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Genetic Diversity Evaluation of *Paraschistura bampurensis* (Nikolskii, 1900) in Shapour and Berim Rivers (Iran) by Using Microsatellite Markers

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Abstract: *Paraschistura bampurensis* is the only species of genus Paraschistura.It is distributed in Iran and Pakistan freshwater. The present study is carried out to investigate the genetic diversity and population structure of *P.bampurensis* in Shapour and Berim rivers basin, Iran. 100 samples, 50 samples from each river, were used. Microsatellite markers have been increasingly used in population genetics studies. In this study, a total of six microsatellite loci and two populations were used. The average number of allele's level in the population accounted for 13, well above the reported values for freshwater fishes.The expected and observed heterozygosity mean were 0.562 and 0.861, respectively. Approximately all of loci showed deviation from Hardy-Weinberg Equilibrium (HWE). The genetic similarity and distance between the two populations were 0.402 and 0.669, respectively. According to the analysis, it seems that *P.bampurensis* has a desirable genetic diversity in the investigated regions.

Key words: *Paraschistura bampurensis* · Genetic Diversity · Microsatellite · Shapour · Berim

INTRODUCTION

Among Iranian freshwater fishes, the Cyprinidae family with 81 species and 48% frequency and the Balitoridae family with 23 species and 11% frequency remain the most dominant species, respectively [1]. As for as the habitat is concerned, Balitoridae fish prefers middle and upper parts of the river, usually cold and oxygen-rich, with rocky substrate and further they are exclusively-active-at-night species [2, 3]. *Paraschistura bampurensis* descends from Nemacheilidae family and as Abdoli [1] pointed out, it is distributed in the East-South and South-West of Iran (Sistan and Baluchestan, Kerman, Khuzestan, Fars and Kohgiluyeh and Boyer-Ahmad).

One of the problems currently running with fish stocks in the world is the loss of genetic diversity that is due to human activities, such as polluting, overfishing, habitat destruction and blocking the migration path [4, 5]. Genetic diversity while enabling environmental adaptation can assure the survival chances of one species or population and accordingly is considered essential for long-term survival of one species [6]. Molecular markers can directly recognize the genetic diversity and distribution [4]. Genetic diversity is achieved through differences in nucleotide sequence of DNA among individuals [7]. In general, the genetic diversity management in animals needs to evaluate the genetic structure and separate reserves the given species [8].

Because of unique features such as high polymorphism, high scope in genome and high mutation, microsatellite markers have wide application in population genetic studies [9]. Microsatellite markers are useful for understanding the population genetic structure, genetic diversity and history of the target species and further they have been detected exclusively for a number of Loach families [10, 11]. To date, not any specific microsatellite markers have been reported for this species. In this study, we investigated the genetic diversity of *Paraschistura bampurensis* and six microsatellites loci specific was determined for this species.

MATERIALS AND METHODS

Sample Collection and DNA Extraction: A total of 100 samples were collected from two rivers of Shapour (29°45' N, 51°33' E) and Berim (30°19' N, 51°15' E), Iran.

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Table 1: Characteristics of northern snakehead microsatellite loci used in this study

MicrosatelliteLoci	Primer sequence	N	Size (bps)	Anneal (°C)
Bbar4	F: ATAATCACAGCCCCGCAGAG R:GGGTGGTGGAATATATTGGAAA	8	84-120	55
Bbar7	F: GAGCAACAGCTGCTGTAGGA R: GTCGGACCAACCTGAAAACT	22	360-492	50
IC228	F: NED- AATACGAAACTACTTGGTAATGGC R: GTGAAAAGGTCCAGTTAAAAGC	14	176-248	48
IC230	F: NED-GGGTATAGGTGAAAAGGTCC R: ATACGAAACTACTTGGTAATGGC	10	180-264	48
IC434	F: 6FAM-TCCACCATGACCATTTTTACATA R: GGTGTCTGGATCTCATCTTGAA	11	224-276	52
IC720	F: NED-CGCAATGCATTCTCCAATCTCAA R: GACCCCACTCATCACTGCCTCTC	15	236-498	62

N: number of Allele

Total genomic DNA was extracted from fin pectoral and fin pelvic tissue by using the traditional proteinase-K digestion and standard phenol/chloroform techniques [12]. DNA extraction were then stored at -20°C until use.

Molecular Analysis: In this study, PCR amplifications were done using six microsatellite loci analyzed: Bbar4, Bbar7 [10], IC228, IC230, IC434 and IC720 [11] (Table 1). Initial denaturation was achieved at 94°C for 3 min followed by 30 cycles of denaturation in 30 seconds at 94°C, 30 seconds at the respective annealing temperatures and extension to 72°C for 1 minute. The final step was extended to 3 minutes at 72°C.PCR products were separated using% 8 polyacrylamide gels stained with silver nitrate [13].

Statistical Analysis: The number of alleles at per Locus, observed heterozygosity (H_o), expected heterozygosity (H_e), the Real number of alleles, the number of observed alleles (N_a), the number of effective alleles (N_e), Hardy–Weinberg Equilibrium (HWE), F_{st} values and number of migrant (N_m) were calculated by Genealex ver.6.5 Software [14]. PopGene ver1.31 software was used to determine the genetic distance and similarity [15] and phylogenetic relationships of populations [16].

