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Genetic analysis of pike-perch, *Sander lucioperca* L., populations revealed by microsatellite DNA markers in Iran

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Abstract

This study was conducted in order to investigate genetic diversity and population structure of pike-perch in the Northern part of Iran. For this purpose, 207 adult pike-perches from four regions of the Caspian Sea watershed (Talesh Coasts, Anzali Wetland, Chaboksar Coasts and Aras Dam) were collected. DNA was extracted and by using 15 pairs of microsatellite primers, Polymerase Chain Reaction (PCR) was conducted. DNA bands were analyzed using Biocapt and GenAlex 6 software package. Out of 15 microsatellite primers, 11 loci were produced, of those, 6 loci were polymorphic and 5 were monomorphic. Analysis revealed that the average number of alleles per locus and observed heterozygosities were not statistically significant (P>0.05) for all four populations. Data indicated an appreciable genetic differentiation, in spite of a low genetic variation, and agreed with the low level of genetic polymorphism already observed for this species in Iran. Deviation from Hardy-Weinberg equilibrium was obvious in most cases, mostly due to the deficiency of heterozygosities. The highest genetic distance was between Anzali Wetland and Aras Dam populations. This investigation represents the first approach to the knowledge of the genetic variability of Iranian populations using microsatellite markers, and reported results could be of interest for future management and conservation programs of this species in Iran.

Key Words: Pike-perch, *Sander lucioperca*, genetic analysis, microsatellite.

INTRODUCTION

The pike-perch, *Sander lucioperca* (Linnaeus, 1785), is a semi anadromous, cool-water percid fish that inhabits both fresh and brackish waters, commonly in estuaries and coastal zones (Koad *et al.*, 2000; Lappalainen *et al.*, 2003). This species seems to prefer salinities lower than 12gL^{-1}. As a predator and commercially valuable species, *S. lucioperca* constitute an important component of the Caspian ichthyofauna, both ecologically and commercially (Abdolmalaki & Psuty, 2007). This species is widely distributed in the Caspian watershed from north to south and west to east including the Ural, Volga, Kura, Sefid Rud Rivers as well as Anzali International Wetland (Berg, 1949; Craig, 2000). It has been suggested that *S. lucioperca* undergoes short migration distances of approximately 15–30 km that may result in the formation of stock units based on different environmental condition (Lappalainen *et al.*, 2003).

The first record for pike-perch catching is about late of 1920 s which indicate that pike-perch catch constituted about one-third of the total taken from the costal zone of the Southern Caspian Sea, some 3000-

4000 tons annually (Razavi, 1999). Shortly thereafter, catches decreased suddenly to some 30 tons annually, and they have never again reached the initial level. The cause of the sudden disappearance of the pike-perch stock from the Southern Caspian Sea has not been determined conclusively but its occurrence is assumed because of the excessive catching that led to overfishing and degradation of spawning grounds and habitats (Kiabi *et al.,* 1999). Historically, the most important spawning area for pike-perch in Iran was Anzali Wetland. Although the precise cause is not known, natural pikeperch spawning in Anzali Wetland stopped completely, and it has not been recorded in last decade. Bearing in mind the size of historical catches of the species, and assuming that the carrying capacity had not changed, the Iranian fisheries Administration in 1990 initiated a pike-perch stock-enhancement program. Since then, Anzali Wetland had been stocked systematically with fingerlings collected from spawners held at Aras Dam, a border reservoir that lies between Iran and Azerbaijan Republic (Abdolmalaki & Ghaninezhad, 1999). So, regarding the fact that this species is being reared in Aras Dam and released into the Caspian Sea and Anzali Wetland for restocking, regular monitoring of genetic variability among the progenies is essential to avoid the loss of current polymorphism due to inbreeding and outbreeding problems.

Microsatellites are hyper variable, codominant nuclear DNA markers in which variation is partitioned in one to five base pair repeat motifs (Zajc *et al.,* 1997; Goldstein & Schlotterer, 1999). In recent years, microsatellite markers as a reliable method, have been applied for many population as well as phylogenetic studies, this is because microsatellites typically have a higher level polymorphism than traditional nuclear loci. In addition, DNA for microsatellites analysis can be easily extracted from tissues obtained by non-lethal sampling (fins, hairs, faces), which is essential when working with threatened or endangered species (McQuown *et al.,* 2003). It has also proved that microsatellite genotyping is a powerful tool for accurate genetic assessment and for sustainable use of wild resources (Barroso *et al.,* 2005; Li *et al.,* 2006; Memis & Kohlmann, 2006).

