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Morphometric variation of the endangered Caspian lamprey, *Caspiomyzon wagneri* (Pisces: Petromyzontidae), from migrating stocks of two rivers along the southern Caspian Sea

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Abstract

Background: Morphological studies on fishes are important from various viewpoints. Studies carried out on the Caspian Sea fishes show that many species possess speciation and population formation microprocess running. Morphological characteristics of the native endangered Caspian lamprey, *Caspiomyzon wagneri* Kessler 1870, from migrating stocks of two major rivers in the southern Caspian Sea were analyzed to investigate the hypothesis population structure and morphologic sexual dimorphism.

Results: Univariate analysis of variance of 180 adult specimens showed significant differences between the means of the two studied groups for 15 standardized morphometric measurements out of 31 ($P < 0.05$). In morphometric trait linear discriminant function analysis, the overall assignments of individuals into their original groups in male and female specimens were 77.1 and 84.0 %, respectively. The discriminant analysis showed a morphological segregation of the studied populations based on the characters predorsal length, interdorsal, interorbital distance, tail length, and first dorsal fin length. The principal component analysis, scatter plot of individual component score between PC1 and PC2, showed the specimens grouped into two areas but with high and moderate overlap between two localities in males and females, respectively.

Conclusions: The present study indicated that there are at least two types of morphological forms of Caspian lamprey that had high morphometric differentiation in the rivers across the southern Caspian Sea, which can be considered in conservational policy of this valuable species.

Keywords: Sexual dimorphism; Morphology; Population structure; Iran

Background

Lampreys (Petromyzontiformes) are a significant ecological, cultural, and economically important fish groups in the world. There are about 43 lamprey species in 9 genera with only 1 recorded from Iran (Coad 2015). Caspian lamprey, *Caspiomyzon wagneri*, is a Eurasian anadromous non-parasitic species (Imanpoor and Abdollahi 2011). The Caspian lamprey is endemic to the Caspian Sea and related river system in its northern, western, and southern watersheds (Holčík 1986) and migrates to the Volga, Ural, Terek, and Kura rivers (Coad 2015; Holčík and Oláh 1992). The Caspian lamprey in the southern Caspian Sea basin migrates to such rivers as Shirud, Talar, Babolrud, Gorganrud, Tajan, Haraz, Sardabrud, Aras, Tonekabon, Polrud, Sefidrud, and Shafarud rivers and the Anzali Lagoon (Imanpoor and Abdollahi 2011; Kiabi et al. 1999; Nazari and Abdoli 2010). This species migrate upstream from the sea where they spend the feeding stage, and when migration starts, lampreys stop growing and begin to mature sexually (Larsen 1980). Adults die after spawning. During the spawning migration, the lamprey undergoes certain morphological changes, some of which have been linked to the sex of the fish (Coad 2015).

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The Caspian lamprey is listed as vulnerable generally in Europe (Renaud 1997) and in Iran because it migrates into rivers which are polluted and dammed and because of its restricted and declining distribution (Coad 2015). Also, Kiabi et al. (1999) consider this species to be near threatened in the southern Caspian Sea basin according to the IUCN criteria. Because of the valuable ecological importance of Caspian lamprey, broad studies are performed, in terms of reproduction (Nazari and Abdoli 2010), age and growth parameters, morphology, endocrinology, and histology (Imanpoor and Abdollahi 2011).

The study of morphological characters, morphometric and meristic, with the objective of defining and characterizing populations, has a long tradition in ichthyology (Almeida et al. 2008). Morphological studies on fishes are important from various viewpoints including evolution, ecology, behavior, conservation, water resource management, and stock assessment (AnvariFar et al. 2011). Suitable and successful management of aquatic organism stock will be gained by the study of genetic stocks of endemic species and identification of populations (Coad 1980). The study of morphological characters with the aim of defining or characterizing fish stock units has for some time been a strong interest in ichthyology (Tudela 1999). Studies carried out on the Caspian Sea fishes show that many species possess speciation and population formation microprocess running, as the Black Sea species (Gholiev 1997). There are several reports on the southern Caspian Sea fishes (e.g., Abdolhay et al. 2010; AnvariFar et al. 2011, 2013) which indicate the existence of morphological variability in different parts of this basin. However, information on population variability and differentiation of Caspian lamprey in the southern Caspian Sea basin is still rather limited.

