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Landmark-based morphometric variation between *Cobitis keyvani* and *Cobitis faridpaki* (Pisces: Cobitidae), with new habitat for *C. faridpaki* in the southern Caspian Sea basin

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Abstract. A 13-landmark morphometric system was used for 617 specimens to investigate the hypothesis of species differentiation among *Cobitis keyvani*, *Cobitis faridpaki* and an unknown *Cobitis* sp. population from the River Babolrud in the southern Caspian Sea basin. Univariate analysis of variance showed significant differences among the means of the three groups for 41 out of 78 standardized morphometric measurements. In discriminant function analysis, the overall assignment of individuals into their original groups was 58.0 %. The proportions of individuals correctly classified into their original groups were 58.1 %, 64.7 % and 44.1 % for *Cobitis* sp., *C. faridpaki* and *C. keyvani*, respectively. Principal component analysis (PCA) and canonical variates analysis (CVA) confirmed the significant difference among these populations (λ de Wilks = 0.42, P = 0). The CVA scatter plot showed 617 specimens grouped into three distinct areas with some overlap. Clustering based on Euclidean distances among the groups of centroids using an UPGMA indicated segregation of the three populations into two distinct clusters: *Cobitis* sp. and *C. faridpaki* in one group and *C. keyani* in other group. Also the unknown population of spined loach from the River Babolrud was recognized as *C. faridpaki* and the river as a new habitat for the species.

Key words: spined loach, truss network system, geometric morphometric, Iran

Introduction

The study of morphological characters, whether morphometric or meristic, with the aim of defining or characterizing fish stock units, has for some time been a strong interest in ichthyology (Tudela 1999, Randall & Pyle 2008, Vishalakshi & Singh 2008, Simon et al. 2010). Morphological characters are most important in the identification and taxonomy of fishes, and the only known facts about many fishes. In addition understanding the function of a morphological structure is a stronghold for practical use in taxonomy and ecology (Bohlen 2008). Spined loaches of the *Cobitis* genus (family Cobitidae) are small benthic freshwater fishes with a wide distribution area covering large parts of Eurasia and Africa (Doadrio & Perdices 2005). Spined loach is of particular interest from a conservation point of view in many areas of its native range (Copp & Wade 2006). Spined loach during the day remains buried in sand, mud or dense weed growths, being active at night, and is mostly solitary (Coad 2013). To date, three valid species of the genus *Cobitis* are represented in Iran. These are *Cobitis linea* Heckel, 1849, *Cobitis faridpaki* Mousavi-Sabet et al., 2011 and *Cobitis keyvani* Mousavi-Sabet et al., 2012. *C. faridpaki* and *C. keyvani* are found in south of the Caspian Sea basin (Mousavi-Sabet et al. 2011b, 2012a). Spined loaches are distributed in the lower reaches of rivers along the Caspian Sea basin (Jolodar & Abdoli 2004, Patimar et al. 2011), but their taxonomic identification is unknown in some rivers of the basin (Esmaeili et al. 2010, Abdoli et al. 2011). *C. faridpaki* and *C. keyvani* are endemic species and they are distributed in the lower streams of Siahrud and Talar rivers, respectively (Mousavi-Sabet et al. 2011b, 2012a). *C. keyvani* is distinguished from the *C. faridpaki* by large, dark and obvious spots along the mid-flank (Mousavi-Sabet et al. 2012a, Coad 2013). Also a population of *Cobitis* sp. is reported from

