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Genetic relationships among collections of the Persian sturgeon, *Acipenser persicus*, in the south Caspian Sea detected by mitochondrial DNA–Restriction fragment length polymorphisms

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ABSTRACT

In the present study, mitochondrial DNA polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was used to assess the population structure and genetic relationships among six Persian sturgeon, *Acipenser persicus* populations from the south Caspian Sea along the Iranian coast. The complete nucleotide dehydrogenase subunit 5 (NADH 5) region of mtDNA amplified by PCR was digested with five restriction enzymes. In total, 154 individuals from six populations including: Guilan (Zone1-2), Mazandaran (Zone 3 and 5), Golestan (Zone 4) and Sefidroud River, from the south Caspian Sea along the Iranian coast were analyzed using five restriction endonucleases (*Rsa* I, *Hinf* I, *Hae*III, *Mbo* I and *Cfr13*I), yielding 17 haplotypes. Samples from Sefidroud River were clearly identified by cluster and molecular variance model (AMOVA) analyses. This collection showed dominant haplotypes that were little in populations from the other geographic areas. The mean haplotype diversity (*h*) and nucleotide diversity (π) were 0.739±0.038 and 0.0105±0.0043, respectively. Based on heterogeneity test haplotype frequencies of Persian sturgeon populations and Monte-Carlo with 1000 replicates in PCR-RFLP method significant differences were seen (χ^2 =37.12, P< 0.0001) and these results showed that haplotype distribution in different location were significant and populations of Sefidroud River were statistically significant (P< 0.0001). This result suggests that the unique genetic structure of Sefidroud River represents a highly valuable genetic resource and should now be treated as demographically independent and managed separately.

Keywords: *Persian sturgeon; Acipenser persicus; mitochondrial DNA; Caspian Sea*

INTRODUCTION

Sturgeons and paddlefishes (Acipenseridae) are highly endangered freshwater fishes. Their eggs (sold as caviar) are one of the most valuable wildlife products in international trade. Concerns of overharvesting and the conservation status of many of the 27 extant species of Acipenseriformes led to all species being included on the CITES Appendices in 1998 (McNeely *et al.,* 1990; Picitch *et al.,* 2005; Ludwig, 2006). Persian sturgeon, *Acipenser persicus* belonging to the family Acipenseridae is distributed throughout the Caspian watershed and also is most common in the Caspian Sea and in the Iranian rivers, Sefidroud and Gorganrud, and to a smaller degree in the Terek, Suli and Tamur Rivers. A small group of individuals live in the Volga, Kura, and Ural Rivers. The Persian sturgeon is concentrated in Iranian waters where sea fishing is permitted. (Vlasenko *et al.,* 1989; Birstein *et al.,* 1997). Commercial fishing of Persian sturgeon has diminished in most of its range, but it continues for certain populations and may increase due to the value of its caviar (Moghim *et al.,* 2006; Pourkazemi, 2006) but little is known about its population structure.

The Persian sturgeon in the past, entered the Sefidroud, Tajan and Gorganrud Rivers in the south and the Volga and Ural Rivers in the north , and also in small numbers the Terek, Samur and Kura Rivers in the west Caspian Sea for spawning (Putilina, 1985).

This species predominately inhabits the southern part of the Caspian Sea but does not form dense concentrations (Tavakoli *et al.,* 2010). Spawning takes place in the southern Caspian Sea rivers from April to June and again from August to September. There is a 2 month interruption in spawning in the Sefidroud River during summer when water temperatures are 26-30°C. There is a period of at least 2-4 years before this species can spawn again. Incubation takes 3-5 days. Lalouie *et al*. (1996) state that most embryos hatch 82-87 hours after fertilization, most of the yolk is absorbed 6 days after hatching and swim-up fry appears from day 7 to 8 at 19-21°C. The timing of passage of fingerlings into the Sea after a hatchery release into Tajan River was found to be 12-72 hours after release (Lalouie *et al.* 1996).

