.02	-	-	-	-	-	-
15	ABAC	2	2.04	2	2.00	-	-	-	-
16	BCAA	2	2.04	2	2.00	1	2.08	-	-
17	BAAB	-	-	2	2.00	1	2.08	-	-
18	BAAC	-	-	-	-	-	-	1	2.08
19	BDAB	-	-	1	1.00	-	-	-	-
20	BABA	-	-	2	2.00	-	-	-	-

Table 4: Haplotype and nucleotide diversity within population

Population	Haplotype Diversity		Nucleotide diversity
	Selfing	Non-Selfing	
Sefid Rud	0.90 ± 0.02	0.90 ± 0.01	0.010
Lamir	0.77 ± 0.05	0.76 ± 0.03	0.007
Shir Rud	0.85 ± 0.04	0.84 ± 0.03	0.009
Tajan	0.74 ± 0.07	0.73 ± 0.05	0.009
Average	0.82 ± 0.00	0.81 ± 0.00	0.009

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Table 5: Restriction fragment patterns of four restriction enzymes. The size of digested PCR products for four rivers calculated with 2500 bp.

HaeIII		*TasI*				*HincII*		*HinfI*		
A	B	A	B	C	D	A	B	A	B	C
490*	490*	460	460	460	630	1350	890*	600	470	600
330	330	385*	385*	385*	385	710	470	420 *	450 *	450 *
300	300	185	230	290	185	410	220	340	340	315
240	170	175	185	185	175			315	315	290
170	150	170	170	170	135			180	190	190
150	140	135	135	135	115			125	125	125
130	110	115	115	115	105			40	90	90
110	80	105*	105	105	82				40	40
60	50	82	82	82	55					
	40	55		55						

*= band repeated

Divergence between Lamir River and Sefid Rud River and Shir Rud was 0.2% and between Lamir and Tajan, and Shir Rud River it was 0.1%. The maximum nucleotide diversity was 0.94%, the minimum was 0.80% and the average was 0.88% (table 6). Table 6 shows nucleotide diversity within population and table 7 nucleotide divergence among populations.

Table 6: Nucleotide diversity (Above Diagonal) and divergence (Below) among population

population	Sefid Rud	Lamir	Shir Rud	Tajan
Sefid Rud		0.0087	0.0094	0.0091
Lamir	0.0002		0.0084	0.0080
Shir Rud	-0.0002	0.0000		0.0089
Tajan	0.0000	0.0001	-0.0001	

Table 7: Nucleotide divergence among populations

population	Sefid Rud	Lamir	Shir Rud	Tajan
Sefid Rud				
Lamir	0.00015273			
Shir Rud	0.000148515	0.00004021		
Tajan	0.000016792	0.00008962	0.00005739	

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Table 8: Probability of exceeding X^2 by chance (Above diagonal) and calculated X^2 value (Below) among populations

Population	Sefid Rud	Lamir	Shir Rud	Tajan
Sefid Rud		0.28	0.89	0.26
Lamir	20.81		0.38	0.26
Shir Rud	11.58	16.75		0.34
Tajan	21.41	18.16	13.5	

The average evolutionary distance was 0.015. Maximum evolutionary distance was 0.294 between ADAA and AAB, ABAB and BDBA, BBAA and AABB and the minimum was between BCBA and BABA. The average number of bases surveyed was 121.2 and the average number of fragments was 30.30.