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the River Babolrud in the same basin which is not identified (Mousavi-Sabet et al. 2011a). In addition, *C. linea* Heckel, 1849 is found in the River Kor and the Hormozgan basins (Bianco & Nalbant 1980).
In recent years, truss network system is increasingly used for morphometric measurements with the purpose of species and/or stock differentiation (e.g. Parsons et al. 2003, Turan & Erguden 2004, Mustafić et al. 2008, Akbarzadeh et al. 2009, Hossain et al. 2010, AnvariFar et al. 2011). The truss network system entails the whole fish body in uniform network, and increases the possibility of extracting morphometric differences within and between species (Turan 2000). Mustafić et al. (2008) described *Cobitis jadovaensis* as a new species and compared it with other *Cobitis* species using the truss network system. The comparative morphological analysis corresponded with mtDNA analysis of the Jadova River population and other Croatian spined loach species (Buj et al. 2008). Geometric morphometrics (GM), a quantitative approach to analysis shape, is widely applied to compare and determine shape variations of biological structures (Sansom 2009). Despite of traditional approaches, in GM, data is obtained from the coordinates of landmark points (Rohlf & Marcus 1993, Adams et al. 2004), which are morphological points of specimens that are of biological interest (Richtsmeier et al. 2002). GM has been used in various studies on fish population biology such as stock identification and discrimination (Cadrin & Friedland 1999). This method, which allow the study of shape and size, offers powerful analytical and graphical tools for the quantification and visualization of morphological variation within and among organisms (Slice 2007).
On the other hand, studies carried out on the Caspian Sea fishes showed that many of species reveals speciation and population differentiation microprocess runs on, as the Black Sea species (Gholiev 1997). The Caspian species have differentiated into subspecies in different parts of the Caspian Sea basin (Gholiev 1997). There are several reports about the southern Caspian Sea fishes indicating the existence of morphological variability between different parts of this basin (e.g. Samaee et al. 2006, 2009, Rahmani & Abdoli 2008, Akbarzadeh et al. 2009, AnvariFar et al. 2011). In respect of the recent descriptions of two new species of *Cobitis* genus from the southern Caspian Sea basin (Mousavi-Sabet et al. 2011b, 2012a), an assessment of morphometric differentiation of indigenous the fish species using multivariate mathematical approach has not been exploited. Variability of these species and their spatial distribution has not been studied in the basin.
Therefore the present investigation aimed (i) at examining the morphometric variability between *C. faridpaki* and *C. keyvani* in south of the Caspian Sea basin, Iran (ii) finding distance measurements that may also be useful characters to differentiate these two closely related species (iii) detection of the unknown *Cobitis* population from the River Babolrud (iiii) determination of the best subset of all available morphometrics by using stepwise discriminant analysis.

Material and Methods

Sample collection

A total of 617 *Cobitis* specimens (which were previously collected monthly during 2009-2010) from three sampling sites, including the River Babolrud (36°24′46.64″ N, 52°42′18.81″ E 188 specimens), the River Siahrud (36°30′36.75″ N, 52°53′50.75″ E 225 specimens) and the River Talar (36°10′57.69″ N, 52°59′58.18″ E 203 specimens) in the southern Caspian Sea basin (Fig. 1) were studied. In order to do morphological analysis, the sampled fishes were fixed in 10 % formaldehyde and sent to the ichthyology laboratory of Guilan University. Sexes of the specimens were determined according to occurrence the Canestrini scale (*lamina circularis*) and examinations of gonad morphology after

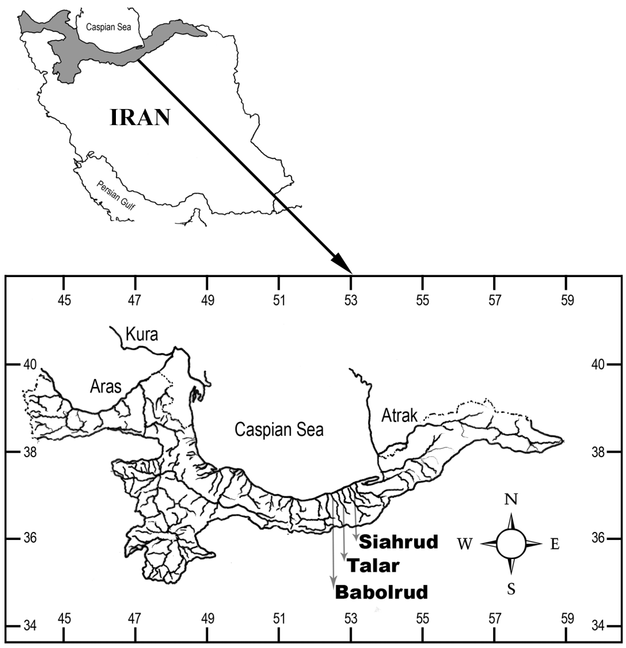

Fig. 1. Map of the Iranian part of the southern Caspian Sea basin showing some important rivers: Aras, Kura, and Atrak rivers and the location of sampling sites including Babolrud, Talar and Siahrud rivers.

