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DISTRIBUTION AND STATUS OF SPINED LOACH POPULATIONS (*Actinopetrigii:* Cobitidae) ALONG THE SOUTHERN CASPIAN SEA BASIN

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ABSTRACT

In order to clarify the distribution pattern of *Cobitis* populations along the southern Caspian Sea basin, 97 specimens collected from five localities were morphologically examined. Univariate analysis of variance showed significant differences (P<0.05) among the means of the five groups for 28 of 33 standardized morphometric measurements and 7 of 10 meristic counts. In the linear discriminant function analysis (DFA) for morphometric characteristics, the overall assignment of individuals into their original groups was high (84.9%), indicating that these populations are highly divergent. The proportion of individuals correctly classified into their original groups were 77.4%, 77.3%, 100%, 100% and 89.5% for *Cobitis* sp. (Gisum River), *Cobitis keyvani* (Sefidroud River), *C. keyvani* (Tonekabon River), *Cobitis faridpaki* (Siahroud River) and *C. keyvani* (Talar River), respectively. Clustering based on Euclidean distances among these groups of centroids using UPGMA and principal component analysis (PCA) indicated that the southeastern Caspian spined loach populations are *C. faridpaki* and the south central ones are *C. keyvani,* and an unknown population, *Cobitis* sp. is distinguished from the southwestern populations of the basin. Also the Tonekabon and Sefidroud Rivers were determined to be the two new habitats for *C. keyvani* in the region.

INTRODUCTION

Morphometric and meristic studies are strong instruments for determining the discreteness of similar species (Turan et al., 2006; Mousavi-Sabet et al., 2011b; Mousavi-Sabet et al., 2012d) and are extensively used to identify differences between fish populations (Tzeng, 2004). They are measurable features that are helpful for separating closely related genera, species and even populations within them (Cadrin, 2000). In order to ensure rational and effective management of fishery resources, it is important to know the stock structure of an explored species, as each stock must be managed separately to optimize their yield (Meng and Stocker, 1984). This should be performed for both large and small fishes such as sturgeons and loaches.

Loaches of the genus *Cobitis* Linnaeus, 1759 (family Cobitidae) have a worldwide distribution. They occupy the streams of Eurasia from England and Iberian Peninsula, as far as the Far East, Japanese Archipelago, Sakhalin Island, Korean

Peninsula, Laos and Vietnam, and are also found in northern Africa, i.e. in Morocco (Economidis and Nalbant, 1996; Freyhof and Serov, 2000; Janko et al., 2005; Vasil'eva and Vasil'ev, 2006; Suzawa, 2006; Erk'akan et al., 2008). Species of this family are small, benthic freshwater fish (Bianco and Nalbant, 1980; Nalbant and Bianco, 1998; Coad, 2013), preferring microhabitat characteristics such as open, shallow areas with slow-flowing or stagnant water (Coad, 2013). A number of these species are popular aquarium fishes (Coad, 2013; Mousavi-Sabet et al., 2012a). Kottelat (2012) recognized 65 species in the genus *Cobitis*, five of which are found in the Caspian Sea basin (*C. amphilekta*, *C. faridpaki*, *C. keyvani*, *C. gladkovi*, and *C. taenia*). Of these, *C. gladkovi* and *C. taenia* are restricted to the northern Caspian Sea basin (Kottelat and Freyhof, 2007), while the remaining three species have been recently described from the southern Caspian Sea basin (Mousavi-Sabet et al., 2011b; Mousavi-Sabet et al., 2012d; Vasil'eva and Vasil'ev, 2012). In Iran, 27 loach species belonging to two families (Nemacheilidae and Cobitidae) and eight genera have been recorded in 19 water basins, of which eleven are endemic to Iran (Nalbant and Bianco, 1998; Coad, 2013). Cobitid loaches consist of two genera (*Cobitis* and *Sabanejewia*) in Iranian freshwater bodies (Coad, 2013). Three valid species of *Cobitis* are recognized in Iran, including *Cobitis linea* Heckel, 1849 which has a restricted distribution in the Kor River and likely the Hormoz basin (Bianco and Nalbant, 1980; Esmaeili et al., 2007), *C. faridpaki* and *C. keyvani* which have been recorded in the southern Caspian Sea basin (Mousavi-Sabet et al., 2011b; Mousavi-Sabet et al., 2012d). The southern Caspian Sea basin has the greatest taxonomic richness of freshwater fishes among the Iranian basins and spined loaches are distributed in the streams and rivers of virtually the entire basin (Abdoli, 2000, Esmaeili et al., 2010). However, the taxonomic status of this group of fishes has not yet been resolved and their exact distribution has not been fully investigated (Esmaeili et al., 2010; Mousavi-Sabet et al., 2011a; 2012a; 2012b; 2012c).

Therefore, this study investigated the distribution pattern of the genus *Cobitis* throughout its distribution range in the river systems of the southern Caspian Sea basin.

