

Full Length Research Paper

# Genetic diversity evaluation of *Paraschistura bampurensis* (Nikolskii, 1900) in Shapour and Berim Rivers (Iran) using microsatellite markers

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*Paraschistura bampurensis* is one of the species of genus *Paraschistura*. It is distributed in Iran and Pakistan freshwater. This paper reports a study carried out on genetic diversity and population structure of *P. bampurensis* in Shapour and Berim river basins, Iran. 100 samples of 50 fish each were collected from each river and were used. Microsatellite markers have been increasingly used in population genetics studies. In this study, a total of six microsatellite loci and two sampling populations were used. The average number of observed alleles in Shapour and Berim population accounted for 13.5 and 12.5, respectively. The average number of allele's level in the population accounted for 13, well above the reported values for freshwater fishes. The mean expected and observed heterozygosity mean were 0.562 and 0.861, respectively. Approximately all loci showed a deviation from Hardy-Weinberg equilibrium. 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 markers, Shapour River, Berim River.

## INTRODUCTION

Among Iranian freshwater fishes, 48% of the Cyprinidae family, with 81 species and 11% of Balitoridae family, with 23 species remain the most dominant species, respectively (Coad, 2012). In the ecosystem, 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 (Abdoli, 1998; Cihar, 1976). *Paraschistura bampurensis* descends from Nemacheilidae family and as Abdoli (1998) 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 provinces).

One of the problems currently facing fish stocks in the

world is the loss of genetic diversity that is due to human activities, such as pollution, overfishing, habitat destruction, and blocking the migration path (Ferguson et al., 1995; Zhao et al., 2005). 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 species (Bataillon et al., 1996). Molecular markers are used to detect the genetic diversity and distribution (Ferguson et al., 1995). Genetic diversity is achieved through differences in nucleotide sequence of deoxyribonucleic acid (DNA) among individuals (Utter, 1991). In general, the genetic diversity management in animals needs to evaluate the genetic structure and separately reserve the given species (Pujolar et al., 2009).

Microsatellite markers are very popular for the study of molecular phylogeography and population genetics

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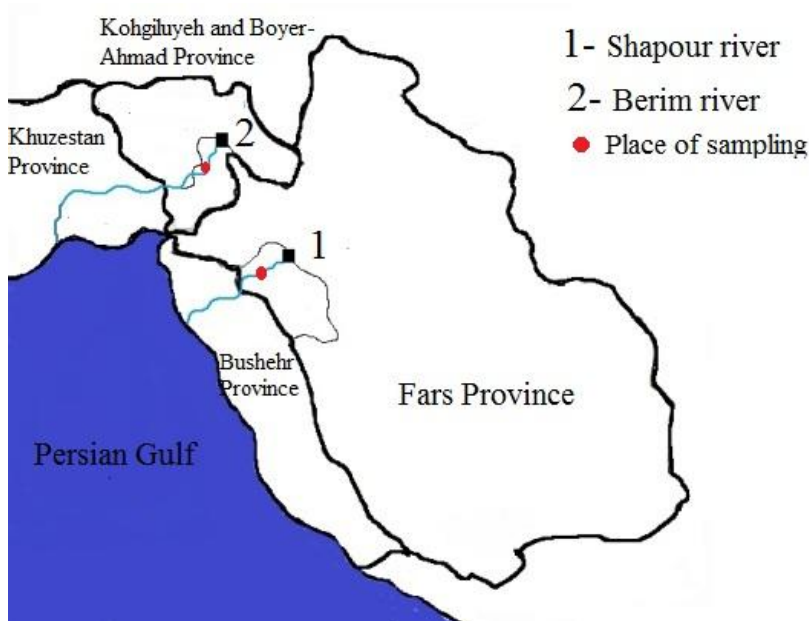


Figure 1. Sampling localities for *Paraschistura bampurensis* in Iran.

because of the advantages of high polymorphism, ease of genotyping and co-dominant inheritance (Sun et al., 2009). Sufficient microsatellite markers are needed to thoroughly understand the genetic population structure, genetic diversity and resource history of a target species, for example a number of Loach families (Taylor et al., 2001; Bang et al., 2009). Considering that these markers are widely used in population genetics studies, we chose these markers for this study.

Our aims were to elucidate the molecular genetic diversity, prepare necessary microsatellite markers and the genetic relationships among the *P. bampurensis* populations. These species are adapted to the rivers and different stream. So, it is important to know the differences among the fish populations adapting different habitats. This is the first study based on microsatellite markers on the population genetic structure of *P. bampurensis* from Iran localities.