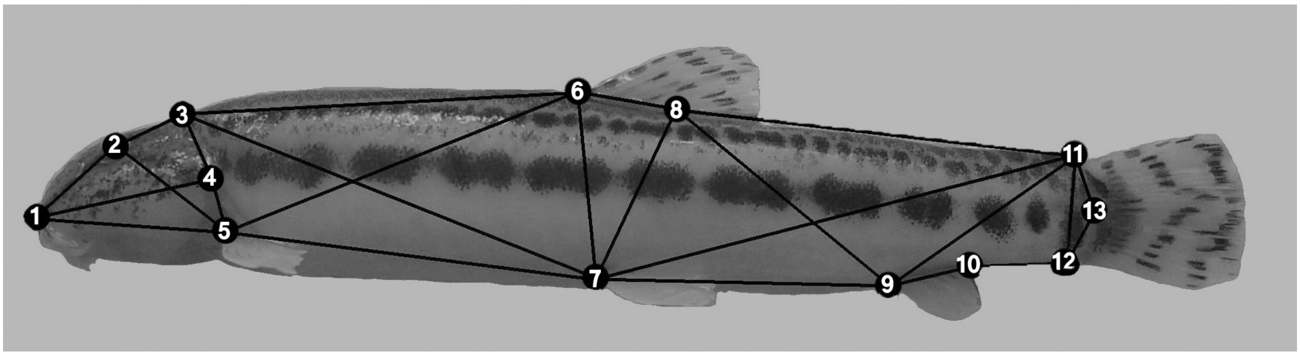

Fig. 2. Digital image of a spined loach depicting the thirteen landmarks and associated box truss used to infer morphological differences among populations. 1. Tip of snout, 2. Center of eye, 3. Forehead (end of frontal bone), 4. End of operculum, 5. Dorsal origin of pectoral fin, 6. Origin of dorsal fin, 7. Origin of pelvic fin, 8. Termination of dorsal fin, 9. Origin of anal fin, 10. Termination of anal fin, 11. Dorsal side of caudal peduncle at the nadir, 12. Ventral side of caudal peduncle at the nadir, 13. End of caudal peduncle.

dissection (Mousavi-Sabet et al. 2011b, 2012a). The *lamina circularis* is an ossified broadened piece at the base of the second (in some species also the first) ray of pectoral fins, which is a secondary sexual character of males (Canestrini 1871, Gengjiao et al. 2010, Mousavi-Sabet et al. 2011b, 2012a). This subset was used in order to reveal sexual dimorphism statistical effects on morphometric characters of the spined loach by ANOVA test.

Laboratory Work

A total of 78 distance measurements between 13 landmarks were surveyed using truss network system according to Strauss & Bookstein (1982) with minor modification for these species (Fig. 2). In order to investigate body shape of the specimens the same 13 homologous landmark-points were used (Fig. 2). The landmark-points were chosen to represent the external shape of the body, which were at the specific points in order to extracting a proper model of fish body shape. Fishes were placed on a white board with dorsal and anal fins erected by pinning. The left body profile of each specimens was photographed (before dissection) in 300-dpi, 32-bit colour by digital camera (Sony Cybershot DSC-F505, Sony, Japan). Images were saved in *.jpg format and analyzed using tpsDig2 software version 2.16 (Rohlf 2005) to coordinates of 13 landmarks. A box truss of 24 lines connecting these landmarks was generated for each fish to represent the basic shape of the fish (Cardin & Friedland 1999).

Statistical analysis

The extracted landmark-points (body shape data) were submitted to a generalized Procrustes analysis (GPA) to remove non-shape data in PAST software. In respect of truss network measurements, 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 size-dependent variation in morphometric characters:

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

where M is original measurement, M_{adj} is the size adjusted measurement, L_0 is the standard length of the fish, Ls 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 performed for each morphometric character to