MATERIAL AND METHODS

Fishes were collected by electrofishing from the southern Caspian Sea basin (Gisum River; 37°41.40′ N, 49°01.05′ E, Sefidroud River; 37°15.46′ N, 049°5.598′ E, Tonekabon River; 36°44.631′ N, 050°50.992′ E, Siahroud River; 36°29.003′ N, 052°53.258′E and Talar River; 36°11.09′ N, 53°00.01′ E) (Figure 1). The collected specimens were fixed in 10% formalin solution at the site and were then transported to the ichthyology laboratory of the Department of Fisheries Sciences, University of Guilan for morphological analysis.

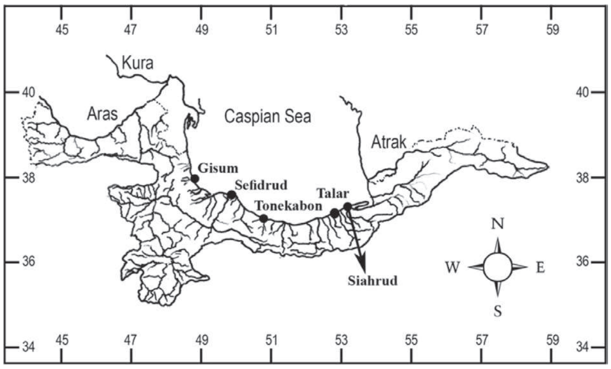

Fig 1. Location of sampling sites in the southern Caspian Sea basin (Gisum, Sefidroud, Tonekabon, Siahroud and Talar rivers) in northern Iran

A total of 35 morphometric characters were measured; these parameters were selected according to Vasil'eva and Vasil'ev (2006), Erk'akan et al. (2008) and Mousavi-Sabet et al. (2011b, 2012d). To ensure that a morphological error is stable, all measurements were performed by one person. Morphometric characters included total body length (TL), standard body length (SL), body depth (H), body width (BW), caudal peduncle length (Cpl), caudal peduncle depth (h), head length (hl), head depth (hd), head width (hw), snout length (prO), eye diameter (Ed), postorbital length (pol), inter orbital length (iol), the first barbel length (Bl1), the second barbel length (Bl2), the third barbel length (Bl3), pre dorsal distance (pD), post dorsal distance (poD), pre ventral distance (pV), post ventral distance (poV), pre anal distance (pA), post anal distance (poA), length of dorsal fin base (lD), length of dorsal fin (lD), dorsal fin depth (hD), length of anal fin base (lA), anal fin depth (Ah), length of anal fin (hA), ventral fin length (Vl), pectoral fin length (Pl), length between the pectoral and ventral fins (PVL), length between the ventral and anal fins (PA), lengths between the dorsal and anal fins (DA, PA) and the diameter of the caudal peduncle (TP). Moreover, ten meristic characters were counted in each sample using a stereomicroscope. Abbreviations used for meristic characteristics are: UnBRDF unbranched dorsal fin rays (spines), BRDF branched dorsal fin (soft rays), UnBRAF unbranched anal fin, BRAF branched anal fin, UnBRVF unbranched ventral fin, BRVF branched ventral fin, UnBRPF unbranched pectoral fin, BRPF branched pectoral fin, UnBRCF unbranched caudal fin, BRCF branched caudal fin. Body weight (g) was measured on a digital scale with 0.01 g accuracy and morphometric measurements were performed using digital calipers with an accuracy of 0.01 mm. After measurement, sex was determined based on external dimorphic characteristics including the occurrence of *lamina circularis* at the base of the second pectoral fin ray in males (Nalbant and Bianco, 1998; Mousavi-Sabet et al., 2011b). This subset was used to statistically test for sexual dimorphism in morphometric characters of *Cobitis* populations using ANOVA. Fish age was assessed based on the annual growth of scales taken from the left side of the body, between the end of the pectoral fin and the beginning of the dorsal fin (Mousavi-Sabet et al., 2012b; Mousavi-Sabet et al., 2012c).

As variation should be attributable to body shape differences, and not related to the relative size of the fish, an allometric method (Elliott et al., 1995) was used to remove a size-dependent variation in morphometric characters:

$$M_{adj} = M\ (Ls / L_0)^{b}$$

where M is the original measurement, M_{adj} is the size adjusted measurement, L_0 is the standard length of the fish, Ls is the overall mean of standard length for all fish from all samples in each analysis, and b was estimated for each character from the observed data as the slope of the regression of log M on log L_0 using all fish in any group.

The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length. Univariate Analysis of Variance (ANOVA) was calculated for all morphometric characters to estimate significant differences among these populations (Zar, 1984). The morphometric characters that were significant at a high level ($P \leq 0.01$) were used for discriminant function analyses (DFA) and principal component analysis (PCA). As a complement to discriminant analysis, morphometric and meristic distances among the populations were assessed using cluster analysis (CA) (Veasey et al., 2001) by taking the Euclidean distance as a measure of heterogeneity and the UPGMA (unweighted pair group method with arithmetical average) technique as the clustering algorithm (Sneath and Soka, 1973). Statistical analyses for morphological data were performed using the SPSS version 16 software package, Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) (Rohlf, 1990) and Microsoft Excel 2010.