Holčík (1986) studied on the morphological characters of this species in the southern Caspian Sea basin. Also, Ginzburg (1936) described ammocoetes from Iran. Yamazaki and Goto (1996) compared the morphometric and meristic characters of the two groups of brook lamprey in Japan. Holcík (1999) stated that dramatic

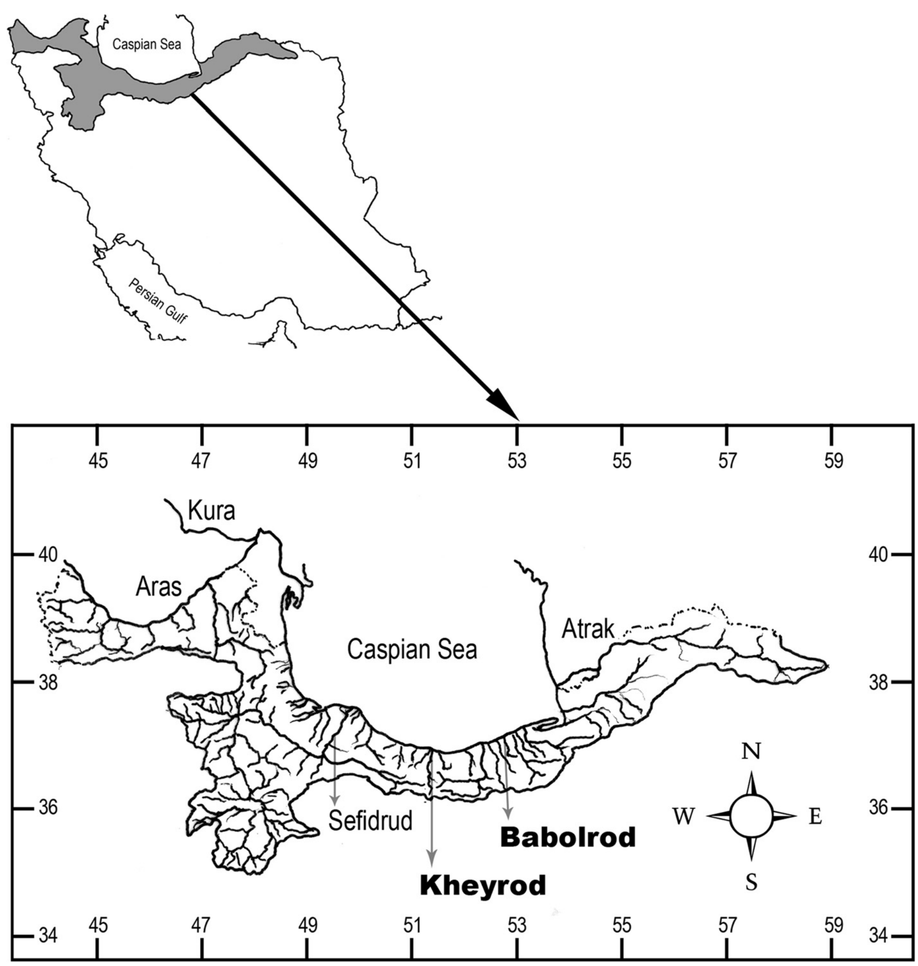

Fig. 1 Map of Iranian south Caspian Sea basin showing some of the most important river systems: Aras, Kura, Sefidrud, and Atrak rivers and the sampling sites including Babolrod and Kheyrod rivers

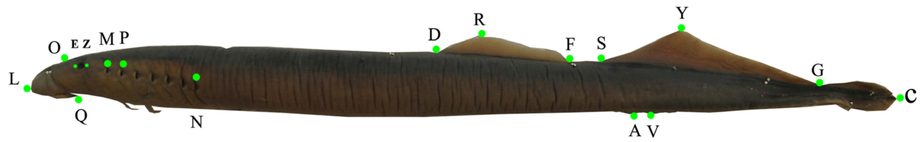

Fig. 2 Morphometric characters measured on Caspian lamprey samples. Landmarks selected based on the studies of Holcik et al. (1986) and Renaud (2011). *LC* total length, *LD* predorsal length, *LF* predorsal distance, *LS* second postdorsal length, *LG* second postdorsal distance, *LN* head length, *LO* prenasal length, *SC* second predorsal fin, *MP* interbranchial opening distance, *ZM* postocular length, *QA* postdisc length, *NC* postbranchial length, *FS* interdorsal, *LA* preanal distance, *VC* tail length, *MN* branchial length, *LM* prebranchial distance, *MC* postbranchial distance, *LE* snout length, *EM* postorbital distance, *LQ* disc length, *AV* urogenital papilla length, *DF* first dorsal fin length, *SG* second dorsal fin length, *R*↓ first dorsal fin height, *Y*↓ second dorsal fin height, *D*↓ body width, *O*↓ head depth, *EZ* eye diameter, *EE*′ interorbital distance