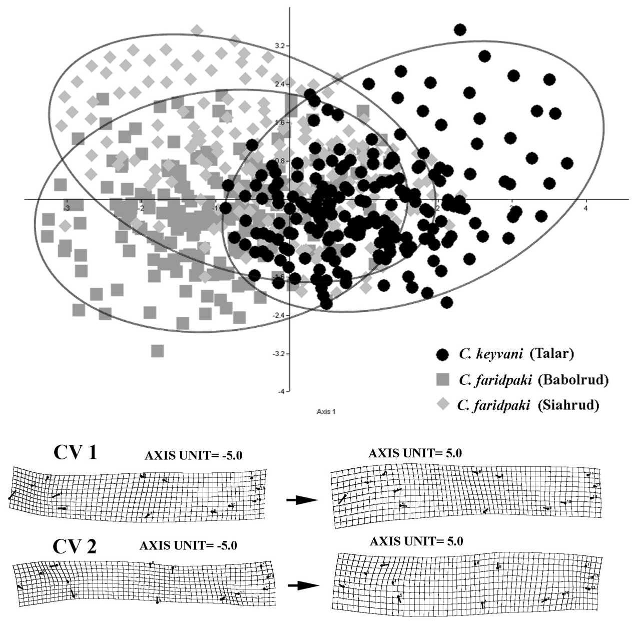

Fig. 3. Canonical variate analysis of each *Cobitis* species (above). The Procrustes deformations between species based on the first two canonical variates (below). Axis units for deformation grids are also shown.

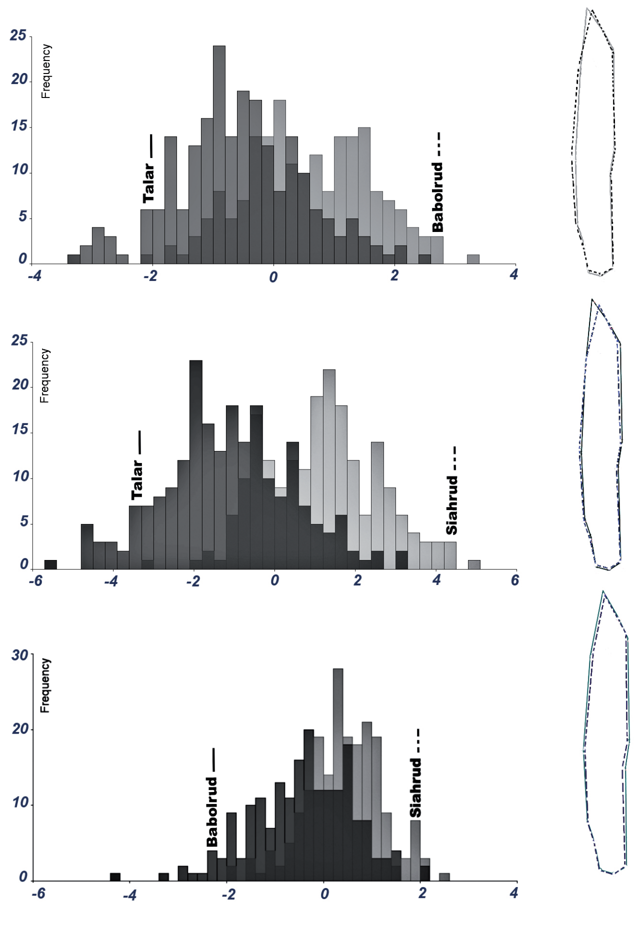

Fig. 4. Histogram of discriminate analysis (DA) functions for pair wise competitions' among *C. keyvani* from the River Talar and two populations of *C. faridpaki* from Siahrud and Babolrud rivers (left). Shape differences on the extremities of each species/population (right).

evaluate the statistical significance of individual morphometric characters among the three groups. The morphometric characters which showed significant variation ($P < 0.05$) only were used for obtaining the stable outcome from multivariate analysis.

Morphometric measurements will undergo a data discarding process using PCA to reduce the dataset (Veasey et al. 2001), to decrease redundancy among the variable (Samaee et al. 2006) and to extract a number of independent variable as well as the important variable in population differentiation (Samaee et al. 2009). The Wilks' lambda was used to compare the difference among all groups. The resultant discriminant function was used to calculate the percentage of correctly classified (PCC) fish. A cross-validation using the leaving one-out procedure was done to estimate the expected actual error rates of the classification functions. As a complement to discriminant analysis, morphometric distances among the individuals of three groups were inferred to cluster analysis (Veasey et al. 2001) by adopting the Euclidean distance as a measure of dissimilarity and the UPGMA (un-weighed pair group method with arithmetical average) method as the clustering algorithm. Patterns of morphometric discrimination were examined by canonical analysis or multiple discriminant analysis (Neff & Marcus 1980). Statistical analyses for morphometric data were performed using the SPSS version 16 software package, past ver. 1.36, numerical taxonomy and multivariate analysis system (NTSYS-pc), MorphoJ and Excel (Microsoft Office 2010).