RESULTS

Descriptive data for the sex ratio, range, mean and standard error (SE) of body length and weight in case of sampled specimens are presented in Table 1.

Table 1. Descriptive data of *Cobitis* populations in the southern Caspian Sea basin (SE: standard error; N: number of specimens)

Locality	Sex	N	Standard Length (mm)		Body Weight (g)	
			Min – Max	Mean ± SE	Min - Max	Mean ± SE
	Males	4	50.96 – 56.99	53.30 ± 1.39	1.08 – 1.74	1.34 ± 0.13
Gisum	Females	27	38.92 – 51.98	45.23 ± 0.95	0.57 – 1.12	0.821 ± 0.02
	Total	31	38.92 – 56.99	46.27 ± 0.97	0.57 – 1.74	0.889 ± 0.04
	Males	5	58.41 – 66.20	62.40 ± 1.27	1.85 – 3.17	2.48 ± 0.22
Sefidroud	Females	17	26.35 – 80.08	39.22 ± 3.77	0.25 – 4.94	0.957 ± 0.31
	Total	22	26.35 – 80.08	44.49 ± 3.59	0.25 – 4.94	1.30 ± 0.27
	Males	-	-	-	-	-
Tonekabon	Females	8	69.22 – 88.82	82.45 ± 2.47	3.15 – 5.60	4.71 ± 0.31
	Total	8	69.22 – 88.82	82.45 ± 2.47	3.15 – 5.60	4.71 ± 0.31
	Males	6	54.01 – 66.96	61.33 ± 2.00	1.77 – 2.70	2.31 ± 0.16
Siahroud	Females	7	45.65 – 75.88	62.73± 4.48	0.93 – 4.45	2.61 ± 0.53
	Total	13	45.65 – 75.88	62.08 ± 2.49	0.93 – 4.54	2.47 ± 0.28
	Males	10	52.73 – 67.13	61.68 ± 1.80	1.17 – 2.29	1.91 ± 0.12
Talar	Females	13	51.61 – 87.76	72.79 ± 3.27	1.06 – 5.59	3.20 ± 0.41
	Total	23	51.61 – 87.76	67.96 ± 2.29	1.06 – 5.59	2.64 ± 0.27

Table 2. Age composition of *Cobitis* populations in relation to sex in the southern Caspian Sea basin (M: male; F: female)

Age	Gisum		Sefidroud		Tonekabon		Siahroud		Talar	
	M	F	M	F	M	F	M	F	M	F
1+	-	-	-	9	-	-	-	-	-	-
2+	-	2	-	5	-	-	-	1	4	2
3+	-	18	5	2	-	1	4	4	3	3
4+	3	7	-	1	-	2	2	1	3	5
5+	1	-	-	-	-	5	-	1	-	3
Total	4	27	5	17	-	8	6	7	10	13

Table 3. Comparison of the relative value (Mean ± SE) of morphometric characters of *Cobitis* populations in the southern Caspian Sea basin