RESULTS

In this study, the use was made of six microsatellite loci. A total of 156 alleles were detected for both populations and allele sizes ranged from 84 bp to 498 bp. The average number of observed alleles in Shapour and Berim population accounted for 14 and 13, respectively. Minimum and maximum observed alleles were 7 and 22 for Bbar4 and Bbar7 Locus for Shapour River, respectively. The mount of observed alleles (N_a), expected alleles (N_e), observed heterozygosity (H_O), expected heterozygosity (H_e) and fixation index (Fis) are shown in Table 2.

Table 2: Genetic variability of six microsatellite loci in two populations for *Paraschistura bampurensis* in Iran.

River		Bbar4	Bbar7	IC228	IC230	IC434	IC720
	N_a	7	22	15	9	11	17
	N_e	5.136	12.477	10.687	6.151	5.977	14.297
Shapour	H_o	0.304	0.739	0.783	0.696	0.348	0.304
	H_e	0.805	0.920	0.906	0.837	0.833	0.930
	F_{IS}	0.622	0.196	0.137	0.169	0.583	0.673
	P_{Hw}	***	**	ns	***	***	***
	N_a	9	21	13	10	10	12
	N_e	3.712	14.901	9.121	6.151	6.260	7.723
Berim	H_o	0.304	0.609	0.826	0.435	1.000	0.391
	H_e	0.731	0.933	0.890	0.837	0.840	0.871
	F_{IS}	0.583	0.348	0.072	0.481	-0.190	0.550
	P_{Hw}	***	**	***	ns	***	***

N_a, number of observed alleles; N_e, Number of effective alleles;Ho, observed heterozygosity; He, Expected heterozygosity; Fis, fixation indices; P_{HW}, Hardy–Weinberg probability test (*P< 0.05, **P <0.01,***P <0.001, n.s., non-significant)

Table 3: number of migrant and F_{st} index of six microsatellite loci in two populations for *Paraschistura bampurensis* in Iran

Loci	Bbar4	Bbar7	IC228	IC230	IC434	IC720	Mean
Nm	6.606	16.897	15.087	6.239	6.103	17.009	11.324
F_{st}	0.036	0.015	0.016	0.039	0.039	0.014	0.027

The mean of expected and observed heterozygosity was obtained 0.562 and 0.861, respectively. The results of Hardy-Weinberg Equilibrium (HWE) almost for all loci showed deviations from Equilibrium (Table 2). The Mean number of migrant (N_m) was obtained 11.324 between regions and the minimum and maximum amount was calculated for loci IC434 (6.103) and IC720 (17.009) (Table3). Furthermore, the analysis of molecular variance and index F_{st} in 99% showed the high genetic diversity (97%) within populations and the low genetic variation among populations (3%). Genetic distance and similarity based on the Nei index was 0.669 and 0.402, respectively and, the UPGMA dendrogram, based on the genetic distance, showed that these two regions are distinctly two different branches.

DISCUSSION

Genetic diversity is important for ecological and evolutionary processes ranging from individual fitness to ecosystem function [17]. Heterozygosity is an important evolutionary indicator in determining the dynamics and survival of populations [18]. In this study, number of observed alleles and heterozygosity (N_a = 13, H_o= 0.562) was higher than the average reported for freshwater fishes (N_a = 7.5, H_o= 0.46) [19]. Information obtained from microsatellite markers showed high genetic diversity within populations and low diversity among populations. However, low observed heterozygosity was indicative of the intra-population genetic structuring of *P. bampurensis* populations in the middle and lower regions of both Shapour and Berim River basins (Wahlund's effect). Both populations showed deviation from Hardy-Weinberg Equilibrium. Considering the fact that Hardy-Weinberg Equilibrium is based on the random mating in a population, deviations from Hardy - Weinberg Equilibrium in wild populations are expected [20]. Natural populations of a wide range of fishes have been reported to exhibit departures from HWE [10, 21-23]. The heterozygote deficit in natural populations may emerge through inbreeding, nonrandom sampling, intra-population structure [21], genetic drift, null alleles [24], fishing pressure [25] or combined impact of the aforementioned factors.

Analysis of molecular variance (AMOVA) is a suitable criterion to assess population structure and determine the differentiation and genetic similarity between populations [26]. According F_{st} index, genetic diversity was calculated between populations 3%. The mean of F_{st} index was calculated about 0.027. This Thread represents the low differentiation between the two populations. According to Wright criteria [27], F_{st} value less than 0.05 indicates the low differentiation among communities. In this study, number of migrant's average was reported as much as 11.324. Li *et al*. [28] noted that when Nm>1 and Nm<1, then genetics differentiation occurred due to number of migrant and gene migration respectively; hence the results of this study reveal that number of migrant was the main reason for genetics differentiation between our communities.

It was demonstrated, by using UPGMA dendrogram, that there were two separated communities in these rivers. Genetic structure of *Paraschistura bampurensis* in this river was probably due to number of migrates occurred during decades. It seems that these two rivers were connected in the past and accordingly this connection caused the genetic similarity among them.

Reproduction behavior and biology of feeding affect the genetic structure of fish [29]. Matured fishes have no long migration during reproduction season and spawn in the vicinity of their habitats. Fries live in the rivers through grasses and stones on the sides. There is no parental care behavior for this species. They spawn in the free spaces between and beneath stones and right in accordance of water flow. Sediments and high water flow rate cause main damages to eggs [30]. Low heterozygosity rises due to inbreeding and decrease of population volume [29]. According to the analysis, it seems that *Paraschistura bampurensis* have favorable genetic diversity through the investigated regions and genetic diversity maintenance is recommended due to its importance ecological roles such as river refinery.

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