Understanding genetic and ecological relationships among populations is important for effective management of natural systems and development of appropriate conservation strategies for declining species (Caughley and Gunn, 1996). In the past management strategies for threatened species have focused primarily on protecting declining populations and on maintaining areas of natural habitat in an attempt to alleviate decreases in population numbers due to demographic and/or environmental stochasticity (Frankham *et al.,* 2002). At this time it was believed that demographic and environmental factors were likely to have a greater influence on extinction probability of natural populations before genetic deterioration imposed a serious threat (Lande, 1988). Consequently, over the last decades substantial efforts have been directed towards conserving the genetic diversity of species and using genetic data to make more informed decisions about how threatened species should be managed (McNeely *et al.,* 1990; Birstein *et al*., 1997)

The application of mtDNA as a genetic marker has become widespread for population genetic studies of sturgeon fishes (Pourkazemi, 1996; Pourkazemi *et al.,* 1999; Rezvani, 2000; Ludwig *et al.,* 2000;; Waldman *et al.,* 2002; Wirgin *et al.,* 2005; Grunwald *et al.*, 2008; Mugue *et al.,* 2008; Wirgin *et al.,* 2009). In most species mitochondrial DNA (mtDNA) is highly variable and is therefore a good marker for detecting possible genetic differentiation. Additionally, mtDNA supplies information which could not have been obtained using only nuclear markers such as allozymes or mini-/microsatellites, due to mtDNA being haploid and maternally inherited. This provides the means of distinguishing between male- and female-mediated gene flow, and, since the effective size of mtDNA is only one-quarter that of nuclear genes (Brown, 2008) and therefore more subject to drift is a good marker for detection of genetic bottlenecks (Avise, 1994). With the development of PCR, RFLP analysis of PCR-amplified segments of the mtDNA has become a common method for population genetic studies (Moritz *et al.,* 1987; Billington, 2003). PCR-RFLP was first introduced for sturgeon genetic stock structure in the Caspian Sea (Pourkazemi, 1996) and also for sturgeon species identification by Ludwig and Kirschbaum (1998).

Several genetic studies have attempted to characterize the genetic variation and population structure of the Persian sturgeon in the Caspian Sea. Rezvani-Gilkolaii (1997) initially investigated the genetic diversity of two wild populations of Persian sturgeon from the western and eastern part of the Caspian Sea using partial sequence analysis of mtDNA *NADH* 5 gene but this study has suffered from limited geographic sampling. In continuance, Ataei *et al*. (2004) reported high genetic variability among three populations of Persian sturgeon from the south Caspian Sea using PCR-RFLP technique. Both Rezvani-Gilkolaii (1997) and Ataei *et al*. (2004) in their studies found its genetic diversity was considerable in its sampled area of distribution, western and eastern part of the Caspian Sea. Furthermore, evidence of significant genetic differentiation in microsatellite allelic frequencies among Persian sturgeon populations suggests they are reproductively isolated and three distinct populations including Sefidroud River, the middle and north Caspian Sea populations were determined (Khoshkholgh, 2007, Pourkazmei, 2008). In the present study, various parts of the Persian sturgeon routes were sampled and restriction fragment length polymorphism analysis of mitochondrial DNA were employed to assess the genetic structure and patterns of

variation within sample localities to further characterize populations of Persian sturgeon, *Acipenser persicus* from south Caspian Sea along the Iranian coast.

MATERIALS AND METHODS

Sample Collection

Fin clip samples of the Persian sturgeon were obtained from 23 sites along the Iranian coast in the south Caspian Sea: Astara-Anzali (Zone1), Kiashahr-Chaboksar (Zone 2), Noshahr-Sari (Zone 3), Miankaleh-Bandare Torkaman (Zone 4) and Chaboksar-Noshahr (Zone 5) and Sefidroud River. The samples were obtained only in several sites in each zone.