Results

Descriptive data for the mean length and standard deviation (SD) and length range of sampled specimens are shown in Table 1. There was no significant correlation between any of the transformed measured morphometric variables and standard length ($P > 0.05$) which indicated that the size effect was removed. Although it is well known that the female and male specimens of the fish have some morphological differences (Mousavi-Sabet et al. 2011a, b, 2012a, b, c), but the interaction between morphometric measurements used in this study by truss network system and sexes were not significant ($P > 0.05$), demonstrating a negligible effect of sex on observed variations. Therefore data for both sexes

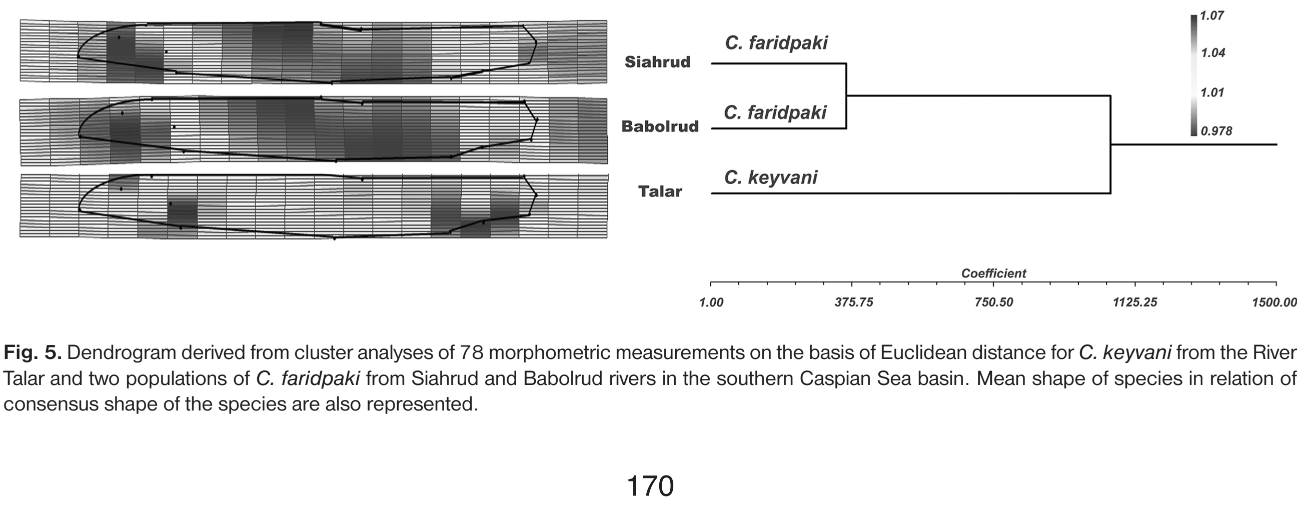

Fig. 5. Dendrogram derived from cluster analyses of 78 morphometric measurements on the basis of Euclidean distance for *C. keyvani* from the River Talar and two populations of *C. faridpaki* from Siahrud and Babolrud rivers in the southern Caspian Sea basin. Mean shape of species in relation of consensus shape of the species are also represented.

Table 1. Descriptive data (Mean ± SD and range) of *C. keyvani* from the River Talar and two populations of *C. faridpaki* from Siahrud and Babolrud rivers.

Sample	Sex	n	Range of standard length (mm)	Mean of standard length (mm) ± SD
C. keyvani (Talar)	males	89	22.655-81.752	51.486 ± 12.589
	females	114	29.081-99.147	59.333 ± 14.174
	Total	203	22.655-99.147	55.410 ± 13.588
C. faridpaki (Siahrud)	males	97	22.468-75.663	49.385 ± 10.154
	females	128	27.310-98.799	57.836 ± 12.848
	Total	225	22.468-98.799	53.611 ± 11.740
C. faridpaki (Babolrud)	males	81	17.645-67.888	46.354 ± 13.499
	females	107	32.346-95.732	53.662 ± 12.485
	Total	188	17.645-95.732	50.008 ± 16.264

Table 2. The results of ANOVA for morphometric measurements of *C. keyvani* from the River Talar and two populations of *C. faridpaki* from Siahrud and Babolrud rivers in the southern Caspian Sea basin.