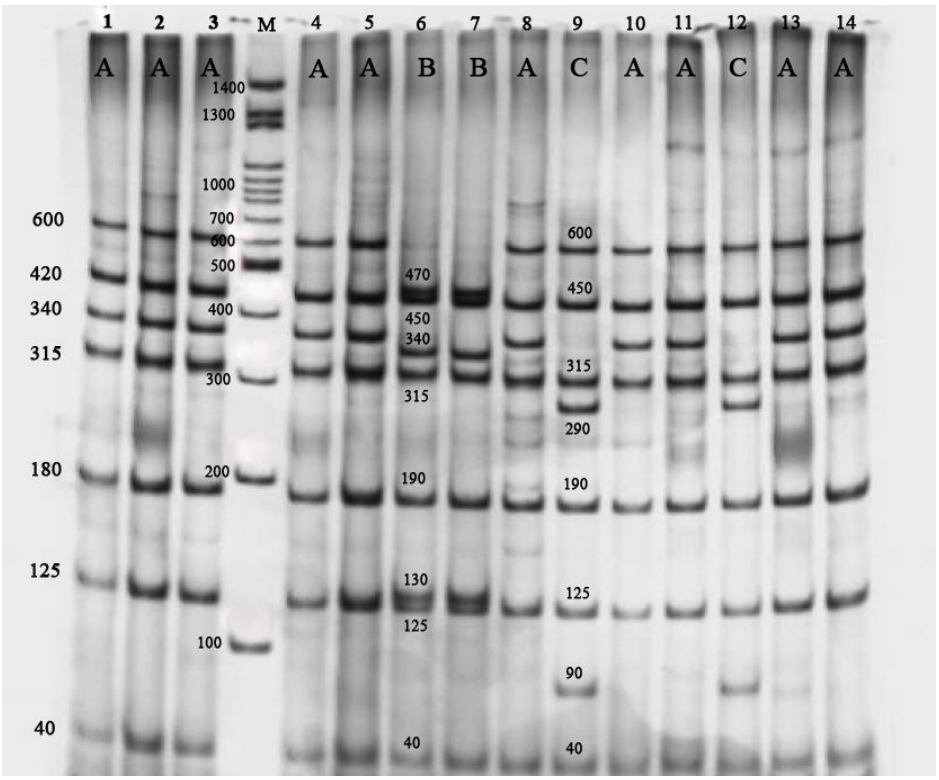

Figure 1: Restriction fragment length polymorphism pattern of PCR product of Mahisefid populations following digestion with *HaeIII* restriction enzyme in Sefid Rud

The distance matrix of interapopulation nucleotide diversity and dendogram using average linkage rescaled distance cluster combine is shown in Figure 2. The populations were clustered in two distinct clads, the first one consists of Shir Rud River and Lamir River and second clad include Tajan River and Sefid Rud River populations, although they are far apart geographically.

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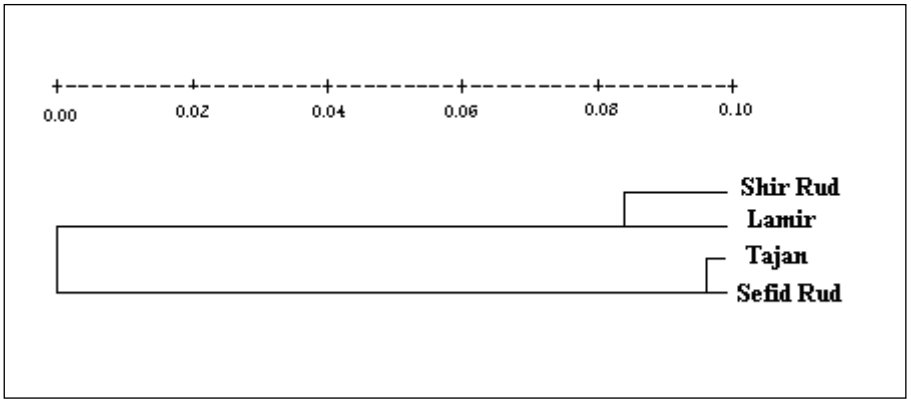