declines in migratory species such as lampreys, sturgeons, salmons, and clupeids were well known in Europe which require attention. Almeida et al. (2008) investigated the morphological variation and differentiation of sea lamprey ammocoetes in Portuguese river basins. Renaud (2011) gave details on the morphology of lampreys of the world. Hence, it is important to understand that this unit population had morphological differentiation. On the other hand, despite the biodiversity and ecological importance of Caspian lamprey, unfortunately there are limited studies available on population differentiation of the fish in the area. Considering the abovementioned facts, the main aims of this study were the following: (1) obtaining information about population differentiation of this species in two most important rivers at the reproductive migration time in the southern Caspian Sea basin, (2) identifying the best set of characters to establish the separation of the eventual groups, and (3) identifying morphometric sexual dimorphism and determining the characters that have sexual dimorphism. The results of this study can be employed in the conservation purpose of this species in the region.

Methods

Collection of samples

A total of 180 adult individuals of Caspian lamprey were collected from two sampling sites from September to October (2011) comprising 106 individuals from Babolrod (36° 42′ N, 52° 38′ E) and 74 individuals from Kheyrod (51° 30′ N, 36° 39′ E) rivers (Fig. 1). Samplings were done by hand netting reproductive migratory fish at nighttime.

Thirty traditional morphometric characters were measured using an electronic digital caliper to the nearest 0.1 mm (Fig. 2). Measurements follow Holcik et al. (1986) and Renaud (2011). All measurements were made by the same person. After measuring, fish were dissected to identify the sex by macroscopic examination of the gonads and external sexual characters (males with well-developed urogenital papillae and females crescent-shaped extra anal fin). Gender was used as the class variable in the analysis of variance (ANOVA) to test for the significant differences in the morphometric characters if any, between males and females of Caspian lamprey.

Statistical analyses

Univariate ANOVA was performed for each morphometric character to evaluate the significant differences among the two locations (Zar 1984), and the morphometric characters which showed significant variation ($P < 0.05$) were only used so as to achieve the recommended ratio of the number of organisms (N) measured to the parameters (P) included in the analysis to be at least 3–3.5 (Kocovsky et al. 2009) for obtaining the stable outcome from the multivariate analysis. In the present study, linear discriminant function analyses (DFA), principal component analysis (PCA), and cluster analysis (CA) were employed to discriminate the two populations. Principal component analysis helps in morphometric data reduction, in decreasing the redundancy among the variables (Anvarifar et al. 2013; Mousavi-Sabet and Anvarifar 2013; Veasey et al. 2001), and in extracting a number of independent variables for population differentiation. Wilks' lambda was used to compare the differences among all individuals of the two groups. The DFA was used to calculate the percentage of correctly classified (PCC) fish. A cross-validation using PCC was done to estimate the expected

Table 1 Descriptive data (mean ± SD and min-max) of Caspian lamprey from sampling sites

Station	Sex	Number	Min-max (length, mm)	Mean ± SD (length)	Min-max (weight, g)	Mean ± SD (weight)
Babolrod	Male	67	281–419	347.71 ± 26.27	49.64–134.12	78.54 ± 16.58
	Female	39	271–419	355.69 ± 26.35	56.77–114.88	86.62 ± 12.53
Kheyrod	Male	38	236–405	351.39 ± 34.95	30.09–146.09	92.30 ± 23.75
	Female	36	275–408	349.91 ± 30.75	60.13–152.21	99.93 ± 22.94

Table 2 Results of ANOVA of morphometric characters of Caspian lamprey samples between stations

Morphometric measurements	F value	P value	Morphometric measurements	F value	P value	Morphometric measurements	F value	P value
LC	0.00	0.99	QA	0.53	0.47	LQ	0.89	0.35
LD	8.13	0.00	MC	3.98	0.05	AV	2.83	0.09
LF	5.53	0.02	FS	13.79	0.00	DF	24.19	0.00
LS	0.30	0.58	LA	0.53	0.47	SG	7.32	0.01
LG	0.84	0.36	VC	12.25	0.00	R↓	1.38	0.24
LN	0.00	0.99	MN	6.64	0.01	Y↓	0.06	0.81
LO	1.48	0.23	LM	0.66	0.42	D↓	0.00	0.97
SC	5.53	0.02	NC	3.90	0.05	O↓	8.69	0.00
MP	1.31	0.25	LE	3.77	0.05	EZ	0.19	0.66
ZM	10.20	0.00	EM	10.20	0.00	EE′	41.86	0.00

actual error rates of the classification functions. As a complement to discriminant analysis, morphometric distances among the individuals of the two groups were inferred to cluster analysis (Veasey et al. 2001) by adopting the Euclidean distance as a measure of dissimilarity and the UPGMA (unweighted pair group method with arithmetical average) as the clustering algorithm.