Several studies have been carried out on the population genetics of Percids species: On *Perca flavesens* (Li *et al.,* 2007; Leclerc *et al.,* 2000; Kapuscinski & Miller, 2000); *Stizostedion vitreum* (Wirth *et al.,* 1999; Zipfel, 2006) & *S. lucioperca* (Bjorklund *et al.,* 2007; Kohlmann & Kersten, 2008; Poulet *et al.,* 2009).

Despite the commercial and conversation importance of this species, information on genetic relationship and diversities of *S. lucioperca* at the molecular level in the Caspian Sea basin is scarce. The only study on morphological variation of the pike-perch *S. lucioperca* in the southern Caspian Sea, using a truss system indicated that the morphometric truss network system was more adequate than meristic characters for separation among the populations (Akbarzadeh et al., 2009).

The development of management plants and implementation of actions to restore pikeperch within its native stocks can be useful from an understanding of the genetic diversity of its populations. This information is helpful in choosing donor populations to use as source of reintroduction and in formulating restoration goals regarding population structure. In general, the role of substructures in populations and their importance in preserving genetic variation is uncertain (Stephenson, 1999), but if there exists some form of substructure in the studied population, this has to be considered in management planning in a way that the risk of losing different population components is minimal. Therefore in this study, the population structure of pike-perch among the major

fishing and spawning grounds in Iran was investigated. The main objectives of this study were to analysis the population genetic structure and genetic diversity among and between populations of *S. lucioperca* based on microsatellite markers. The value of the Caspian Sea as a unique environment has been widely recognized and preserving the genetic diversity of the fish populations should be one of the main priorities at all levels of decision making. Detailed knowledge of the genetic structure of the exploited fish populations is a prerequisite for their successful long-term management. The aim of this study was to provide better tools for the evaluation of different fisheries management alternatives in coastal areas by describing microsatellite DNA variation in Iranian pikeperch populations and to assess the level of genetic diversity and especially the potential differentiation between coastal and freshwater populations. The genetic structure devised from neutral marker variation indicates the general level of isolation and the time that has been available for adaptive evolution.

MATERIALS AND METHODS

Samples Collection

Totally 207 samples of adult *S. lucioperca* were caught from four regions including 57 samples from Aras Dam, 50 samples from the Talesh Coasts, 50 samples from Anzali Wetland and 50 samples from the Chaboksar Coasts in 2007-2008 from the Southwest Caspian Sea watershed in Iran (Fig. 1). For each sample, 2-3 g dorsal fin tissues was collected and conserved in absolute alcohol for subsequent DNA extraction and amplification.

DNA Extraction

Total genomic DNA was extracted from fin tissue using proteinase-K digestion, phenol: chloroform; isoamylalcohol precipitation as described by Pourkazemi *et al,* (1999). The quality and concentration of DNA were assessed using agarose gel electrophoresis and spectrophotometry (model NanoDrop, ND-1000). Finally DNA was stored at -20°C until use.

PCR Profiles and Primer Sequences

Nuclear DNA was amplified using 15 microsatellites primers (YP13, YP17, YP41, YP60, YP68, YP78, YP110, YP111, YP113, P*fla* L1, P*fla* L2, P*fla* L3, P*fla* L8, P*fla* L9, P*fla* L10) designed for *Perca flavescens* (Leclerc *et al.,* 2000; Li *et al.,* 2007, Table 1). The polymerase chain reaction (PCR) conditions, especially annealing temperatures, were optimized for the microsatellite loci as necessary to produce scorable amplification products. Polymerase chain reaction was performed in a 20 µL reaction volume containing 100-150 ng of template DNA, 10 pmol of each primer, 200µM each of the dNTPs, 1U of *Taq* DNA polymerase (Cinnagen, Tehran, Iran), 1.5 mM $MgCl_2$ and 1x PCR buffer. The temperature profile consisted of 2-min initial denaturation at 94°C, followed by 35 Cycles of 30s denaturation at 94°C, 30s annealing at a locus-specific temperature (Table 2), 30 s extension at 72°C and a final 5-min extension at 72°C for the first 9 microsatellite primers (YP13, YP17, YP41, YP60, YP68, YP78, YP110,YP111 & YP113). For the rest microsatellite primers (P*fla* L1, P*fla* L2, P*fla* L3, P*fla* L8 & P*fla* L9, P*fla* L10) the temperature profile consisted of 3- min initial denaturation at 96°C, 30s annealing at a locus-specific temperature (Table 2), 1 min extension at 72°C and a final 5-min extension at 72°C. Polymerase chain reaction products were separated on 6% polyacrylamide gels and stained by silver nitrate. Alleles were sized using BioCapt software and each gel contained an allelic ladder (100 bp) to assist in consistent scoring of alleles.