Character	Gisum	Sefidroud	Tonkabon	Talar	Siahroud
TL (mm)	46.27 ± 0.96	44.49 ± 3.51	82.45 ± 2.31	84.25 ± 1.50	62.08 ± 2.39
SL (mm)	39.07 ± 0.68	38.31 ± 3.16	71.73 ± 3.03	71.58 ± 1.30	52.79 ± 2.27
Standard Length %					
Total length	118.55 ± 0.71	116.82 ± 0.43	114.95 ± 0.58	115.69 ± 0.39	117.97 ± 0.83
Head length	23.37 ± 0.19	22.59 ± 0.38	19.52 ± 0.27	19.80 ± 0.00	21.68 ± 0.34
Head depth	12.55 ± 0.22	13.15 ± 0.09	11.62 ± 0.26	12.83 ± 0.01	13.57 ± 0.24
Predorsal distance	53.15 ± 0.36	52.82 ± 0.41	52.55 ± 0.59	52.09 ± 0.03	50.98 ± 0.66
Postdorsal distance	38.61 ± 0.30	39.00 ± 0.39	40.48 ± 0.59	39.26 ± 0.35	39.50 ± 0.34
Body depth	14.93 ± 0.16	15.26 ± 0.36	15.57 ± 0.24	16.44 ± 0.53	17.47 ± 0.27
Preanal distance	79.94 ± 0.08	80.67 ± 0.39	80.83 ± 0.66	80.23 ± 0.39	81.77 ± 0.66
Caudal peduncle height	8.97 ± 0.14	8.91 ± 0.23	8.87 ± 0.45	9.68 ± 0.11	10.09 ± 0.21
Caudal peduncle length	13.56 ± 0.20	14.04 ± 0.28	14.51 ± 0.42	13.19 ± 0.24	12.96 ± 0.25
Dorsal fin length	18.18 ± 0.28	18.95 ± 0.37	14.83 ± 1.50	18.11 ± 0.28	19.29 ± 0.25
Dorsal fin height	9.44 ± 0.29	8.25 ± 0.23	9.26 ± 0.29	9.75 ± 0.26	11.67 ± 0.44
Anal fin length	14.00 ± 0.24	13.59 ± 0.25	12.79 ± 0.39	13.45 ± 0.18	14.54 ± 0.02
Anal fin height	6.78 ± 0.19	6.40 ± 0.15	6.59 ± 0.20	6.68 ± 0.17	7.85 ± 0.21
Pectoral fin length	13.91 ± 0.31	14.51 ± 0.31	13.08 ± 0.58	14.78 ± 0.47	16.54 ± 0.60
Ventral fin length	12.36 ± 0.19	11.99 ± 0.27	11.67 ± 0.31	12.16 ± 0.29	13.71 ± 0.34
Pectoventral distance	32.63 ± 0.32	31.81 ± 0.50	33.43 ± 1.63	33.62 ± 0.42	32.46 ± 0.66
Ventral-anal distance	26.42 ± 0.21	26.60 ± 0.33	27.34 ± 0.54	26.47 ± 0.23	28.09 ± 0.47
Head Length %					
Snout length	38.43 ± 0.63	36.46 ± 0.90	41.15 ± 0.71	40.44 ± 0.68	39.12 ± 1.14
Eye diameter	17.50 ± 0.40	18.30 ± 0.69	18.64 ± 0.59	19.62 ± 0.29	19.16± 0.52
Postorbital length	53.44 ± 1.13	53.38 ± 1.21	45.88 ± 1.05	42.03 ± 0.05	46.74 ± 0.94
Head depth	53.71± 0.82	58.58 ± 0.94	59.51 ± 1.07	64.94 ± 0.10	62.62 ± 0.91
Interorbital length	10.01 ± 0.75	17.77 ± 0.49	21.18 ± 0.47	17.56 ± 0.29	20.45 ± 0.25
First barbel length	14.11 ± 0.37	11.94 ± 0.50	13.88 ± 0.78	15.44 ± 0.63	15.50 ± 0.50

The age of the samples ranged from 1^+ to 5^+ years. The age composition of *Cobitis* populations in relation to sex is presented in Table 2.

The morphometric and meristic characters are presented in Table 3 and Table 4, respectively.

Testing the interaction between variables and sexes from 97 sex-recorded fish revealed that morphometric measurements did not differ significantly, demonstrating a negligible effect of sex on observed variation. Only hw, pD, PL, pA and DA measurements showed significant differences between male and female specimens (P<0.05). Therefore the data were standardized and analysed without distinguishing the sexes. Following allometric transformation, there was no significant correlation between standard length and morphometric measurements, indicating that the size effect was effectively removed from the data with the allometric transformation (Table 5).

Univariate analysis of variance (ANOVA) revealed significant differences (P<0.05) with varying degrees among the means of the samples from the

Table 4. Comparison of meristic character values (Mean ± SE) of *Cobitis* populations in the southern Caspian Sea basin

Character	Gisum	Sefidroud	Tonkabon	Talar	Siahroud
UnBRDF	1.87 ± 0.06	1.00 ± 0.00	1.00 ± 0.00	2.07 ± 0.06	1.15 ± 0.10
BRDF	7.29 ± 0.08	6.95 ± 0.04	6.87 ± 0.11	6.06 ± 0.05	6.61 ± 0.16
UnBRAF	1.80 ± 0.07	1.04 ± 0.04	1.25 ± 0.15	2.11 ± 0.05	1.53 ± 013
BRAF	5.74 ± 0.07	5.27 ± 0.09	6.37 ± 0.16	4.96 ± 0.08	5.07 ± 0.07
UnBRPF	1.00 ± 0.00	1.00 ± 0.00	1.12 ± 0.11	1.00 ± 0.00	1.00 ± 0.00
BRPF	7.09 ± 0.08	6.86 ± 0.07	6.37 ± 0.16	5.10 ± 0.06	6.69 ± 0.16
UnBRVF	1.00 ± 0.00	1.09 ± 0.06	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
BRVF	6.58 ± 0.08	5.72 ± 0.09	6.12 ± 0.11	6.93 ± 0.05	5.84 ± 0.18
BRCF	14.93 ± 0.13	14.18 ± 0.10	14.37 ± 0.16	14.66 ± 0.18	14.38 ± 0.13

Table 5. The results of ANOVA for morphometric measurements between sexes of *Cobitis* populations in the southern Caspian Sea basin