Size-dependent variation was corrected by adapting an allometric method as suggested by Elliott et al. (1995):

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

where M is the original measurement, M_{adj} is the size-adjusted measurement, L_0 is the standard length of the fish, L_s is the overall mean of the standard length for all fish from all samples in each analysis, and b is estimated for each character from the observed data as the slope of the regression of log M on log L_0 using all fish from both groups. The results derived from the allometric method were confirmed by testing the significance of the correlation between transformed variables and standard length (Turan 1999).

Statistical analyses for morphometric data were performed using the SPSS version 16 software package, Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc), and Excel (Microsoft office, 2010).

Results

Descriptive data for the sex ratio, range (minimum-maximum), and mean and standard deviation (SD) of length and weight in the case of sampled specimens are shown in Table 1. The correlation between transformed morphometric variables and standard length was non-significant ($P > 0.05$) that confirmed size or allometric signature on the basic morphological data was accounted. Morphological differences ($P < 0.05$) were observed between two populations of Caspian lamprey in Babolrod and Kheyrod in the southern Caspian Sea basin for 15 out of 30 morphometric characters (Table 2), and these significant variables were used further for multivariate analysis (PCA, DFA, and CA). The ANOVA revealed effective morphologic characters on sexual dimorphism ($P < 0.05$) in 14 out of the 30 studied measurements which include LC, LD, LS, SC, QA, LA, LE, LQ, AV, R↓, Y↓, D↓, O↓, and EE′. Therefore, the analyses of morphometric characters were conducted with the sexes separated.

A common problem with many fish morphology studies that use multivariate analysis is potentially inadequate

Table 3 Results of ANOVA for sexual dimorphism of morphometric characters in Caspian lamprey samples

Morphometric measurements	F value	P value	Morphometric measurements	F value	P value	Morphometric measurements	F value	P value
LC	7.81	0.01	QA	5.08	0.03	LQ	29.93	0.00
LD	4.32	0.04	MC	1.74	0.19	AV	37.24	0.00
LF	3.49	0.06	FS	2.81	0.10	DF	0.35	0.55
LS	9.22	0.00	LA	5.08	0.03	SG	0.05	0.82
LG	0.80	0.37	VC	0.74	0.39	R↓	10.65	0.00
LN	0.05	0.82	MN	0.06	0.81	Y↓	4.30	0.04
LO	1.29	0.26	LM	1.18	0.28	D↓	51.30	0.00
SC	5.00	0.03	NC	1.82	0.18	O↓	12.66	0.00
MP	1.46	0.23	LE	8.07	0.01	EZ	0.35	0.55
ZM	3.04	0.08	EM	3.04	0.08	EE′	5.22	0.02

Table 4 Eigenvalues, percentage of variance, and percentage of cumulative variance for different sexes

Factor	Male			Female		
	Eigenvalue	Percentage of variance	Percentage of cumulative variance	Eigenvalue	Percentage of variance	Percentage of cumulative variance
PC1	4.02	26.78	26.78	4.00	26.65	26.65
PC2	1.94	12.96	39.74	2.18	14.52	41.17
PC3	1.62	10.79	50.54	1.72	11.50	52.67
PC4	1.50	10.00	60.53	1.63	10.88	63.55
PC5	1.27	8.45	68.98	1.19	7.95	71.49
PC6	–	–	–	1.11	7.37	78.86

sample size. For decades, authors of theoretical works on PCA and DFA 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 (Kocovsky et al. 2009). In this study, for multivariate analysis, we used only morphometric characters that were significant at a high level ($P < 0.05$), and under these circumstances, the *N*:*P* ratio was 12 (180/15) for these traits including LD, LF, SC, ZM, MC, FS, VC, MN, NC, LE, EM, DF, SG, O↓, and EE′.