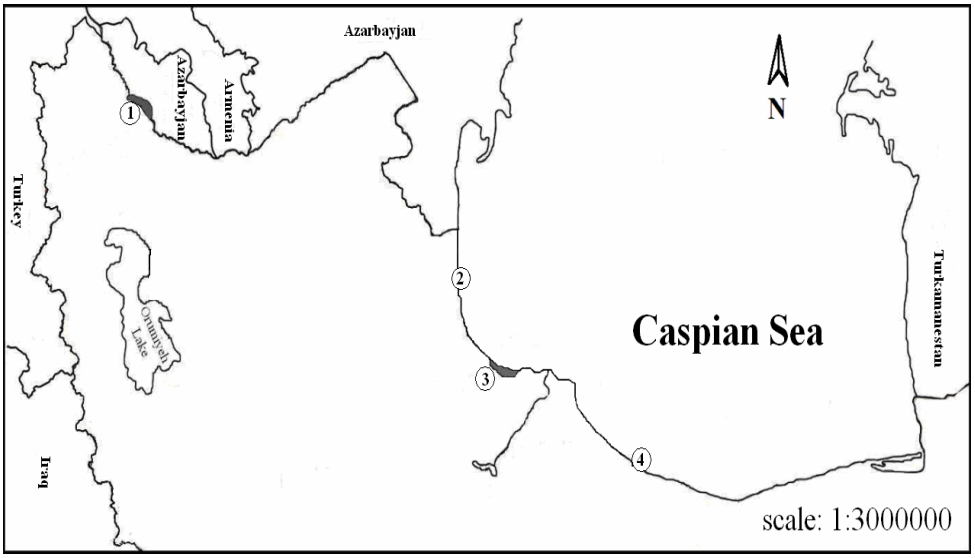

Fig. 1: Map showing sampling regions of four populations of *S. lucioperca*: Aras Dam (1), Talesh Coasts (2), Anzali Wetland (3) and Chaboksar Coasts (4)

DNA Analysis

Allelic frequency, observed and expected hetrozygosities, genetic distance (Nei, 1972) and genetic identity (Nei, 1972) were computed in GenAlex 6.0 software (Peakall & Smouse, 2005). This package was also used to calculate F_{ST} and R_{ST}, Nm, Hardy-Weinberg (HW) tests of equilibrium and AMOVA (Analysis of Molecular Variance).

RESULTS

Amplification and Banding Patterns

Out of 15 sets of microsatellite primers, 4 sets (YP68, YP113, Pfla L1 & Pfla L10) have not shown any flanking sites on pike-perch genome. Eleven sets of primers were successfully amplified where 5 sets (YP17, YP41, YP78, YP111 & Pfla L3) showed monomorphic pattern in all samples. Therefore totally 6 sets of primers produce polymorphic bands. Loci YP13, YP60 and P*fla* L8 had the highest numbers of alleles (4), while the other loci had the lowest (3).

Genetic Variation within Sampling Regions

The average number of alleles found per locus in Aras Dam, Talesh Coasts, Anzali Wetland and Chaboksar Coasts samples were 2.5, 3.5, 2.7 and 3, respectively.

The average observed hetrozygosities in these four regions were 0.51, 0.53, 0.56 and 0.49 respectively (Table 3). The observed heterozygosity of all four regions at YP110 & P*fla* L9 were significantly higher than the corresponding expected heterozygosity ($P<0.01$). The differences between all four sampling area were not statistically significant ($P>0.05$), neither for the average number of alleles nor for the observed heterozygosities.

Significant deviations from Hardy-Weinberg equilibrium at the locus level are shown in Table 3. Aras Dam, Talesh Coasts, Anzali Wetland & Chaboksar Coasts regions deviated at two, four, two and three loci, respectively, mostly due to the deficiency of heterozygosities.

Genetic Variation among Sampling Regions

Genetic distance calculated between each pair of collections ranged from 0.034 (between Talesh and Chaboksar Coasts) to 0.110 (between Aras Dam and Anzali Wetland, Table 4). The highest range of genetic difference were observed between Aras Dam and Anzali Wetland (F_{ST}= 0.093, R_{ST}= 0.106, P≤0.01) and the lowest between Talesh and Chaboksar Coasts (F_{ST}= 0.022, R_{ST}= 0.012, P≤0.01) (Table 5).