Morphometric measurements	F value	P value	Morphometric measurements	F value	P value
H	2.645	0.108	poA	2.789	0.099
BW	0.248	0.620	pV	2.656	0.107
Cpl	0.442	0.508	poV	0.656	0.420
h	0.101	0.752	Dl	0.086	0.770
hl	3.410	0.068	hD	0.818	0.368
***hw**	**4.858**	**0.030**	lD	1.151	0.286
hd	1.527	0.220	hA	1.270	0.263
prO	0.000	0.995	Al	0.001	0.974
Ed	0.001	0.977	Ah	0.997	0.321
Pol	0.503	0.480	Vl	0.023	0.880
iol	0.321	0.572	**Pl**	**5.431**	**0.022**
BL1	0.100	0.752	PVL	2.194	0.142
BL2	0.106	0.746	**DA**	**4.642**	**0.034**
BL3	0.296	0.588	PA	0.939	0.335
pD	**6.627**	**0.012**	AD	0.000	0.989
poD	0.139	0.710	TP	0.049	0.826
pA	**5.913**	**0.017**			

*Highlighted data shows significant differences between the sexes (P<0.05)

Table 6. The results of ANOVA for morphometric measurements among *Cobitis* populations in the southern Caspian Sea basin

Morphometric character	F value	P value	Morphometric character	F value	P value
H	15.922	0.000	poA	3.953	0.006
BW	4.816	0.002	pV	10.994	0.000
Cpl	3.552	0.010	poV	1.398	0.242
h	7.318	0.000	Dl	6.242	0.000
hl	5.614	0.000	hD	10.174	0.000
hw	5.322	0.001	lD	3.923	0.006
hd	10.321	0.000	hA	4.436	0.003
prO	5.067	0.001	Al	4.684	0.002
*Ed	1.995	0.103	Ah	11.233	0.000
Pol	2.011	0.101	Vl	7.887	0.000
iol	0.564	0.690	Pl	17.510	0.000
BL1	5.767	0.000	Pvl	6.699	0.000
BL2	4.902	0.001	DA	3.621	0.009
BL3	5.494	0.001	PA	4.215	0.004
pD	24.960	0.000	AD	5.983	0.000
poD	0.536	0.710	TP	7.416	0.000
PA	15.318	0.000			

* Highlighted data show there is no significant difference among the stations (P>0.05)

five localities for 28 of 33 standardized morphometric measurements (Table 6). The traits that were highly significant (P≤0.01) were used for DFA, PCA and CA analysis.

To determine whether the number of samples is sufficient for PCA and DFA analysis, researchers of theoretical works on PCA and DFA have recommended that the ratio of the number of organisms measured (N) relative to the parameters included (P) in the analysis is at least 3–3.5 (Johnson, 1981; Kocovsky et al., 2009). Small N values may fail to adequately capture covariance or morphological variation, which may lead to false conclusions regarding differences among groups (Mcgarigal et al., 2000). In this study, morphometric characters that were highly significant at the level (P≤0.01) were used for the multivariate analysis, and under these conditions the N:P ratio was 4.40 (97/22). The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy is another useful method to show the appropriateness of data for factor analysis. KMO coefficients greater than 0.6 indicate that the PCA method is suitable for the data (Kasier, 1974). In this study, the KMO value obtained was 0.768 for the morphometric data. This suggests that the sampled data are appropriate to proceed with a factor analysis procedure.

In order to determine which morphometric measurements most effectively discriminate among these populations, the contributions of variables to the principal components (PCA) were examined. Principal component analysis of 22 morphometric measurements extracted six factors with eigenvalues >1, explaining 64.457% of the variance (Table 7).

Table 7. Eigenvalues, percentage of variance and percentage of cumulative variance for the 6 PC for morphometric measurements for *Cobitis* populations in the southern Caspian Sea basin

Factor	Eigenvalues	Percentage of variance	Percentage of cumulative variance
PC1	5.921	26.915	26.915
PC2	2.284	10.380	37.295
PC3	1.960	8.909	46.204
PC4	1.607	7.303	53.507
PC5	1.286	5.844	59.351
PC6	1.123	5.105	64.457

The principal component analysis of 22 morphometric characters for PC1 and PC2 explained 26.915% and 10.380% of the variation, respectively (Table 7). The most significant loadings on PC1 were from BL1, BL2, BL3, hD, Ah, VL and PL, and on PC2 were from H, h, hd, Ed, DL, hA, VL and PL (Table 8).

Table 8. Results of factor extractions in PCA after Varimax normalized rotation

Morphometric measurements	Component					
	PC1	PC2	PC3	PC4	PC5	PC6
H		0.659				
CPL						0.728
h		0.792				
hl				0.669		
hd		0.686				
prO				0.871		
Ed		0.527				-0.500
Pol				0.610		
BL1	0.651					
BL2	0.748					
BL3	0.755					
pD			-0.482	0.401		
Dl		0.579				
hD	0.630					
lD			0.485		0.462	
hA		0.460	0.575			
AL			0.747			
Ah	0.783				0.765	
Vl	0.456	0.442	0.446		0.752	
PL	0.456	0.622				

The rotated (Varimax) component loadings for the six components (factors) are presented in Table 8. Visual examination of plots of PC1 and PC2 scores revealed that the specimens were deviated, with some degree of overlap among populations of the same species (Figure 2).