Table 1. Persian sturgeon samples collected for population genetic analysis. Sampling localities (1-23) are from the south Caspian Sea along the Iranian coast

Region	Map no.	Sampling site	Sample size
Zone 1, Guilan	1	Astara	8
	2	Lemir	3
	3	Hevigh	5
	4	Kapour-Chal	6
	5	Golshan	4
	6	Jefroud	5
Sefidroud River, Guilan	7	Sefidroud	27
Zone 2, Guilan	8	Yousef-Abad	10
	9	Sefidroud	3
	10	Ghasem-Abad	8
Zone 5, Mazandaran	11	Iezdeh	8
	12	Nevisi	4
	13	Miroud	5
	14	Khazar-Abad	3
	15	Amir-Abad	5
	16	Shiroud	8
Zone 3, Mazandaran	17	Nashtaroud	4
	18	Noursar	5
	19	Chalous	4
	20	Hasan-Abad	6
Zone 4, Golestan	21	Turkeman &Faridpak	7
	22	Mian-Ghaleh	9
	23	Khajeh-Nafas	7
Total			154

Fig. 1. The locations of the Persian sturgeon sampling sites. Sampling localities (1-23) from south Caspian Sea along the Iranian coast (Fisheries Zone 1- 5).

All the sampled individuals were mature and in spawning condition. The location of the sampling areas is shown in Fig. 1. Between 21 and 31 individuals were

collected and analyzed from each locality (Table 1). All the specimens were obtained by the stock assessment department of the International Sturgeon Research Institute. For mtDNA analysis, *c.* 3–5 g of fin tissue was stored in ethanol (96%) at room temperature in the field and at -20°C in the laboratory.

MtDNA amplification and RFLP analysis

Mitochondrial DNA variation was analyzed by RFLPs, performed on PCR-amplified segments that contained mtDNA ND5 gene. DNA was extracted from each specimen according to Hillis and Moritz (1990) with some modifications (Pourkazemi, 1996). Persian sturgeon-specific primer 5′-CCAAGTAGAAGCTATGCATTCA-3′ (forward) and 5′-GGAGGCGAATATTT GTTGA-3′ were designed to amplify mtDNA ND5 gene.

Double strand DNA amplifications were performed in 50 µL volumes, containing; 5 µl of 10× buffer (100 mM Tris-HCl, pH 8.3, 15 mM MgCl2, 500 mM KCl); 2 µl of each primer (100 pmol); 5 µl of a 2.5 mM solution of each deoxyribonucleoside triphosphate (dNTP); 2.5 units of *Taq* DNA polymerase (Vio Taq^{Tm} VT1001, Fermentase). Enough ultrapure water was added to each sample to make a solution of 50 µl. PCR amplification conditions were as follows: 35 cycles of strand denaturation at 94° C for 1 min, annealing at 58° C for 1 min and extension at 72° C for 1 min (5 min for the last extension only). The amplified segments from each specimen were subsequently screened for polymorphism with the following 25 restriction endonucleases: *Acc*I, *Ava*II, *Bsp1407*I, *Cfr*131 (*Asu*I), *Dra*I (*Aha*III), *Eco*RI, *Hae*III, *Hinc*II, *Hind*III, *Hinf*I, *Hin*6I (*Hha*I), *Mbo*I (*Sau3*AI), *Mbo*II, *Nco*I, *Nde*I, *Nsp*I (*Xce*I), *Pvu*II , *Rsa*I , *Sma*I , *Taq*I, *Tas*I (*Tsp*EI), *TaiI* (*Mae*II), *Vsp*I ,*Xba*I, *Xho*I. The digested samples were separated electrophoretically on 6% poly-acrylamide gels.

Statistical analysis

To each different fragment pattern produced by a restriction endonuclease a specific letter identified each distinct single endonuclease pattern. Thus, each composite mtDNA haplotype was designated by a multi-letter code. MtDNA restriction sites were inferred from restriction fragment patterns, considering each enzyme separately. A presence/absence restriction site matrix was constructed for each composite haplotype. Based on the high amount of polymorphism initially found in the 1837bp mtDNA ND5 gene, five restriction endonucleases (*Rsa* I, *Hinf* I, *Hae*III, *Mbo* I and *Cfr13*I) were chosen in the present study for extensive screening. These restriction enzymes were among those revealing the highest polymorphism.