Table1: Loci, repeat motif, primers sequence, gene bank number and primer source used at present study

No.	Loci	Repeat motif	Primer sequence	Gene bank No.	Primer source
1	YP13	$(GTA)_{11}$	F-GGCACCCAAACTACCACT R-CAGTCGGGCGTCATCATCAAACAAGCCCCATACA	DQ826683	Li *et al.,* (2007)
2	YP17	$(TAG)_{10}TANGTG(TAG)_2$	F- CAGTCGGGCGTCATCACAGCGTTTCCACAGTATTGACC R- GGGTTTTACACTGTTGATGGGAT	DQ826686	
3	YP41	$(TCTT)_{11}$	F-CGCTCCCTCCCTCTATCC R- CAGTCGGGCGTCATCATTGCTGTGCTGCCATTTC	DQ826692	
4	YP60	$(AGAA)_{10}$	F- ATGTGTTATTGCTTTGCGTA R- CAGTCGGGCGTCATCAGCTGTTCCTGTAATGTGTTG	DQ826697	
5	YP68	$(AC)_5GCACGC(AC)_5AT(AC)_9$	F- GACAGAAAGCAAGAAGGGAA R- CAGTCGGGCGTCATCAATCCTTTTCTCCAATCCTGA	DQ826701	
6	YP78	$(GTA)_{13}$	F- GCAGCCCCTACAATGGTT R- CAGTCGGGCGTCATCAGCCTTCTTCTGTTATTTTTCC	DQ826705	
7	YP110	$(TTG)_{18}$	F- CAGTCGGGCGTCATCATTCAGACCCCTTCACTTTTG R- ATCAGAGCAATGACCAAGCC	DQ826719	
8	YP111	$(CTA)_{16}(ATA)_{18}$	F- CAGTCGGGCGTCATCATGTGTATGGCTATTGTGCTC R- TTTGTTCAGTGTTTTTTCGC	DQ826720	
9	YP113	$(GT)_{17}$	F- CAGTCGGGCGTCATCACGGTTGGGACACAGAGACAC R- TGGTGTGGATTGGGGCAT	DQ826721	
10	P*fla* L1	$(GA)_{27}$	F- AAGCAGCCTGATTATATATC R- CAGACAATTAAACATGCAAC	AF211826	Leclerc *et al.,* (2000)
11	P*fla* L2	$(CA)_{27}$	F-GTAAAGGAGAAAGCCTTAAC R-TAGCATGACTGGCAAATG	AF211827	
12	P*fla* L3	$(TG)_{18}$	F-GCCGAATGTGATTGAATG R-CGCTAAAGCCAACTTAATG	AF211828	
13	P*fla* L8	$(TG)_{39}$	F-GCCTTATTGTGTGACTTATCG R-GGATCTTTCACTTTTTCTTTCAG	AF211833	
14	P*fla* L9	$(TG)_{24}$	F-GTTAGTGTGAAAGAAGCATCTGC R-TGGGAAATGTGGTCAGCGGC	AF211834	
15	P*fla* L10	$(TG)_{14}$	F- TCCACCCTTTGATAAGGGAC R- ACAAATCTCCTGTCAAACGC	AF211835	

Table 2: Polymorphic amplified locus, allele size (bp) and annealing temperature on *S. lucioperca*

Locus	Observed allele size (bp)	annealing temperature(˚C)
YP13	274-307	60
YP60	205-245	59
YP110	461-473	59
P*fla* L2	212-230	53
P*fla* L8	170-198	57
P*fla* L9	188-244	61

Table 3: Variability of six microsatellite loci in four populations of *S. lucioperca* from Iran (A, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; P, P-value of χ^2 tests for Hardy-Weinberg equilibrium)