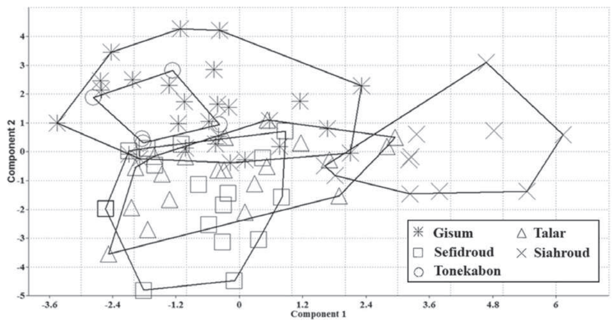

Fig 2. Plot of the factor scores for PC1 and PC2 of morphometric measurements for *Cobitis* populations in the southern Caspian Sea basin (Gisum: *Cobitis* sp.; Sefidroud, Tonekabon and Talar: *C. keyvani*; Siahroud: *C. faridpaki*)

In this analysis, only characteristics with eigenvalues exceeding 1 were included (Table 8). Eigenvalues were plotted against the factors arranged in descending order along the X-axis. Figure 3 shows the scree diagram for the selection of the principal components.

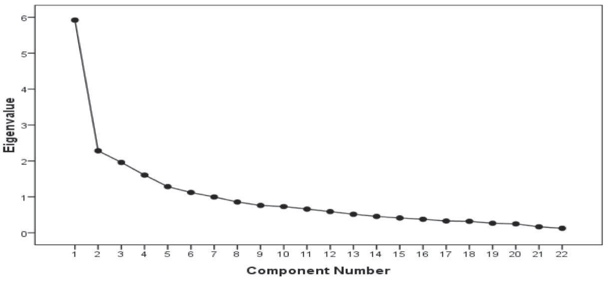

Fig 3. Screen plot of the principal components for morphometric measurements for *Cobitis* populations in the southern Caspian Sea basin

The dendrogram derived from the cluster analysis of Euclidean distances among the groups of centroids showed that there were three different main morphological types of *Cobitis* in the southern Caspian Sea basin which are related to the identified and misidentified species in the basin, including *Cobitis* sp. (Gisum) from the southwestern areas, *Cobitis keyvani* (Sefidroud, Tonekabon and Talar) from the south central areas and *Cobitis faridpaki* (Siahroud) from the southeastern areas, that were partly distinct from one another with respect to morphometric characters (Figure 4).

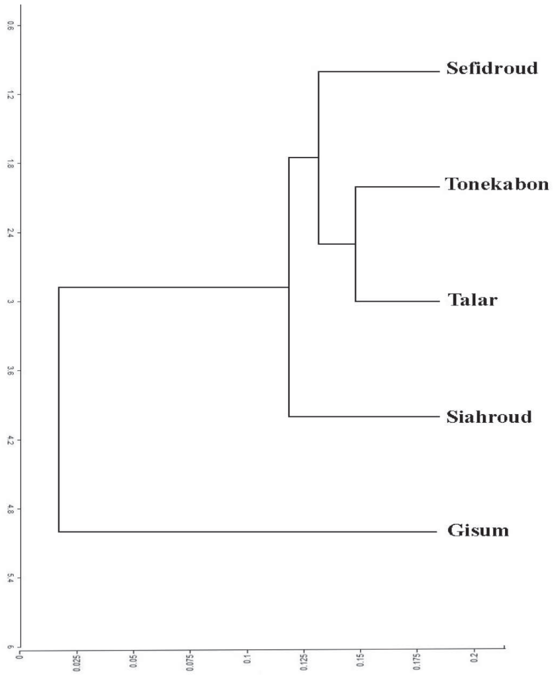

Fig 4. Dendrogram derived from cluster analyses of 22 morphometric variables on the basis of Euclidean distance for *Cobitis* populations in the southern Caspian Sea basin (Gisum: *Cobitis* sp.; Sefidroud, Tonekabon and Talar: *C. keyvani*; Siahroud: *C. faridpaki*)

The Wilks' lambda test of discriminant analysis was used to compare the means of each variable among groups that indicated significant differences in morphometric characters of the five populations, with a significant level set at $P \leq 0.01$. Statistically significant differences were found among the studied populations according to DFA analysis (Figure 5).