A composite mtDNA haplotype, consisting of five letters that represent the fragment pattern generated by each of the restriction endonucleases, was compiled for each individual. The nucleotide diversity (π) in a population was computed by: $\pi = 2\sum d_{ij}[n(n\text{-}1)]^{-1}$ (Nei &Tajima, 1981), where d_{ij} is an estimate of the number of nucleotide substitutions per site between DNA sequences i and j, n is the number DNA sequences and also haplotype diversity (h) was calculated in each population. The equation of Nei &Tajima (1981) was used to estimate haplotype diversity: $h = 2n\,(1\text{-}\sum x_i^2)(2n\text{-}1)^{-1}$, where n is the sample size, and x_i is the frequency of the haplotype in each population.

The main genetic variation indices were estimated using the REAP (McElroy *et al*., 1992) and ARLEQUIN version 2.0 (Schneider *et al*., 2000) software packages. Heterogeneity of haplotype frequencies between each sample pair was evaluated using the Monte Carlo method (1000 pseudorandom replicates (Roff and Bentzen, 1989) and F_{ST} statistics (Weir and Cockerham, 1984). The estimates of nucleotide divergence (Nei &Tajima, 1981) between the mtDNA haplotypes and the samples examined were taken as standard genetic distances. The estimates were used for phylogenetic analysis performed with an unweighted pair group method with arithmetic mean (UPGMA) algorithm. Clustering robustness was estimated using bootstrap (100 iterations) (Felsenstein, 1985). Estimates of nucleotide divergence and dendrogram topology were made using the PAUP version 4.0b10 software package (Swofford, 2002). Quantitative estimates of the geographic subdivision of mtDNA variation were performed using the AMOVA method, where molecular variance was partitioned into three hierarchical levels, including the between-groups, between-population within groups, and among haplotype within population

components (Excoffier *et al.,* 1992). To test statistical significance of the hierarchic components of variance, the corresponding F-statistics criteria were calculated (Weir and Cockerham, 1984).

RESULTS

The amplified segment of the Persian sturgeon mtDNA ND5 gene had an approximate length of 1837 base pair. RFLP analysis with five enzymes revealed a total of 17 restriction patterns. Three to four restriction patterns were detected using each restriction enzyme (3, 3, 3, 3, and 4 for *Rsa*I, *Hinf* I, *Hae*III, *Mbo* I and *Cfr13*I, respectively; Table 2). Table 2 shows the restriction band sizes of Persian sturgeon mtDNA ND5 gene digested with the five restriction enzymes. For example, the restriction enzyme *Rsa*I showed three different genotypes, A, B and C. The genotype A had two different bands with a size range from 1008 to 829 bp and the genotypes B and C also showed three different bands (Table 2). The fragment sizes do not sum perfectly to 1837 bp because of limiting resolution power of the gels and thus it was possible to analyze the data according to presence or absence of restriction sites rather than bands.

Table 2. Fragment patterns and size estimates (in base pairs) from the PCR–RFLP analysis of Persian sturgeon *A. persicus* mtDNA ND5 gene using five restriction endonucleases.

Rsa I			*Hinf* I			*Hae*III			*Mbo* I			*Cfr*13I			
A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*D*
1008	1008	1008	612	514	592	950	855	814	744	645	706	767	767	1200	1837
829	726	551	516	450	395	336	525	546	701	556	559	748	546	300	
	100	272	362	362	362	308	308	308	347	347	347	205	205	205	
			348	311	328	150	139	169	48	279	234	132	164	137	
				200	170	93							151		

A total of seventeen composite haplotypes were observed in the 154 specimens of natural population of Persian sturgeon from the south Caspian Sea. The composite haplotypes were created by combination of individual enzyme genotypes (Table 3). The most common haplotype AAAAA was shared between six geographic regions and was found in 46.7% of the Persian sturgeon samples. Frequent of this haplotype ranged from 0.35 in the Sefidroud River to 0.88 in Zone 1 (Astara-Anzali). The other common haplotype (AAABA) was also observed in all populations; these two common haplotypes were observed in 57.7% of all the samples. The haplotype AACAB predominated in Sefidroud River (0.58). Two haplotypes, BAACA and BACCB were observed only in the specimens from Zone 4, albeit at low frequencies and also the rare haplotype ABBCA observed only in collections from Zone 3.