Figure 2: Dendogram using average linkage rescaled distance cluster combine

Discussion

The aim of the present study was to estimate the level of genetic divergence among Mahisefid populations using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis of mitochondrial NADH-5,6 dehydrogenase (ND-5/6) genes. mtDNA polymorphisms have been shown to be a powerful tool for the assessment of phylogeographic patterns in freshwater and anadromous fishes (Avise, 1994; Bernatchez and Wilson, 1998). One of the main advantages of using mtDNA markers is a lack of recombination, which, together with maternal inheritance, enables identification of maternal lineages and allows inferences about time divergence from a common ancestor to be calculated assuming a molecular clock. DNA of mitoconderial is specific to determine genetic diversity for aquatic animals especially for haplotye diversity and can be very useful. Some scientists used different parts of fish for sampling study (Alam and Islam, 2005; Dahle et al., 2006) used blood for study, but caudal fin for this study when don't need to kill the fish and very easy to cut. N/D 5/6 gene of mtDNA Mahisefid was amplified similar to *Asipencer stellatus* (Porkazemi, 1996). *Acipenser nudiventris* (Gasemi, 2003; Qasemi et al., 2006), *Acipense stellatus* (Shabani et al., 2005), *Common carp* (Gross et al., 2002) and catfish (Krieg et al., 2000). It had 2500 bp length that confirmed the PCR reproduced. The results of present RFLP study on N/D 5/6 gene mtDNA of Mahisefid showed low genetic diversity. The average haplotype of AAAA was 29.93%, 36.73% frequency in Lamir River, 20% in Sefid Rud River, 27 % in Shir Rud River and 39.58% in Tajan River. The average haplotype of BAAA was 27.55%, 28.57% frequency in Lamir River, 29.00% in Sefid Rud River, 27.08 % in Shir Rud River and 22.92% in Tajan River populations. It showed that there was a specific haplotype based on

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Monte-Carlo simulation with 1000 repeats, however, the four rivers had no significant difference in (P>0.05) in the haplotype frequency. Similarly, studies on Monte-Carlo 1000 simulations (P>0.05) by Qasemi et al. (2006) also showed no significant difference (P>0.05) between ship populations in the south of the Caspian Sea. Other studies on sevroga by Shabani (2005), and *Rutilus rutilus caspicus* by Rezvani Gilkholahi et al. (2006) also indicated that there were no significant differences (P>0.05) among the populations. Unique haplotypes of Mahisefid populations should be given special attention especially in terms of conservation programs for this species. Krieg et al., (2000) reported nucleotide diversity of *S.glanis* and it was between 0.00 - 0.029, Triantafyllidis et al. (1999) reported nucleotide diversity in *S.glanis* 0.17 and 0.66 in *S.aristotelis* and the low level of diversity was due to small effective population size. Krieg (1999) reported nucleotide diversity in *S.salar* that was 0.1 and in Caspian Sea Acipenseridae (Pourkazemi, 1996), *A.stellatus* (Gasemi, 2003; Shabani, 2005) and *Abramis.brama* (Khara, 2004) it was low. Ataee (2002) reported nucleotide diversity in *A. persicuse* and it was high (0.044) and four times more than ship sturgeon (Ataee, 2002). Artificial propagation and effective population size can reduce diversity (Skibinski, 1998). In this study the haplotypes of AAAA and BCBA in Sefid Rud River, ABAC, ADAA in Lamir River, BAAB in Shir Rud river and BACC in Tajan River and the haplotype showed gene flow between the sampling site and genetic diversity in different rivers, each river obtained during the past. Shabani (2005) found 18 haplotypes in *A. persicuse,* Gasemi et al. (2006) found 10 haplotypes in ship sturgeon (*A.nudiventris*) and Khara (2004) found 6 haplotypes in bream (*Abramis brama*). Daud (1995) used 5 enzymes in shrimp (*Penaeus monodon and Penaeus merguiensis*), Khara (2004) used 15 enzymes to study bream and 5 of them showed polymorphic, Gasemi et al. (2006) used 39 enzymes to study of ship sturgeon (*Acipenser nudiventris*) and 5 enzymes showed polymorphic, Shabani (2005) used 31 enzyme to study of sevrouga (*Acipenser stellatus*) and only 5 of them showed polymorphic and Gross et al., (2002) used 10 enzymes and 6 of them showed polymorphic. In the present study out of 20 enzymes only 4 restriction enzymes showed polymorphism and only 20 base pairs have survived. There are many reasons for low genetic variability in the four studied rivers, natural reproduction reduced to minimum level (even zero in some rivers), most of the Mahisefid came from artificial reproduction which causes low genetic diversity, 3-4 years are required for the first maturation and during this period adult fish such as broodstock may migrate in various regions therefore cause gene flow, there is no physical barrier between sampling sites in the sea (Abdolhay et al., 2010; Afraei Bandpei et al., 2010). In this study, haplotype (AAAA) and haplotype (BCBA) were observed in Sefid Rud River