Locus	Parameters	Aras Dam	Talesh coasts	Anzali Wetland	Chaboksar coasts
YP13	A	3.0	4.0	3.0	4.0
	H_o	0.470	0.560	0.520	0.480
	H_e	0.590	0.678	0.625	0.573
	P	0.000*	0.000*	0.000*	0.003*
YP60	A	2.0	4.0	2.0	2.0
	H_o	0.440	0.380	0.480	0.460
	H_e	0.500	0.608	0.461	0.380
	P	0.354 ns	0.000*	0.768 ns	0.109 ns
YP110	A	2.0	3.0	2.0	2.0
	H_o	0.300	0.260	0.300	0.327
	H_e	0.250	0.233	0.255	0.273
	P	0.186 ns	0.818 ns	0.212ns	0.178 ns
P*fla* L2	A	3.0	3.0	3.0	3.0
	H_o	0.510	0.500	0.660	0.571
	H_e	0.480	0.499	0.503	0.506
	P	0.367 ns	0.706 ns	0.117*	0.513 ns
P*fla* L8	A	2.0	4.0	3.0	4.0
	H_o	0.400	0.640	0.660	0.327
	H_e	0.380	0.620	0.552	0.653
	P	0.631 ns	0.000*	0.267 ns	0.000*
P*fla* L9	A	3.0	3.0	3.0	3.0
	H_o	0.930	0.880	0.800	0.918
	H_e	0.570	0.648	0.631	0.622
	P	0.000*	0.000*	0.000*	0.000*
Average number of alleles per locus		2.5	3.5	2.7	3.0
Average Ho		0.520	0.540	0.570	0.517
Average He		0.500	0.548	0.504	0.501

*Significant at $P< 0.05$

Table 4: Pairwise population of genetic distance (below diagonal) and genetic identity (above diagonal) (Nei, 1972) detected at 6 loci in pike-perch samples

		Genetic Identity			
	Samples	**Aras Dam**	**Talesh coasts**	**Anzali Wetland**	**Chaboksar coasts**
Genetic Distance	Aras Dam	-	0.945	0.896	0.918
	Talesh coasts	0.057	-	0.958	0.966
	Anzali Wetland	0.110	0.043	-	0.922
	Chaboksar coasts	0.085	0.034	0.081	-

Table 5: Pairwise estimates of genetic differentiation detected at 6 loci in pike-perch samples, using unbiased R_{ST} (below diagonal) and F_{ST} values (above diagonal)

		F_{ST} (N_m)			
	Samples	**Aras Dam**	**Talesh coasts**	**Anzali Wetland**	**Chaboksar coasts**
	Aras Dam	-	0.034* (7.11)	0.093* (2.43)	0.070* (3.31)
R_{ST}	Talesh coasts	0.029 * (8.43)	-	0.032 * (7.44)	0.022 * (11.27)
(N_m)	Anzali Wetland	0.106* (2.11)	0.025* (9.80)	-	0.064* (3.63)
	Chaboksar coasts	0.064* (3.64)	0.012* (21.14)	0.55 * (4.30)	-

Probabilities of R_{ST} or F_{ST} determined by AMOVA tests, at $P \leq 0.01$
*Significant at $P < 0.01$

DISCUSSION

The long-term persistence of an endangered fish species could be investigated by allelic diversity, gene diversity, effective population size and population structure (Yue *et al.,* 2004). Despite the importance of pike-perch as a highly commercially important fish in Iran, natural populations of this fish have been declined. Information about these populations is pivotal for their conservation and sustainable use. Unfortunately, the knowledge on the molecular population genetic structure of this species has not conducted yet.

In this study we have employed 6 polymorphic microsatellite loci to assess the genetic relationship among populations of pike-perch from four regions in Iran along the Southwest Coasts of the Caspian Sea and Aras Dam. According to the results all of the sampling regions had low number of alleles but Aras Dam samples showed the lowest number of alleles (2.5 in average). Among the six primers used in this study, the first three primers have not been used for other pikeperch populations yet. But these primers have shown high number of alleles in the studied species (Li *et al.,* 2007). So these primers were used in this study because of their polymorphic character .The rest of primers were used in a few individuals (6-8) of *S. lucioperca* (Leclerc *et al.,* 2000). Their results showed that the average number of alleles per locus, similar to our study, was low, but the number of individuals was insufficient for population genetic statistical analysis and their results were not comparable with our study. In another study of genetic structure of pike-perch in Rhone delta (Poulet *et al.,* 2009) 5 alleles were produced in P*fla* L2 locus, which is higher than our produced alleles (3 in average) for this locus. Due to insufficient control and planning of the releases and the low number of commercial pikeperch hatcheries, practically all stocking programs have relied on only three to four different parent populations, all of them with a common origin (Aras Dam). This practice has probably resulted in a large-scale loss of the original genetic diversity within the species in comparison to previous studies.