## MATERIALS AND METHODS

### Sample collection and DNA extraction

A total of 100 samples fish were collected from two places, in the river Shapour (29° 45' N, 51° 33' E) and Berim (30° 19' N, 51° 15' E), Iran (Figure 1). Total genomic DNA was extracted from fin pectoral and pelvic tissue by using the traditional proteinase-K digestion and standard phenol/chloroform techniques (Hillis et al., 1996).

Approximately 100 mg tissue was treated with 25  $\mu$ l proteinase K (10 mg/ml) and 50  $\mu$ l sodium dodecyl sulfate (SDS) (10%) in 500  $\mu$ l

Sodium Chloride-Tris- Ethylenediaminetetraacetic acid (STE) buffer (0.1 M NaCl, 0.05 M Tris and 0.01 M Na<sub>2</sub>EDTA, pH: 8.0) overnight at 37°C. After incubation, DNA was isolated by two steps of phenol-chloroform (25 phenol: 24 chloroform: 1 isoamyl alcohol), followed by precipitation with cold absolute ethanol. DNA extraction were then stored at -20°C until usage.

### Molecular analysis

In this study, six microsatellite markers were amplified by polymerase chain reaction (PCR) using the following primers: Bbar4, Bbar7 (Taylor et al., 2001), IC228, IC230, IC434 and IC720 (Bang et al., 2009) (Table 1). Initial denaturation was achieved at 94°C for 3 min followed by 30 cycles of denaturation for 30 s at 94°C, 30 s at the respective annealing temperatures, and extension to 72°C for 1 min. The final step was extended to 3 min at 72°C. PCR products were separated using 8% polyacrylamide gels stained with silver nitrate (Bassam et al., 1991).

### Statistical analysis

The number of alleles per locus, observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), 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 (Peakall and Smouse, 2012). PopGene ver.1.31 software was used to determine the genetic distance and similarity (Nei, 1978), and phylogenetic relationships of populations (Yeh et al., 1999). Scoring errors, large allele dropout and null alleles were checked employing the program MICROCHECKER (Oosterhout et al., 2004).

**Table 1.** Characteristics of *P. bampurensis* microsatellite loci used in this study.

Microsatellite loci	Primer sequence	N	Size (bps)	Anneal (°C)
Bbar4	F: ATAATCACAGCCCCGCAGAG R: GGGTGGTGAATATATTGGAAA	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.

## RESULTS

In this study, structure and genetic diversity of *P. bampurensis* was studied at six microsatellite loci for two samples locations in Iran. A total of 156 alleles were detected for both populations and allele sizes ranged from 84 to 498 bp (Table 1). The average number of observed alleles in Shapour and Berim population accounted for 13.5 and 12.5, respectively (Table 2). Minimum and maximum observed alleles were 7 and 22 for Bbar4 and Bbar7 Locus for Shapour River, and 9 and 21 for Berim River, respectively. The observed  $N_a$ ,  $N_e$ ,  $H_o$ ,  $H_e$  and fixation index (Fis) are shown in Table 2. The average  $H_o$  and  $H_e$  are 0.529 and 0.872 for samples from Shapour river and 0.594 and 0.850 for samples from Berim river, respectively, and the average  $H_o$  range for both samples is 0.529 to 0.594).

The results of HWE almost for all loci showed deviations from equilibrium (Table 2). The mean  $N_m$  was obtained as 11.324, between Shapour and Berim river, and the minimum and maximum amount calculated for loci IC434 and loci IC720 were 60103 and 17.009, respectively (Table 3). These values were calculated according to the formula  $[Nm = [(1 / Fst) - 1] / 4]$  between population. At the loci Bbar4, IC434 and IC720, null alleles might have appeared. Furthermore, the analysis of molecular variance and index Fst in 99% showed the high genetic diversity (97%) within populations and the

low genetic variation among populations (3%). Loci IC434 showed deficit of heterozygotes in Berim. Genetic distance and similarity based on the Nei index was 0.669 and 0.402, respectively and the Unweighted pair group method with arithmetic mean (UPGMA) dendrogram, based on the genetic distance, showed that these two regions are distinctly two different branches (Figure 2).