Morphometric measurements	F value	P value	Morphometric measurements	F value	P value	Morphometric measurements	F value	P value
1-2	10.42	0.00	3-7	1.32	0.27	6-9	0.88	0.42
1-3	6.21	0.00	3-8	7.68	0.00	6-10	0.94	0.39
1-4	4.28	0.01	3-9	13.64	0.00	6-11	3.61	0.03
1-5	0.02	0.98	3-10	11.28	0.00	6-12	5.25	0.01
1-6	5.57	0.00	3-11	1.56	0.21	6-13	5.06	0.01
1-7	0.06	0.94	3-12	4.71	0.01	7-8	15.23	0.00
1-8	4.24	0.01	3-13	3.57	0.03	7-9	3.01	0.05
1-9	2.62	0.07	4-5	2.16	0.12	7-10	9.17	0.00
1-10	16.15	0.00	4-6	13.04	0.00	7-11	2.28	0.10
1-11	0.25	0.78	4-7	1.35	0.26	7-12	0.12	0.88
1-12	0.48	0.62	4-8	10.55	0.00	7-13	1.02	0.36
1-13	32.19	0.00	4-9	5.67	0.00	8-9	1.19	0.31
2-3	1.94	0.14	4-10	13.13	0.00	8-10	1.68	0.19
2-4	3.77	0.02	4-11	3.84	0.02	8-11	2.49	0.08
2-5	0.11	0.90	4-12	1.33	0.27	8-12	3.28	0.04
2-6	9.63	0.00	4-13	3.73	0.02	8-13	3.16	0.04
2-7	0.19	0.82	5-6	7.53	0.00	9-10	4.31	0.01
2-8	7.35	0.00	5-7	0.12	0.89	9-11	4.52	0.01
2-9	5.72	0.00	5-8	5.42	0.00	9-12	3.63	0.03
2-10	11.07	0.00	5-9	2.13	0.12	9-13	2.32	0.10
2-11	0.78	0.46	5-10	6.26	0.00	10-11	4.97	0.01
2-12	0.99	0.37	5-11	1.61	0.20	10-12	10.21	0.00
2-13	1.27	0.28	5-12	0.07	0.93	10-13	5.81	0.00
3-4	2.29	0.10	5-13	0.81	0.44	11-12	0.40	0.67
3-5	0.04	0.96	6-7	10.10	0.00	11-13	0.82	0.44
3-6	9.27	0.00	6-8	0.21	0.81	12-13	1.92	0.15

Table 3. Eigenvalues, percentage of variance and percentage of cumulative variance for the nine principal components in case of morphometric variables for *C. keyvani* from the River Talar and two populations of *C. faridpaki* from Siahrud and Babolrud rivers.

Factor	Eigenvalues	Percentage of variance	Percentage of cumulative variance
PC 1	12.780	31.170	31.170
PC 2	7.389	18.021	49.191
PC 3	5.715	13.938	63.129
PC 4	2.930	7.146	70.275
PC 5	2.372	5.785	76.061
PC 6	1.929	4.705	80.766
PC 7	1.335	3.256	84.022
PC 8	1.189	2.899	86.921
PC 9	1.120	2.732	89.653

were pooled for all subsequent analyses. Differences ($P < 0.05$) among three populations of spined loach in the Babolrud, Talar and Siahrud river systems in the southern Caspian Sea basin were observed for 41 out of 78 morphometric characters (Table 2) and these variables were used further for multivariate analysis (PCA, DFA and CVA).

In order to determine which morphometric measurement affected populations differentiates mostly, the contributions of variables to principal components (PC) were examined. The PCA of 41 morphometric measurements showed that PC I accounts for 31.17 % of the variation and PC II for 18.02 % (Table 3) and the most significant weightings

Table 4. Correlations between the measured morphometric variables and the linear discriminant functions for *C. keyvani* from the River Talar and two populations of *C. faridpaki* from Siahrud and Babolrud rivers in the southern Caspian Sea basin.