To examine the suitability of the data for principal component analysis, Bartlett's test of sphericity was performed, and it was significant ($P < 0.01$). In order to determine which morphometric measurement made most effectively differentiates between the populations, the contributions of variables to principal components (PC) were examined. Principal component analysis of 15 morphometric measurements extracted five factors with eigenvalues >1, explaining 68.98 % of the variance in male, and six factors with eigenvalues >1, explaining 78.86 % of the variance in female (Table 3). The first principal component (PC1) accounted for 26.78 and 26.65 % of the variation and the second principal component (PC2) for 12.96 and 14.52 % in males and females, respectively (Table 4). The most significant loadings on PC1 in males were ZM, MN, and EM and in females were the same. Also, the most significant loadings on PC2 in males were MC and NC and in females were the same. Visual examination of plots of PC1 and PC2 scores revealed that the male specimens were grouped into two areas but with high overlap between two stations. Also, in female visual examination of plots of PC1 and PC2 scores, specimens were grouped into two areas with moderate overlap between two stations (Fig. 3). In this analysis, the characteristics with an eigenvalue exceeding 1 were included and others discarded. It is worth mentioning out

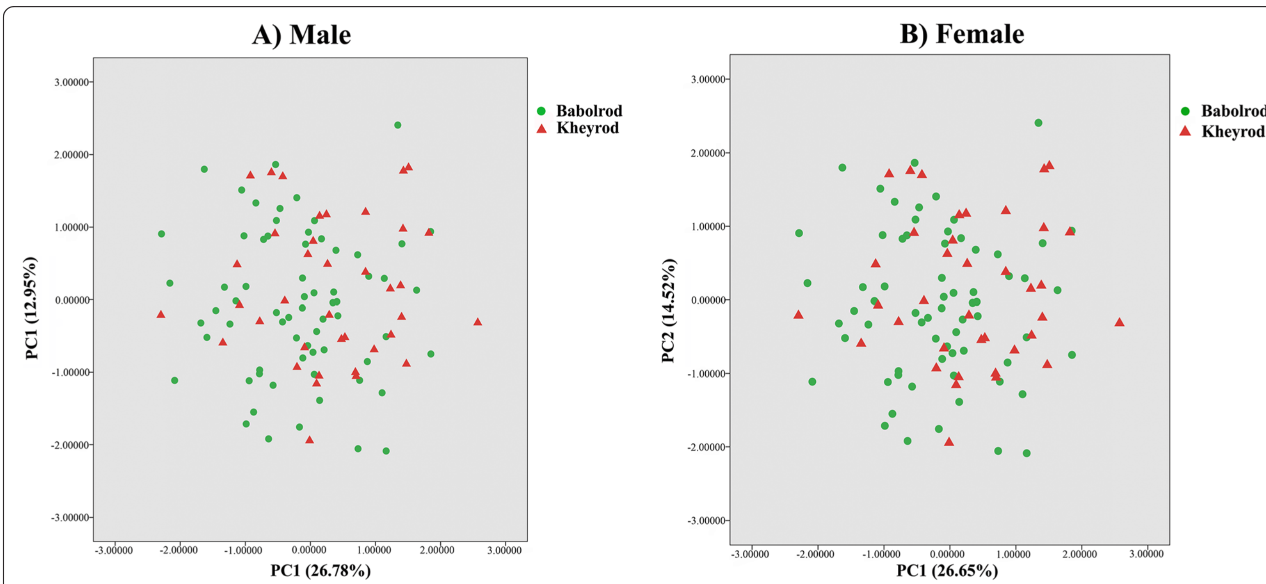

Fig. 3 Plot of the factor scores for PC1 and PC2 of all morphometric measurements for different sexes of Caspian lamprey samples. **a** Male. **b** Female

Table 5 Results of Wilks' lambda test for verifying differences between populations of Caspian lamprey in both sexes

	Test of functions	Wilks' lambda	Chi-square	*df*	Significance
Male	1	0.695	36.950	3	0.000
Female	1	0.527	45.813	3	0.002

here that a factor loading greater than 0.30 is considered significant, 0.40 is considered more important, and 0.50 or greater is considered very significant (Nimalathasan 2009). According to Mousavi-Sabet and Anvarifar (2013), for parsimony, in this study, only those factors with loadings above 0.7 were considered significant.