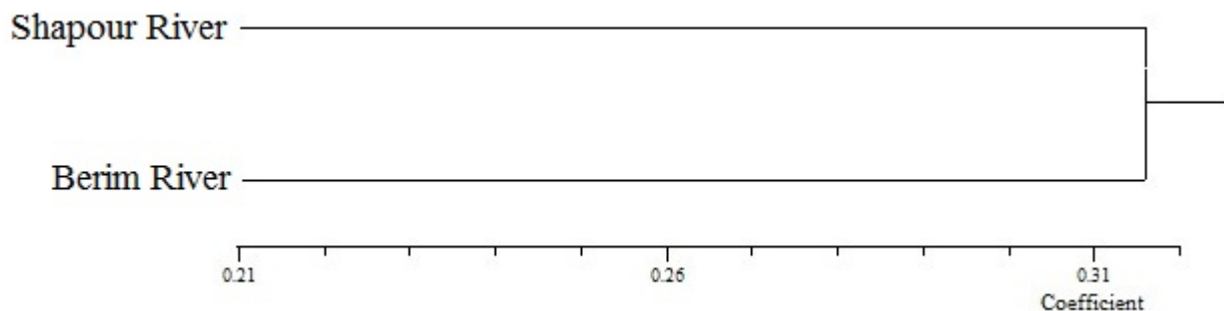
## DISCUSSION

Genetic diversity is important for ecological and evolutionary processes ranging from individual fitness to ecosystem function (Rezvani et al., 2012). Heterozygosity is an important evolutionary indicator in determining the dynamics and survival of populations (Reed, 2009). 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 ( $N_a = 7.5$ ,  $H_o = 0.46$ ) (DeWoody and Avise, 2000). Information obtained from microsatellite markers showed high genetic diversity within populations and low diversity among populations. Both populations showed deviation from HWE. Considering the fact that HWE is based on the random mating in a population, deviations from HWE in wild populations are expected (Dixon et al., 2008). Wild populations of a wide range of fishes have been reported to exhibit departures from HWE (Castric et al., 2002; Yue

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

River	Parameter	Bbar4	Bbar7	IC228	IC230	IC434	IC720
Shapour	N <sub>a</sub>	7	22	15	9	11	17
	N <sub>e</sub>	5.136	12.477	10.687	6.151	5.977	14.297
	H <sub>o</sub>	0.304	0.739	0.783	0.696	0.348	0.304
	H <sub>e</sub>	0.805	0.920	0.906	0.837	0.833	0.930
	F <sub>IS</sub>	0.622	0.196	0.137	0.169	0.583	0.673
	P <sub>HW</sub>	***	**	ns	***	***	***
Berim	N <sub>a</sub>	9	21	13	10	10	12
	N <sub>e</sub>	3.712	14.901	9.121	6.151	6.260	7.723
	H <sub>o</sub>	0.304	0.609	0.826	0.435	1.000	0.391
	H <sub>e</sub>	0.731	0.933	0.890	0.837	0.840	0.871
	F <sub>IS</sub>	0.583	0.348	0.072	0.481	-0.190	0.550
	P <sub>HW</sub>	***	**	***	ns	***	***

N<sub>a</sub>, number of observed alleles; N<sub>e</sub>, number of effective alleles; H<sub>o</sub>, observed heterozygosity; H<sub>e</sub>, expected heterozygosity; F<sub>IS</sub>, fixation indices; P<sub>HW</sub>, Hardy-Weinberg probability test (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, n.s, non-significant).

**Figure 2.** UPGMA dendrogram based on Nei's genetic distance, summarizing the data on differentiation between two populations of *P. bampurensis*, according to microsatellite DNA marker analysis.

et al., 2004; Lucentini et al., 2006; Bang et al., 2009). Examination of genotyping errors using Microchecker revealed no evidence for large allele dropout or stutter-band scoring at any of the six loci. The heterozygote deficit in natural populations may emerge through inbreeding, non-random sampling, intra-population structure (Castric et al., 2002), genetic drift, null alleles (Angel et al., 2006), fishing pressure (Bergh and Getz, 1989) 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 (Grassi et al., 2004). According F<sub>st</sub> index, genetic diversity between populations was 3%. The mean of F<sub>st</sub> index was about 0.027. This issue represents the low

differentiation between the two populations. According to Wright (1987), F<sub>st</sub> value less than 0.05 indicates the low differentiation among communities. In this study, number of migrant's average was reported as 11.324. Li et al. (2007) noted that when N<sub>m</sub> > 1 and N<sub>m</sub> < 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 *P. bampurensis* in these rivers was probably due to number of migrates which occurred during decades. It seems that these two rivers were connected in the past, and accordingly this connection

**Table 3.** Number of migrant and  $F_{st}$  index of six microsatellite loci in two populations for *P. 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

caused the genetic similarity among them.

Reproduction behavior and biology of feeding affect the genetic structure of fish (Abbas et al., 2010). Mature fishes have not migrated 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 (Wei et al., 1997). Low heterozygosity rises due to inbreeding and decrease of population volume (Abbas et al., 2010). According to the analysis, it seems that *P. 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. This information should be taken into consideration for any genetic conservation and stock improvement plan. However further study involving low numbers of populations covering all parts of the country with additional microsatellite loci is recommended to reveal detailed genetic structure of this important fish species in Iran.

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