Morphometric measurements	DF1	DF2	Morphometric measurements	DF1	DF2	Morphometric measurements	DF1	DF2
1-2	–0.35*	0.13	3-9[a]	0.43*	0.17	6-12[a]	–0.18*	0.14
1-3[a]	–0.24*	–0.03	3-10	0.36*	0.14	6-13[a]	–0.16*	0.04
1-4	–0.17	0.24*	3-12	0.05	0.39*	7-8	0.17	–0.67*
1-6[a]	0.08	0.10*	4-6[a]	0.20*	–0.15	7-9[a]	0.23*	–0.12
1-8[a]	0.11*	–0.05	4-8[a]	0.23	–0.25*	7-10[a]	0.28*	–0.14
1-10[a]	0.46*	–0.04	4-9[a]	0.29*	–0.22	8-12[a]	–0.17	0.21*
1-13	0.61*	0.27	4-10[a]	0.37*	–0.20	8-13[a]	–0.14*	0.13
2-4	–0.12	0.29*	4-11	0.11*	–0.07	9-10[a]	0.15*	–0.04
2-6[a]	0.20*	0.13	4-13[a]	0.15	–0.20*	9-11[a]	–0.15*	–0.10
2-8[a]	0.22*	0.00	5-6[a]	0.21	–0.26*	9-12[a]	–0.23*	0.10
2-9[a]	0.34*	–0.03	5-8[a]	0.23	–0.34*	10-11[a]	–0.26*	–0.11
2-10[a]	0.38*	–0.01	5-10[a]	0.35*	–0.24	10-12[a]	–0.33*	0.12
3-6[a]	0.19	0.25*	6-7	–0.10	–0.57*	10-13[a]	–0.28*	–0.05
3-8[a]	0.21*	0.14	6-11[a]	–0.14*	0.13			

* Largest absolute correlation between each variable and any discriminant function. [a] This variable was not used in the analysis.

Table 5. Percentage of specimens classified in each group and after cross validation for morphometric data.

		Species (station)	Predicted group membership			
			Siahrud	Talar	Babolrud	Total
Original	Count	*C. faridpaki* (Siahrud)	146	40	39	225
		C. keyvani (Talar)	27	123	53	203
		C. faridpaki (Babolrud)	53	47	88	188
	%	*C. faridpaki* (Siahrud)	64.9	17.8	17.3	100
		C. keyvani (Talar)	13.3	60.6	26.1	100
		C. faridpaki (Babolrud)	28.2	25.0	46.8	100
Cross-validated	Count	*C. faridpaki* (Siahrud)	144	40	41	225
		C. keyvani (Talar)	30	118	55	203
		C. faridpaki (Babolrud)	54	51	83	188
	%	*C. faridpaki* (Siahrud)	64.0	17.8	18.2	100
		C. keyvani (Talar)	14.8	58.1	27.1	100
		C. faridpaki (Babolrud)	28.7	27.1	44.1	100

on PC I were from 1-2, 1-4, 2-4, 4-11, 4-13 and on PC II were from 1-10, 2-10, 3-10, 4-10, 10-11, 10-12 and 10-13. If the Kaiser-Meier-Olkin coefficient (KMO) exceeded 0.6, it was assumed that PCA method will suitable for our data (AnvariFar et al. 2011). In this study the obtained KMO coefficient was 0.63 that is explaining of appropriation of this test at good level about these measurements. In this analysis the characteristics with an eigenvector of 1 were included and others discarded.

Canonical variates analysis confirmed the significant difference among the populations (Wilks' lambda = 0.42, P = 0). The scores of the two canonical variables for each population (Fig. 3) revealed that specimens grouped into three distinct areas while there was a relativity high degree of overlap among these populations. Conspecific populations had much more overlap than heterospecific populations.

The Wilks' lambda tests indicated differences among the three populations when their morphometric measurements were compared by means of discriminant analysis. In this test all functions were highly significant ($P \leq 0.01$). For the discriminant analysis, the average of PCC was 58.0 % for morphometric characters. Medium classification success rates were obtained for *C. faridpaki* from the River Siahrud (64.7 %) and the River Babolrud (58.1 %), and for *C. keyvani* from the River Talar (44.1 %), indicating a moderately correct classification of specimens into their original populations. The histogram of discriminant functions for pairwise groups is shown in Fig. 4. In this analysis there was a slight degree of separation among three populations. Correlations between the measured morphometric variables and the discriminant functions for the fish specimens are shown in Table 4 and the measurements used in this analysis included 1-2, 1-4, 1-13, 2-4, 3-10, 3-12, 4-11, 6-7 and 7-8. The cross-validation testing procedure was exactly the same as the PCC results (Table 5).