Table 3. Geographic distribution of composite mtDNA haplotypes among *Acipenser persicus* samples. Letters denote mtDNA fragment patterns produced by digestion of PCR of PCR products with the following polymorphic restriction endonucleases: *Rsa* I, *Hinf* I, *Hae*III, *Mbo* I and *Cfr13*I

No.	Haplotypes	Collection						Total
		Zon1 (31)	**Zon2** (21)	**Zon3** (25)	**Zon4** (23)	**Zon5** (27)	**Sefidroud** (27)	154
1	AAAAA	15	12	14	11	14	6	72
2	AAABA	5	3	2	2	4	1	17
3	AAAAB	4	2	1	1	3	-	11
4	AACAB	1	1	-	2	1	10	15
5	AABBB	-	1	2	-	2	-	5
6	AABAB	3	-	3	-	2	1	9
7	BAACA	-	-	-	2	-	-	2
8	ABBAA	2	2	-	1	-	-	5
9	AACAA	-	-	-	-	-	2	2
10	ABAAB	-	-	1	1	-	1	3
11	AACBA	-	-	-	-	-	1	1
12	ABCAA	-	-	-	-	-	2	2
13	BAAAA	1	-	1	2	1	-	5
14	BBBAB	-	-	-	-	-	1	1
15	BACCB	-	-	-	1	-	-	1
16	ABBCA	-	-	1	-	-	1	2

17	BACAB	-	-	-	-	1	1

The mean Haplotype and nucleotide diversity averaged 0.739 and 0.0059, respectively. Higher diversity values were found at Sefidroud River and Zone 4. The haplotype number per sample size calculated for two population Zone 2 and Zone 3 showed nearly similar results (Table 4).

The estimated F_{ST} values for the 15 pairs of 6 sampling localities ranged from 0.0010 between Zone1 and Zone 2 to 0.6136 between Zone 2 and Sefidroud River (Table 5).The five pairs formed with the site in Sefidroud river had higher F_{ST} values than the other pairs, ranging from 0.3231 for the combination Sefidroud River –Zone 5 to 0.6136 for Sefidroud River –Zone 2 and were all significant (P< 0.0001). The one pair formed by the combination of sites in Zone 4 with those in the Zone 1 (0.0264) also had marginally significant F_{ST} values (P = 0.0451). In contrast, values for remaining pairs generally low (0.0010–0.0264) and all of combinations were not significant except for Zone 4 - Zone 1(Table 5).

Table 4. Haplotype and nucleotide diversity within six locations Of Persian sturgeon

Sample localities	Haplotype diversity (h ± SE)	Nucleotide diversity (π ± SE)
n1 (Astara-Anzali)	0.683±0.027	0.0034±0.0024
n2 (Kiashahr-Chaboksar)	0.716±0.037	0.0048±0.0024
n3 (Noshahr-Sari)	0.721±0.028	0.0059±0.0046
n4 (Chaboksar-Noshahr)	0.764±0.031	0.0088±0.0064
n5 (Miankaleh-Bandare Torkaman)	0.737±0.054	0.0061±0.0038
idroud River	0.814±0.056	0.0153±0.0064
tal	0.739±0.038	0.0105±0.0043

Table 5. Pairwise F_{ST} values are illustrated above the diagonal. Abbreviations for collections are defined in Fig. 1. Pairwise estimates of mtDNA divergence (% of nucleotide substitutions) are illustrated below the diagonal

Location	Zon1	Zon2	Zon3	Zon4	Zon5	Sefidroud
Zon1	-	0.0010	0.0096	0.0264	0.0081	0.6031
Zon2	0.00214	-	0.0013	0.0097	0.0040	0.6136
Zon3	0.00201	0.00149	-	0.0025	0.0041	0.4802
Zon4	0.00215	0.00318	0.00264	-	0.0065	0.4307
Zon5	0.00178	0.00133	0.00441	0.00378	-	0.3231
Sefidroud	0.01456	0.01344	0.01272	0.01176	0.01109	-