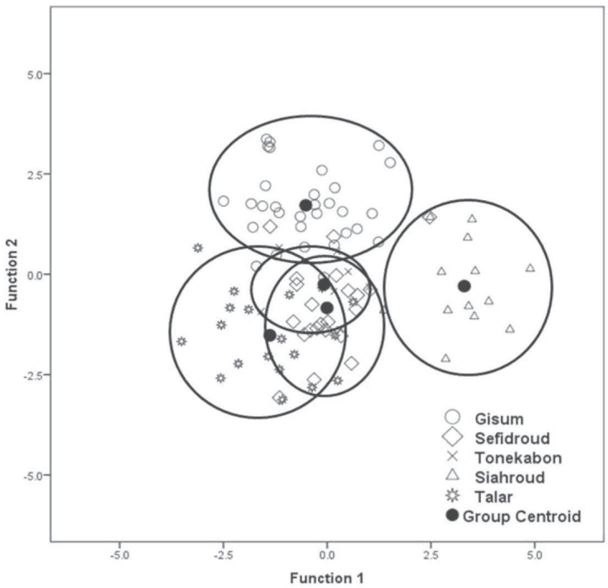

Fig 5. Representation of the canonical discriminant functions 1 and 2 (Gisum: *Cobitis* sp.; Sefidroud, Tonekabon and Talar: *C. keyvani*; Siahroud: *C. faridpaki*)

The overall random assignment of individuals into their original groups was high (84.9%). The proportions of individuals correctly classified into their original groups were highest for *C. keyvani* (Tonekabon) and *C. faridpaki* (Siahroud) specimens (100%), while 89.5% of *C. keyvani* (Talar) specimens were correctly classified. These high values indicate that these populations are highly divergent from one another (Table 9).

Results of one-way analysis of variance (ANOVA) for meristic data (between sexes and among localities) showed that the characteristic branched anal fin rays were significantly different between the sexes ($p<0.05$). Statistically significant differences were found among the studied populations ($p<0.05$) for all meristic characteristics, except the unbranched pelvic fin.

DISCUSSION

In this study, the ANOVA test showed that the relations between morphometric characteristics and sexes were significant in five characters, i.e. hw, pD, PL, pA and DA ($p<0.05$). Males and females often

Table 9. Percentage of specimens classified in each group and after cross validation for morphometric data

Locality	Morphometric				
	Gisum (%)	Sefidroud (%)	Tonekabon (%)	Siahroud (%)	Talar (%)
Original					
Gisum	77.4	12.9	9.7	0.0	0.0
Sefidroud	9.1	77.3	0.0	4.5	9.1
Tonekabon	0.0	0.0	100.0	0.0	0.0
Siahroud	0.0	0.0	0.0	100.0	0.0
Talar	0.0	10.5	0.0	0.0	89.5
Cross Validated					
Gisum	67.7	16.1	9.7	3.2	3.2
Sefidroud	9.1	68.2	4.5	4.5	13.6
Tonekabon	12.5	25.0	50.0	0.0	12.5
Siahroud	0.0	7.7	0.0	92.3	0.0
Talar	5.3	21.1	0.0	0.0	73.7

exhibit broadly parallel patterns of population divergence along environmental evolution (Quinn et al., 2001; Alves and Belo, 2002; Gilchrist et al., 2004) and yet this is not always the case (Butler and Losos, 2002). Water flow was the second most important environmental gradient for both sexes. At high flow sites, males and, to a lesser extent, females had smaller heads. This may be adaptive as these traits increase thrust and reduce drag (Walker, 1997; Langerhans et al., 2004). The dorsal fin location is related to the vertical position in the water column, with posteriorly-placed dorsal fins representing adaptations to surface habitats in non-flowing waters (Matthews, 1998). In some species, smaller fins may be favoured in stream-dwelling individuals as the reduction of drag during swimming more than compensates for their reduced power and propulsion efficiency in a current. Smaller fin size in stream-dwelling Centrarchids may be related to their body shape, or to their low usage of fast-moving water within the streams they inhabit (Brinsmead et al., 2002). In fish, a direct relationship exists between swimming behaviour and feeding habits, and the development of body shape is dependent on the type and the manner in which it is obtained (Keast and Webb, 1966).

The obtained results from the ANOVA analysis showed that 28 of 33 transformed morphometric data were significantly different ($p<0.05$) among the five populations located along the southern Caspian Sea basin, which demonstrates high phenotypic variation among them. It is commonly accepted that morphological variation has both environmental and genetic components, though stable differences in shape between groups of fish may reveal different growth, mortality or reproductive rates that are relevant for the definition of stocks (Swain and Foote, 1999; Cadrin, 2000). Morphometric features may also vary among cohorts, because the environment at the time of spawning and juvenile development changes between years (Meng and Stocker, 1984; Austin et al., 1999). Samaee et al. (2006) reported that *Capoeta capoeta gracilis* located in the Aras, Sefidroud, Shiroud, Tonekabon, Haraz and Gorganroud Rivers in Iran were separated, and they stated that different environmental and habitat conditions, such as temperature, turbidity, food availability, water depth and flow in different rivers, caused differentiation between populations. The inter-basin differences in maximum length and weight of the fish may be due to different availability of food resources, growth rate and natural selection that favours larger size in some rivers (Patimar et al., 2009). Hence, the effects of some environmental factors, such as temperature, salinity, food availability and migration distance, can potentially determine the morphometric segregation of fish (Turan et al., 2006).