Genetic differentiation within and between populations of species is affected by several general factors, such as common history, current and past gene flow, as well as local population-specific processes, such as genetic drift and adaptive selection. The role of geographic distance, and implicit gene flow, for the spatial pattern of genetic variation is well known both theoretically and empirically (Epperson, 2003; Rousset, 2004). If the accumulation of mutations since divergence is the only factor that determines the amount of genetic differentiation, measured as F_{ST}, there will be a strong correlation between F_{ST} and the number of generations since divergence so that F_{ST} increases linearly with time (Slatkin, 1995). However, additional factors influence differentiation, making the correlation less than perfect. For example, genetic drift causes the populations to deviate from the expected line of divergence in a random fashion so that some populations are less and others are more genetically different than expected. However, gene flow will act in a systematic way making populations in close proximity more similar to each other than more distant populations. In the present study the F_{ST} value in all four sampling regions was low but significant ($P<0.01$), suggesting that the four populations are genetically differentiated and don't represent a single panmictic population. The lowest of F_{ST} value on AMOVA was between Talesh and Chaboksar Coasts populations with the high gene flow.

However there is no information about the main origin of the Talesh and Chaboksar Coasts populations. Perhaps these populations are related to other rivers (e.g. Ural, Volga, Kura, and Sefid Roud) or neighboring Coasts.

The genetic distance between these four populations were in the range of 0.034-0.110, which indicates that the genetic difference among the studied populations is not pronounced. Regarding the fishery returning, it is possible that the caught samples are a combination of the various generation and different birth places which have formed a gathering to feed.

Heterozygote deficits were found in some of the samples. This may have several causes, one being technical errors, such as allelic dropout or null alleles. As in several other studies of fishes where heterozygote deficits have been found (Brown & Brown, 1996; Pouyaud *et al.*, 1999; Gerlach *et al.*, 2001; Krause *et al.*, 2001; Castric *et al.*, 2002), the results in this study show that part of this heterozygote deficit can be attributable to the sampling of relatives. Even if individuals are more related than expected in a random sample, the samples do not consist of siblings, but of related individuals with a shared ancestor a few generations back.

The data generated in this study showed that there are four different populations of pikeperch in the studied regions of Iran. This information can be applied for future genetic improvement by selective breeding and to design suitable management guidelines for the genetic materials. However, in order to have better conservational policy and restocking programs, further studies are recommended on determining the other different populations of this important species in other regions of Iran.

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تجزیه و تحلیل ژنتیکی جمعیت های سوف سفید (*Sander lucioperca L.*) ایران با استفاده از نشانگرهای DNA ریز ماهواره

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چکیده

این مطالعه به منظور بررسی تنوع و ساختار ژنتیک جمعیت ماهی سوف سفید در بخش شمالی کشور ایران انجام شد. به این منظور 207 نمونه ماهی سوف سفید بالغ از چهار منطقه واقع در حوضه دریای خزر (سواحل تالش، تالاب انزلی، سواحل چابکسر و سد ارس) انتخاب شدند. **DNA** ماهیان استخراج، و با استفاده از 15 جفت آغازگر ریزماهواره، واکنش زنجیره ای پلیمراز (**PCR**) انجام شد. باند های **DNA** به دست آمده با استفاده از نرم افزارهای **Biocapt** و **GenAlex 6** مورد تجزیه و تحلیل قرار گرفت. از 15 آغازگر ریزماهواره، 11 لوکوس ایجاد شد که 6 تای آنها پلی مورفیک و 5 تای دیگر مونومورفیک بودند. تجزیه و تحلیل ها نشان داد که تعداد متوسط آلل ها و هتروزیگوسیتی مشاهده شده در در هر لوکوس برای تمامی جمعیت ها از لحاظ آماری معنی دار نیست (**P >**0/05). داده های به دست آمده به رغم تغییرات ژنتیکی کم، حاکی از تمایز ژنتیکی قابل ملاحظه ای در این گونه بود و نشان داد که پلی مورفیسم ژنتیکی این گونه در ایران در سطح پایینی قرار دارد. انحراف از تعادل هاردی- واینبرگ در بسیاری از موارد مشهود بود که علت عمده آن نبود هتروزیگوسیتی بود. بیشترین فاصله ژنتیکی بین جمعیت های تالاب انزلی و سد ارس مشاهده شد. این بررسی با استفاده از نشانگرهای ریز ماهواره اطلاعات اولیه ای را در ارتباط با تنوع ژنتیکی جمعیت های ایرانی سوف سفید فراهم نمود و نتایج حاصل از آن در مدیریت آتی و حفاظت این گونه در ایران قابل استفاده است.

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