The nucleotide sequences divergence values between pairs of populations ranged 0.00133–0.01456 (Table 5, below diagonal). The divergence matrix of the Persian sturgeon populations based on nucleotide sequences is presented in Table 5. The maximum difference (about 0.014% of nucleotide substitutions) was recorded between the Sefidroud River samples and the Zone 1 samples. A high divergence in the UPGMA tree generated from the pairwise population genetic distances was indicated between the Sefidroud River populations and those of the other locations from the south Caspian Sea (Fig. 2).

Table 6 presents the results of the heterogeneity test (χ^2) of the Persian sturgeon populations to estimate the genetic differentiation of the populations examined. Significant differences were found in 9 of the 15 pairwise comparisons, most involving the Sefidroud River samples versus those of the other locations. The χ^2 test of haplotype frequencies also revealed that the population of Sefidroud River is significantly differentiated from the others (P< 0.0001). The significances of differences between all population pairs are given in Table 6. As shown in Table 6, no significant difference was found between most of the pairs compared. It was shown that the main contribution to the heterogeneity of the population set is only made by the Persian sturgeon samples from the Sefidroud River.

Table 6. Pairwise comparison heterogeneity test (χ^2) in the samples of Persian sturgeon from 10 Caspian Sea locations (*P*-values in parentheses).

cation	Zon1	Zon2	Zon3	Zon4	Zon5	efidroud
n1	-					
n2	1.32 (0.5504)	-				
n3	7.56 (0.4456)	8.32 (0.4124)	-			
n4	24.53 (0.0451)	18.74 (0.0948)	15.17 (0.1563)	-		
n5	14.89 (0.1982)	1.02 (0.5689)	20.00 0.0805	18.63 (0.0927)	-	
idroud	106.8 (0.0000)	94.5 (0.0000)	89.62 (0.0000)	77.14 (0.0000)	58.65 (0.0000)	-

The hierarchical analysis of genetic variation based on an AMOVA indicated that 65.47% of the total molecular variance occurred among haplotypes within localities (Table 7). A significant portion (31.18%) was attributable to differences between localities (P<0.0001). All pairwise comparisons of population differentiation by Sefidroud River were statistically significant (Table 6). Geographic heterogeneity analysis revealed significant differences in the distribution of Persian sturgeon composite haplotypes over locations (χ^2 =37.12, P<0.0001) indicating that population structure does exist in the range of the Persian sturgeon populations sampled. The dendrogram derived from the matrix (Table 6) is shown in Fig. 2.

Table 7. Hierarchic search for haplotype differences in the Persian sturgeon.

Source of variation	df	Percentage of variation	Fixation indices	*P*
Among groups	1	31.18	F_{SC} =0.0112	<0.001
Among populations Within groups	4	3.35	F_{CT} =0.0009	>0.167
Among haplotype Within population	148	65.47	F_{ST} =0.0267	<0.001
Total	153			

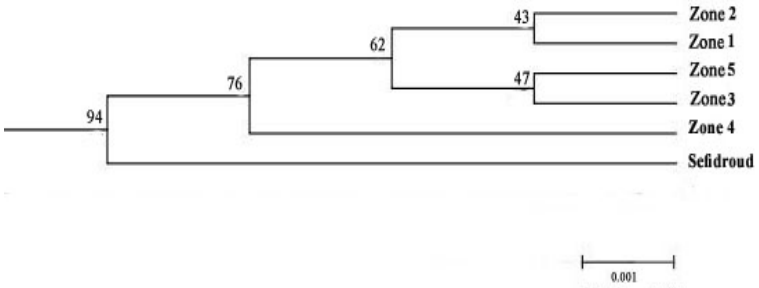

Fig. 2: UPGMA dendrogram of the population genetic distances for the mtDNA ND5 data from Persian sturgeon of 6 regions in the south Caspian Sea. Bootstrap values are given at each node.