PCA is another multivariate analysis that confirmed these results. The visual examination of plotted PC1 and PC2 scores for each sample revealed the distinctiveness of the populations, with some degree of overlap. This deviation might be related to the geographic isolation, which may have resulted in restricted intermingling and subsequent morphological differentiation. The overlap of characters of different populations of the same species in discriminant space may suggest a sufficient degree of intermingling among these localities to homogenize populations, and absence of sufficient time to generate phenotypic differentiation among the populations, since the fish habitats have similar environmental conditions.

The UPGMA tree based on Euclidian Distance coefficients for morphometric characters showed that the populations of *Cobitis* in this region deviated. It seems that allopatric speciation caused the population differentiation of the genus *Cobitis* in the southern Caspian Sea basin. When a species has a more or less continuous distribution across a range, the balance between gene flow and the forces responsible for population differentiation, such as genetic drift or differential selection, may result in clines, whereby genetic differentiation increases with geographic distance (Borsa et al., 1997; Pinheiro et al., 2005). According to morphometric data in the UPGMA tree, all five populations were grouped into two main branches. In the first branch, the specimens of the Talar and Tonekabon Rivers (*C. keyvani*) were clustered as closest taxa, as a sister group to the specimens of the Sefidroud River (*C. keyvani*). In the second branch, the specimens of the Siahroud River (*C. faridpaki*) were clustered as separate species. Moreover, the specimens of the Gisum River (*Cobitis* sp.) were clustered as most differentiated species from all other localities.

The meristic results also confirm that *Cobitis* populations from the southern Caspian Sea basin are distinct from one another. Environmental differences that isolate stocks and cause limited intermingling between areas may have contributed to the detected differentiation; for example, southern Turkish waters (northeastern Mediterranean) have higher temperatures than northern Turkish waters (Aegean, Marmara and Black Seas), and Fahy (1980) reported a temperature effect on the number of dorsal fin rays developing in *Fundulus majalis*.

In conclusion, the results of the present study revealed that habitat fragmentation by geographical factors and change in environmental conditions lead to this complicated distribution pattern. The distribution of *Cobitis* species in the southern Caspian Sea basin includes: southeastern populations - *C. faridpaki*, the south central ones - *C. keyvani* and an unknown population, *Cobitis* sp., distinguished from the southwestern parts of the basin. The Tonekabon and Sefidroud Rivers were found to be the two new habitats for *C. keyvani* in the region. The present study provides basic information about the morphometric differentiation of *Cobitis* populations in the basin and suggests that the observed morphological variations should be considered in fisheries management and the preservation of these fishes.

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Sažetak

RASPODJELA I STATUS VIJUNA (*Actinopetrigii*: Cobitidae) U JUŽNOM SLIVU KASPIJSKOG JEZERA

Kako bi se razjasnila raspodjela populacije vrste *Cobitis* u južnom dijelu Kaspijskog jezera, morfološki je ispitano 97 jedinki te vrste, prikupljenih s pet različitih lokaliteta. Jednosmjerna analiza odstupanja pokazala je značajne razlike (P<0,05) između srednjih vrijednosti pet skupina u 28 od 33 standardiziranih morfometrijskih mjerenja, te 7 od 10 merističkih izračuna. Prilikom linearne diskriminantne funkcijske analize (DFA) prema morfometrijskim karakteristikama, ukupna podjela jedinki u njihove izvorne skupine bila je visoka (84,9%), što ukazuje na to da se te populacije znatno razlikuju. Udio pojedinačnih vrsta koje su ispravno klasificirane u svoje izvorne skupine bio je 77,4% kod vrste *Cobitis* sp. (rijeka Gisum), 77,3% kod vrste *Cobitis keyvani* (rijeka Sefidroud), 100% kod vrste *C. keyvani* (rijeka Tonekabon), 100% kod vrste *Cobitis faridpaki* (rijeka Siahroud) i 89,5% kod vrste *C. keyvani* (rijeka Talar). Grupiranjem na temelju euklidskih udaljenosti između ovih pet skupina centroida, koristeći rezultate UPGMA i analize glavnih sastavnica (PCA), utvrđeno je da *C. faridpaki* spadaju u jugoistočne kaspijske populacije vijuna, da *C. keyvani spadaju* u centralni južni dio jezera te da se nepoznata populacija, *Cobitis* sp., razlikuje od jugozapadnih populacija sliva Kaspijskog jezera. Rijeke Tonekabon i Sefidroud su dva nova staništa za vrstu *C. keyvani* u regiji.

Ključne riječi: *Cobitis*, morfometrijske razlike, meristika, obrazac raspodjele, Kaspijsko jezero

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