The dendrogram derived from the cluster analysis of Euclidean distances among groups of centroids showed that the three populations of spined loach segregated from each other into two distinct clusters, *C. keyvani* from the River Talar appeared in one cluster while *C. faridpaki* populations from the Siahrud and Babolrud rivers belonged to the other clusters (Fig. 5).

The differences in body shape between each studied species are presented in Fig. 5. Distribution of the grids in Fig. 5 and body shape differences (Fig. 4), shows a longer snout, shallow body and head depths, and elongated body for *C. keyvani*, vs. relatively short snout, high body and head depths and stout body for both *C. faridpaki* populations.

Discussion

This is the first report on differentiations among the spined loach populations in the southern Caspian Sea basin by using landmark-based morphology. The results of multivariate analysis demonstrated that the two described species are correctly separated from each other, and two populations of Faridpak's spined loach are classified as one cluster and morphometrically closer together. Obtained results for the ANOVA analysis showed that 41 out of 78 transformed morphometric measurements were significantly different in these groups of *Cobitis* living in the southern Caspian Sea basin (Table 2), which demonstrates a high phenotypic variation among them.

Discriminant function analysis (DFA) could be a useful method to distinguish different species of the same genus or different stocks of the same species, with respect to stock management programs (Karakousis et al. 1991). The results of DFA obtained in present study indicated that 58.0 % of the individuals were correctly classified into their original groups on average, demonstrated a high differentiation among the populations of spined loach in the studied areas. This relative segregation was confirmed by another multivariate analysis, CVA, where the visual examination of the plotted CV 1 and CV 2 scores for each sample (Fig. 3) revealed that these species (*C. faridpaki* and *C. keyvani*) were clearly distinct from each other. The obtained results about body shape differentiation between these studied species agreed with Mousavi-Sabet et al. (2012a) who reports *C. keyvani* is distinguished from *C. faridpaki* by elongated body, snout shape and low body and head depths.

It is well known that morphological characteristics can show high plasticity in response to differences in environmental conditions (Swain et al. 1991). Therefore, the distinctive environmental conditions of these rivers may underlie the morphological differentiation among the populations from these locations. Such kind of discrimination has been reported among six populations of *Capoeta capoeta gracilis* located in the Aras, Sefidrud, Shirud, Tonekabon, Haraz and Gorganrud river systems in Iran (Samaee et al. 2006).

The morphological differences may be solely related to body shape variation and not to size effects which were successfully accounted for by allometric transformation. Literature shows that factor of size account more than 80 % of variation among a set of variables in morphometric studies. On the other hand, factor of size plays a predominant role in morphometric analysis and makes result in erroneous status if it cannot be removed in statistical analyses of data (Tzeng 2004). In the present study, size effect was removed successfully by the allometric transformation, so any significant differences indicated by the ANOVA and multivariate analysis, are caused by the body shape variation.

The causes of morphological differences between populations are often quite difficult to explain (Cadrin & Friedland 1999, Cadrin 2000, Poulet et al. 2004). It has been suggested that the morphological characteristics of fish are determined by an interaction between genetic and environmental factors (Swain & Foote 1999, Poulet et al. 2004, Salini et al. 2004, Pinheiro et al. 2005). The environmental characteristics prevailing during the early development stages when individuals are more phenotypically influenced by the environment are of particular importance (Pinheiro et al. 2005). The phenotypic variability may not necessarily reflect population differentiation at the molecular level (Ihssen et al. 1981, Tudela 1999). The influences of environmental parameters on morphometric characters are considered by several authors in the course of fish population segregation already (e.g. Swain & Foote 1999, Cardin 2000, Turan 2000). Different rivers in the same basin have various conditions that can change the feeding habits and food items, growth pattern and reproductive strategy of individual species (AnvariFar et al. 2011). The importance of such factors on producing morphological differentiation in fish species is well known (Akbarzadeh et al. 2009).

The present findings revealed the potential power of the landmark-based methods for the identification of spined loach stocks. The present study provides basic information about the differences of *Cobitis* species and populations in the southern Caspian Sea basin and it suggests that observed morphological variation should be considered in stock management programs and commercial exploitation of these species as an ornamental fish in aquarium trade (Mousavi-Sabet 2012d). *C. faridpaki* is recorded from area different from their type locality, so a new site of occurrence is found (the River Babolrud). To determine the contribution of genetic differentiations in the morphological findings, further exploration is necessary using DNA techniques.

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