Figure 2 shows the cluster analysis based on nucleotide divergence values between populations (Table 3, below diagonal).This analysis clearly identified some clusters that show approximate correspondence with the main geographic regions. The first cluster included populations with minimum level of divergence, those from Zone 2 and the samples from Zone 1 in the south Caspian Sea, whereas the second one comprised the Zone 3 and Zone 5. The most differentiated cluster included specimens of the Sefidroud River. This structure was

confirmed by AMOVA since a lower part of the variation in haplotype frequencies among the 6 populations was accounted for by the variation between the geographic groups (31.18% of variance, $P < 0.001$, Table 7). The remaining sources of variation in haplotype frequency were those between individuals (65.47%, $P< 0.001$, Table 7), and between populations within each of the geographic groups (3.35%, $P = 0.167$).

DISCUSSION

In this study, intraspecific population structure was demonstrated between Persian sturgeon localities using RFLP in mtDNA ND5 gene. The number of haplotypes revealed by RFLP in mtDNA ND5 gene clearly shows a high level of polymorphism in this region of mitochondrial DNA. In our study, we found nearly similar number of haplotypes (17), using five restriction enzymes, than Ataei et al. (2004), who found 18 haplotypes in Persian sturgeon in a fragment of the mtDNA control region using four enzymes. The difference in haplotype frequencies between Sefidroud River and the other collections of the Persian sturgeon is marked and significant. This result contrasts with the study by using RFLP analysis of mtDNA control region (Ataei *et al.,* 2004) in which no significant differentiation in haplotype frequencies was observed between populations from the different regions in the south Caspian Sea. This finding agrees well with previous findings based on microsatellites (Khoshkholgh, 2007; Pourkazemi, 2008). A possible explanation for the contrasting results between mtDNA control region and mtDNA ND5 gene could be the differential effects of natural selection on different mtDNA segments (Avise, 1994). In addition, several differences have been reported among sturgeon species that inhabit the Caspian Sea by researchers using the nucleotide variation of mtDNA ND5/6 segments. This segment is also considered useful for the establishment of population analysis among sturgeon species (Rezvani-Gilkolaei, 2000; Shabani *et al.,* 2003; Qasemi *et al.,* 2004)

We observed high haplotype diversity in the Persian sturgeon. Although different methods and mtDNA regions had been used to analyze this species, diversity values were similar (Rezvani-Gilkolaei, 1997; Ataei *et al.,* 2004). The high diversity found in these populations may be associated with an abundant and stable population that exists along the Iranian coast (Tavakoli *et al.,* 2010). High levels of haplotype diversity have been reported in other sturgeon in the Caspian Sea. Pourkazemi (1996) found a high value (0.913) for the ND5/6 gene region for the average haplotype diversity of stellate sturgeon collections from four geographic regions in the south Caspian Sea, but Shabani et al. (2004) reported lower value for the same gene between Volga and Gorganroud ivers (0.736). Similarly, Rezvani-Gilkolaii (2000) found high levels of haplotype diversity (0.964) for the ND5/6 gene region between Russian sturgeon populations in southwest and southeast areas of the south Caspian Sea. The level of haplotype diversity between collections reported in this study is also in agreement with an earlier study of Persian sturgeon (Ataei *et al.,* 2004).

RFLP analysis of Persian sturgeon mtDNA ND5 gene, had revealed haplotype frequency differences among collections in the southwest and southeast regions of the Caspian Sea to be non-significant ($P >0.05$). This lack of a geographic effect is in full agreement with previous results based on mitochondrial RFLP variation of no evidence for population substructure within southeast and southwest (Ataei *et al.,* 2004). Having sampled more extensively and used a more informative and genealogical neutral genetic marker the present study establishes this conclusion firmly. Our results are probably a consequence of genetic flow existing within the species in the southwest and southeast part of the Caspian Sea, resulting in a single panmictic population. Juvenile dispersion is not as clearly understood as in this species, but movements of Persian sturgeon adults might be the main form of genetic interchange. Sturgeons often migrate over great distances in search of food, suitable spawning habitats or simply to avoid seasonally unfavorable conditions (Bemis and Kynard 1997). Although adult Persian sturgeons are observed in non-spawning

habitats (Moghim, 2006), several studies suggest that they prefer depths of 20 to 40 m during summer but will readily move to much deeper water in cooler months (Putilina and Artyukhin. 1985; Ivanov *et al*., 1999). However low level of genetic differentiation in this species from fisheries zones 1-5 could be associated with high migration rates of this fish