Wilks' lambda tests of discriminant analysis indicated significant differences in 15 morphometric characters of the two populations in both sexes. In this test, one function in male and female was highly significant ($P < 0.01$) (Table 5). The linear discriminant analysis in male gave an average PCC of 77.1 %. Medium classification success rates were obtained for Babolrod (73.1 %) and Kheyrod (84.2 %) that indicate a high correct classification of specimens into their original populations (Table 6). The discriminant analysis in female gave an average PCC of 84.0 % for morphometric characters. The proportion of individuals correctly classified into their original groups was 84.6 and 83.3 % in Babolrod and Kheyrod, respectively, indicating a high rate of correct classification of individuals into their original populations (Table 6). In both male and female, the cross-validation testing procedure was exactly the same as the PCC results. Figures 4 and 5 indicate the coordinates of two populations in the first two axes of DFA. In this analysis, there was a high degree of separation between Caspian lamprey specimens in the southern Caspian Sea basin. The measurements that were used in this analysis for males included LD, FS, and EE′ and for females were VC, DF, and EE′.

Clustering analysis based on Euclidean square distances between the groups of centroids using an UPGMA resulted two main clusters: Babolrod (male), Babolrod (female), and Kheyrod (female) in one group and Kheyrod (male) in the other group. Also, the results of this analysis demonstrated Babolrod (male and female) closed together and far from Kheyrod (male), although they are far apart geographically (Fig. 6).

Discussion

The aim of the present study was to investigate the hypothesis population differentiation and morphologic sexual dimorphism in Caspian lamprey populations using traditional method. The morphometric analysis was planned, whereas the previous studies revealed non-significant differences for meristic between the fish populations (Nazari et al. 2010). Our study results demonstrate that there is significant phenotypic variation between the two studied populations, also between the sexes. Discriminant function analysis could be a useful method to distinguish different stocks of the same species (Karakousis et al. 1991). In the present study, a high classification of individuals that were correctly classified into their respective groups by DFA was achieved (Fig. 4), and this segregation was partly confirmed by PCA. Although, there were some ranges of overlap somewhat in all of the characters examined between two groups. Yamazaki and Goto (1996) compared the morphometric and meristic characteristics between two groups of brook lamprey and reported some ranges of overlap in multivariate analysis. This survey indicated that the population differentiation which resulted from different multivariate analyses in females was higher than that in males. Abdolhay et al. (2010) have shown that the average coefficient of variation (CV %) of raw data, morphometric characters, and ratio in females were higher than those in males of Mahisefid populations. Almeida et al. (2008) stated that the discriminatory power of the meristic characters was comparatively weaker, being responsible for the reduced separation between groups of sea

Table 6 Percentage of specimens classified in each group and after cross-validation for morphometric data

		Station	Predicted group membership					
			Male			Female		
			Babolrod	Kheyrod	Total	Babolrod	Kheyrod	Total
Original	Count	Babolrod	49	18	67	34	5	39
		Kheyrod	5	33	38	6	30	36
	%	Babolrod	73.1	26.9	100.0	87.2	12.8	100.0
		Kheyrod	13.2	86.8	100.0	16.7	83.3	100.0
Cross-validated[a]	Count	Babolrod	49	18	67	33	6	39
		Kheyrod	6	32	38	6	30	36
	%	Babolrod	73.1	26.9	100.0	84.6	15.4	100.0
		Kheyrod	15.8	84.2	100.0	16.7	83.3	100.0

[a]Cross-validation is done only for those cases in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that case