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population, haplotype ABAC and ADAA in Lamir River population, haplotype BAAB in Shir Rud River population and haplotype BACC in Tajan River population. These haplotypes showed there is gene flow between rivers because they share some components of haplotype. Similarly, Tsipas et al. (2009) studied on common carp had detected two haplotypes in *C. carpio* populations and two haplotypes in *C. gibelio* populations with a high nucleotide divergence between the two species and two genetically distinct *C. gibelio* populations. In most hatcheries, broodstocks are obtained from various rivers, and some may not have records which cause genetic mixture and thus this subsequently may reduce genetic variability of the species. Low genetic variability may also be due to low levels of genetic survey (only 20 enzymes), thus by using more enzymes and other genes or direct sequencing, high genetic variation may be identified. Similarly, the study by Kitada et al. (2009) on red sea bream, artificial propagation and stocking had reduced genetic diversity. Although the population size of Mahisefid is high in rivers, if no attention is paid to manage them with proper selection program of broodstocks, it may affect natural populations of Mahisefid in near future.

The study suggests that there was a low genetic variability in four populations of Mahisefid in the south of Caspian Sea. The Mahisefid population can be divided into two main clusters, the first clade consists of Shir Rud River and Lamir River populations and the second clade consists of Tajan River and Sefid Rud River populations. The clustering of Mahisefid populations was not in accordance with their geographical areas or river systems.

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تنوع ژنتیکی ماهی سفید (Kamenski 1901) *Rutilus frisii kutum* در رودخانه حوزه جنوبی دریای خزر با روش PCR-RFLP

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چکیده

ماهی سفید از نظراقتصادی با ارزشترین ماهی در شمال ایران است . آنالیز mt DNA برای مطالعه تکامل بیولوژی جمعیت ماهی سفید در جنوب دریای خزر انجام شد . 294 نمونه از رودخانه‌های سفیدرود (100نمونه) ، لمیر(98نمونه) ، شیرود(48نمونه) و تجن (48نمونه) در فصل تخم‌ریزی جمع‌آوری گردید. 24 آنزیم آزمایش شد که 4 آنزیم به نام HinfI TasI, HaeIII و HincII برای این مطالعه انتخاب شد.تنوع هاپلوتایپ و نوکلوئتید ماهی سفید در چهار رودخانه مهم که در آن بچه ماهی تولید می‌شود با روش PCR-RFL-P در ناحیه ND5/6 میتوکندری DNA انجام شد. 20 هاپلوتایپ مطالعه شد که هاپلوتایپ‌های AAAA و BAAA بیشترین فراوانی را داشت . متوسط فراوانی هاپلوتایپ AAAA 29/93 درصد و متوسط فراوانی هاپلوتایپ BAAA 27/55 درصد بود. حداکثر تنوع نوکلوئید 0/94 درصد و حداقل تنوع نوکلوئید 80٪ درصد بود و متوسط آن 0/88 درصد بود. انشقاق بین لمیر و سفیدرود و شیرود 0/2 درصد بود و بین رودخانه‌های تجن و شیرود 0/1 درصد بود. متوسط فاصله تکاملی 0/15 بود و حداکثر فاصله تکاملی بین هاپلوتایپ ADAA ، AAAB ، ABAB و BDBA ، BBAA و AABB 294/ بود. متوسط تعداد باز 121/2 بود و متوسط رشته 30/30 بود.این مطالعه پیشنهاد داشت ارزش ژنتیکی بین چهارجمعیت رودخانه جنوب دریای خزر کم بود. جمعیت ماهی سفید می‌تواند به دوشاخه تقسیم شود که اولین شاخه شیرود و لمیر می‌باشد و دومین شاخه شامل تجن و سفیدرود می‌باشد که این شاخه‌بندی در جمعیت ماهی سفید بر اساس مناطق جغرافیایی در رودخانه‌ها نمی‌باشد.

واژگان کلیدی: ماهی سفید، خصوصیات ژنتیکی، میتوکندری DNA ،ایران

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