The largest portion of the genetic diversity revealed in this study is distributed among Sefidroud River populations. To maintain the total evolutionary potential of this endangered species, many of these populations must be preserved. However, as it may not be possible to conserve all populations, particular attention for genetic conservation programs should be focused on the populations showing the highest levels of haplotypic diversity. Given that mtDNA variants are neutral, mtDNA polymorphisms reveal nothing directly about adaptive changes in evolution (Moritz, 1994). However, mtDNA analysis is clearly useful and informative for the analysis of genetic diversity and population structure.

In summary, this study confirms the genetic differentiation of Persian sturgeon populations in the south Caspian Sea previously observed with microsatellites. It also shows that mtDNA ND5 gene of this species is a potential genetic marker for use in aquaculture genetics (*i.e.* maintaining stock diversity).To fully understand population genetic structure, future efforts should analyze samples from the distribution limits of the species, especially, to compare specimens from the southern, middle (Azerbaijan and Turkmenistan) and northern (Russian and Kazakhstan) parts of the Caspian Sea.

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بررسی روابط ژنتیکی بین نمونه های تاس ماهی ایرانی *Acipenser persicus* مناطق جنوب دریای خزر با استفاده از تکنیک هضم آنزیمی (PCR-RFLP)

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چکیده

در مطالعه حاضر، به منظور تعیین ساختار ژنتیک جمعیت تاس ماهی ایرانی (*Acipenser persicus*) دریای خزر روش هضم آنزیمی (PCR-RFLP) ژن ND5 در DNA میتوکندریایی مورد استفاده قرار گرفت. در مجموع تعداد ۱۵۴ نمونه باله تاس ماهی ایرانی از ۶ منطقه مختلف سواحل جنوبی دریای خزر جمع آوری گردید. کمیت DNAنمونه ها به روش اسپکتروفتومتری و کیفیت آن از طریق الکتروفورز ژل آگارز و رنگ آمیزی با اتیدیوم بروماید تعیین شد. هضم آنزیمی ژن ND5 با استفاده از ۵ آنزیم برشگر پلی مورفیک شامل *Mbo* I، *Cfr*13I، *Hae*III، *Hinf* I و *Rsa* I انجام گرفت و الگوی هضم آنزیمی با استفاده از الکتروفورز ژل پلی اکریل آمید ۶٪ و رنگ آمیزی نیترات نقره مشاهده شد. در مجموع بین ۱۵۴ نمونه مورد مطالعه ۱۷ هاپلوتیپ متفاوت بدست آمد. تنوع هاپلوتایپی و نوکلئوتیدی به ترتیب ۰/۰۳۸ ± ۰/۷۳۹ و ۰/۰۰۴۳ ± ۰/۰۱۰۵ بدست آمد. آنالیز واریانس مولکولی (AMOVA) نشان داد در رودخانه سفیدرود از سواحل جنوبی دریای خزر اختلاف معنی داری مشاهده گردید ($P < ۰/۰۰۱$) و در نهایت وجود یک جمعیت مشخص گردید و نشان داده شد که توزیع هاپلوتیپ‌ها در بین مناطق مختلف نمونه‌برداری اختلاف معنی داری دارد ($\chi^2= 37.12$, $P < 0.0001$). با توجه به نتایج روش به کار گرفته شده مشخص گردید که در دریای خزر جمعیت های مستقلی از تاسماهی ایرانی بوده بطوریکه نمونه های رودخانه سفیدرود جمعیت مستقلی از این گونه را تشکیل داده و از نظر ژنتیکی منبع بسیار ارزشمندی محسوب می شوند و بایستی به صورت جداگانه مدیریت شوند.

*مولف مسئول