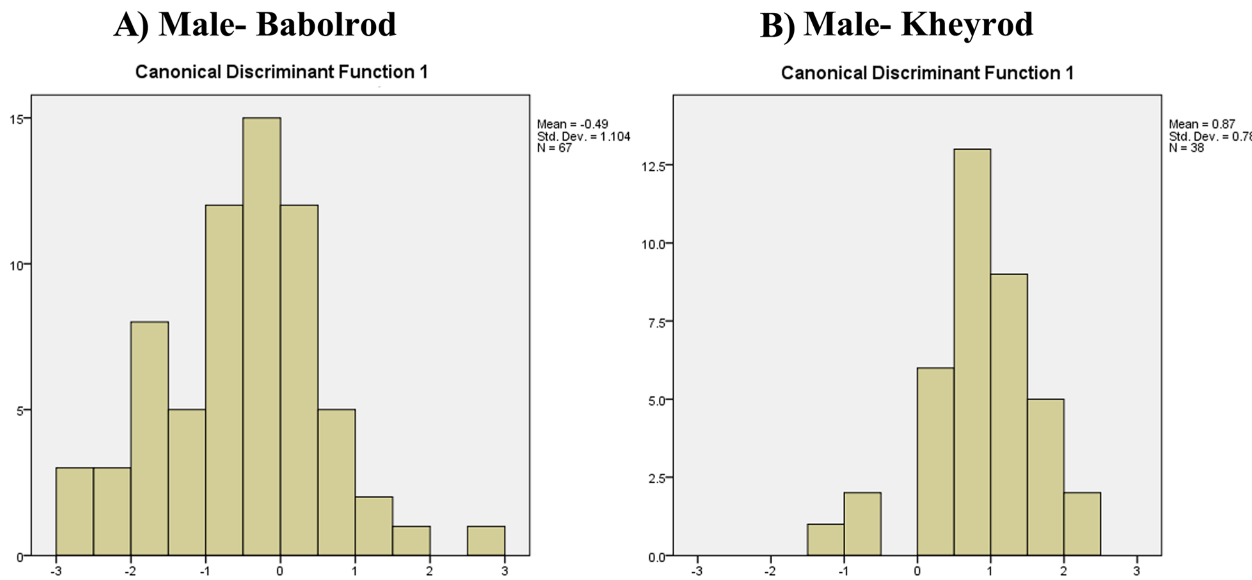

Fig. 4 Coordinate plot for male specimens of the two studied populations of Caspian lamprey from the southern Caspian Sea according to the first two discriminant functions from morphometric data analysis. **a** Babolrod. **b** Kheyrod

lamprey. The PCA and DFA showed a morphological segregation of the studied populations based on the characters postocular length, branchial length, postorbital distance, postbranchial distance, postbranchial length, predorsal length, interdorsal distance, interorbital distance, tail length, and first dorsal fin length. Almeida et al. (2008) reported that the DFA showed a morphological segregation of the sea lamprey ammocoete populations in Portuguese river basins based on the characters head, tail, and branchial length.

During the spawning migration, the lamprey undergoes certain morphological changes, some of which have been linked to the sex of the fish. Coad (2015) reported that females of Caspian lamprey reach larger sizes than males and have a smaller urogenital papilla. Also, the teeth become blunt, fin size increases, the dorsal fins become almost united at the base in males, and there is a change in color. The results of our survey have shown that half of the morphometric characters have differences between females and males which revealed sexual

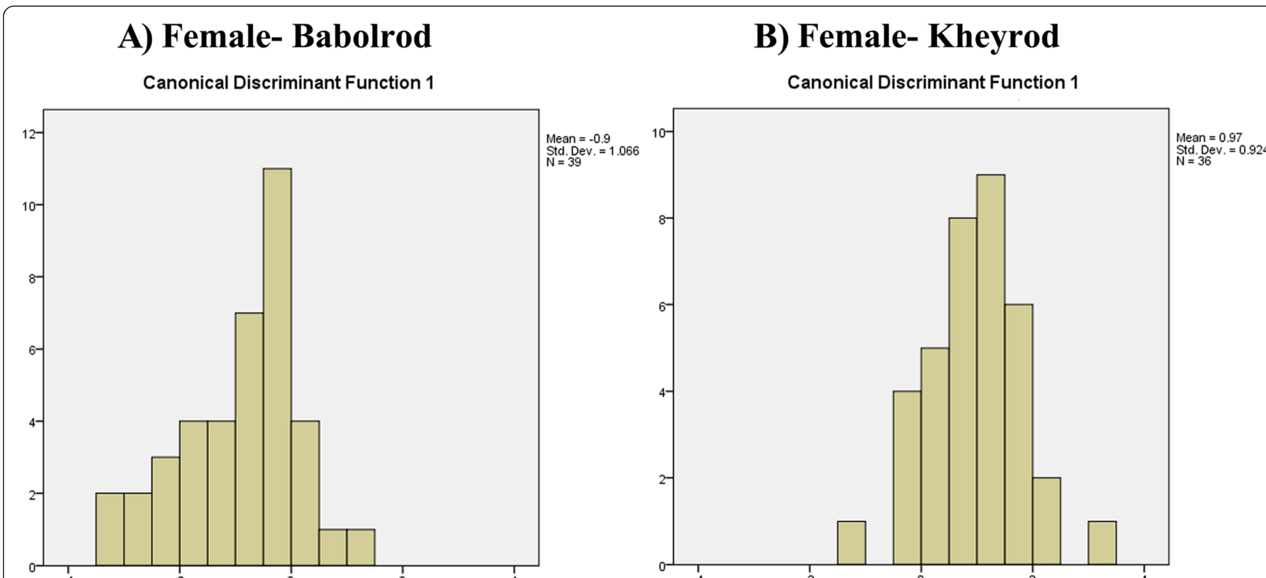

Fig. 5 Coordinate plot for female specimens of the two studied populations of Caspian lamprey from the southern Caspian Sea according to the first two discriminant functions from morphometric data analysis. **a** Babolrod. **b** Kheyrod

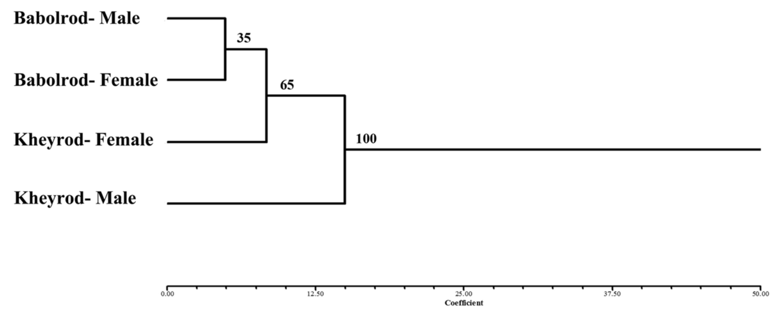

Fig. 6 Dendrogram derived from cluster analyses of morphometric measurements for different sexes of Caspian lamprey specimens in the southern Caspian Sea basin

dimorphism. These characters include LC, LD, LS, SC, QA, LA, LE, LQ, AV, R↓, Y↓, D↓, O↓, and EE′.

These morphological differences may be solely related to body shape variation and not to size effect which was successfully accounted by allometric transformation. On the other hand, size-related traits play a predominant role in morphometric analysis, and the results may be erroneous if not adjusted for statistical analyses of data (Tzeng 2004). In the present study, the size effect had been removed successfully by allometric transformation, and the significant differences between the populations are due to the body shape variation when tested using ANOVA and multivariate analysis. The causes of morphological differences between populations are often quite difficult to explain (Poulet et al. 2004). It has been suggested that the morphological characteristics of fish are determined by genetic, environment, and the interaction between them (Pinheiro et al. 2005; Poulet et al. 2004; Swain and Foote 1999). The environmental factors prevailing during the early development stages, when the individual's phenotype is more amenable to environmental influence, are of particular importance (Pinheiro et al. 2005). The influences of environmental parameters on morphometric characters are well discussed by several authors in the course of fish population segregation (e.g., Swain and Foote 1999). The habitat of this species in the southern Caspian Sea proper is unknown although some specimens have been caught in the Caspian at 600–700 m (Jolodar and Abdoli 2004; Coad 2015). It seems that isolation by distance and different environmental conditions such as variability of food items, growth pattern, and abiotic characteristics between two stations such as temperature, oxygen, turbidity, and water quality are the mechanisms responsible for population differentiation of Caspian lamprey in the southern Caspian Sea basin.

Conclusions

The present study showed that each sampling site represents an independent population, and there are at least two types of morphological forms of Caspian lamprey that had high morphometric differentiation in the southern Caspian basin. The results can be interesting for management and conservation programs of this valuable endangered species in this region. A detailed study involving the molecular genetics and environmental aspects may further confirm the present findings unambiguously. However, in order to have better conservational policy, further studies are recommended on determining other possible populations of this species in other regions of the Caspian Sea.

Abbreviations

LC: total length; LD: predorsal length; LF: predorsal distance; LS: second postdorsal length; LG: second postdorsal distance; LN: head length; LO: prenasal length; SC: second predorsal fin; MP: interbranchial opening distance; ZM: postocular length; QA: postdisc length; NC: postbranchial length; FS: interdorsal; LA: preanal distance; VC: tail length; MN: branchial length; LM: prebranchial distance; MC: postbranchial distance; LE: snout length; EM: postorbital distance; LQ: disc length; AV: urogenital papilla length; DF: first dorsal fin length; SG: second dorsal fin length; R↓: first dorsal fin height; Y↓: second dorsal fin height; D↓: body width; O↓: head depth; EZ: eye diameter; EE′: interorbital distance.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SV provided the materials and drafted the manuscript. HM-S made comments on the manuscript and revised it. MR-M provided the materials and drafted the manuscript. HA carried out the experiments and drafted the manuscript. AH measured the specimens. All authors read and approved the final manuscript.

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