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Morphological Differentiation of *Vimba persa* (Pisces: Cyprinidae) along the southern Caspian Sea Basin, Iran

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ABSTRACT

Morphological characteristics of the native Caspian vimba Vimba persa *(Pallas, 1814) from five major rivers along the southern Caspian Sea basin were analyzed using 25 morphometric and 10 meristic characters in order to investigate the hypothesis differentiation and clarify its taxonomic status. Univariate analysis of variance of 288 adult specimens showed significant differences between the means of the five groups for all standardized morphometric measurements (p<0.05) and 5 out of 10 meristic traits. In morphometric traits linear discriminant function analysis, the overall assignments of individuals into their original groups between morphometric were 98.3% and between meristic were 55.4%. The principal component analysis, scatter plot of individual component score between PC1 and PC2 showed that the specimens grouped into five areas in morphometric but in meristic characters these populations have high overlap. The present study indicated there is high morphometric differentiation among the populations of Caspian vimba that can be considered in restocking programs, management and conservational policy of this valuable species in the across of the southern Caspian Sea basin.*

Keywords: Caspian vimba, Morphometric differentiation, Meristic, Population, Iran

INTRODUCTION

Caspian vimba, *Vimba persa* (Pallas, 1814), is one of the valuable stocks in the Caspian Sea that is benthopelagic species living in river systems of the Caspian, Azov, Baltic and Black seas as well as in western Europe [1, 2]. This species has great economic importance. The vimba catch over the whole Caspian Sea basin for Iranian waters in 2009-2010 was 474 tones [3]. This species also considered as sport fishery species in Iran [4]. Also, Aquaculture of this species has been investigated in Iran [5] and Fisheries organizations of Iran every year perform propagation and releasing this species to Caspian Sea [6]. Caspian vimba has a semi-migratory form that enters fresh water for reproduction in spring and after spawning, it migrates to estuaries and brackish water for feeding until the next reproductive season [7]. Fishing, rivers regulation, pollution, destruction of habitat and blockage of migration routes have resulted in the extinction of this fish species in the Caspian Sea [8]. Kiabi *et al*. [9] consider this species to be near threatened in the south Caspian Sea basin according to IUCN criteria.

Because of most valuable commercial and ecological importance of Caspian vimba, broadly studied is performed; in terms of reproduction [10, 11], age and growth parameters [3, 4, 12, 13], pathology [14], physiology and endocrinology [15, 16] and Karyology [17].

Abbasi *et al*. [18] and Hosseini *et al*. [19] measured some morphometric and meristic characters of migrant Caspian vimba to Sefidrod River and Anzali Lagoon, respectively. Rahmani and Abdoli [20] compared populations from the Gorgan River, Shirud River and Anzali Lagoon and found morphometric and meristic differences among them. Mohamadian *et al*. [21] used microsatellite markers on fish from Havighrod River and Gorganrod River and showed significant population structuring, with enormous diversity in the past. Mohamadian *et al*. [22] compared genetically population of Havighrod River with Anzali Lagoon and stated conservation, sustainable harvest and restocking of these populations should be considered. Also, Mohamadian *et al*. [23] identified four population of *Vimba persa* along the Iranian coast and reported population of Caspian vimba in three province of the Southern Caspian Sea were genetically differentiated from each other. However, information on population morphologically differentiation of these specimens in the southern Caspian Sea basin is still rather limited. In addition, it is important to understand that this population had morphological differentiation or not.

The study of morphological characters, morphometric and meristic, with objective of defining and characterizing populations, has a long tradition in ichthyology [24]. Morphological studies on fishes are important from various viewpoints including evolution, ecology, behaviour, conservation, water resource management and stock assessment [25]. Suitable and successful management of aquatic organisms stock will be gained by study of genetic stocks of endemic species and identification of populations [26]. 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 [27]. Studies carried out on the Caspian Sea fishes show that Caspian vimba possess speciation and population formation microprocess running, as the Caspian and black seas species [28]. There are several reports on the southern Caspian Sea fishes including e.g. Samaee *et al*. [29]; Akbarzade *et al*. [30]; Abdolhay *et al*, [31]; AnvariFar *et al*., [23, 32]; and Mohamadian *et al*. [22, 23] which indicate the existence of morphological variability in different parts of this basin. Gholiev [28] reported there are 3 populations of Caspian vimba in Caspian Sea. However, information on population variability and differentiation of Caspian lamprey specimens in the southern Caspian Sea basin is still rather limited.

Considering the above mentioned facts, main objectives of this study were: 1) obtain information about population differentiation of this species along the Iranian coast of the southern Caspian Sea basin using analysis of morphometric characters and meristic counts 2) identify the best set of characters to establish the separation of the eventual groups 3) morphometric sexual dimorphism and determine characters that have sex dimorphism. Results of this study can runs to be employed in the stock management, restocking and conservation programs of this valuable species in the Caspian Sea.

MATERIALS AND METHODS

Sampling. A total of 288 adult individuals of the Caspian vimba were collected from five sampling sites, from October to November 2007, that comprising 39 individuals from Astara (36°42'N, 52°38'E), 46 individuals from Bandar-Anzali (37°28'N, 49°26'E), 76 individuals from Tonekabon (36°49'N, 50°51'E), 72 individuals from Sari (36°44'N, 52°50'E) and 55 individuals from Bandar-Torkaman (37°02'N, 54°00'E) (Fig. 1). The specimens caught by beach seine.

Laboratory Work. 25 traditional morphometric characters were measured using a digital caliper to the nearest 0.01 mm (Fig. 2). Also, 10 meristic variables were counted in each specimen by direct observation. Counts and measurements follow Holcik *et al*. [33]; Samaee *et al*. [34] and Anvarifar *et al*. [32]. Abbreviations used for meristic characteristics are: L1, lateral line scales; Squ.sup, scales rows between lateral line and dorsal fin origin; Squ.inf, scales rows between lateral line and anal fin origin; D1, dorsal fin spine; D2, dorsal fin branched rays; A1, anal fin spine; A2, anal fin branched rays; Gr1, anterior Gill rakers; Gr2, outer Gill rakers; Vn, vertebrae. To avoid human error all morphological measurement were performed by the same person. After measuring, fish were dissected to identify the sex by macroscopic examination of the gonads. Gender was used as the class variable in ANOVA to test for the significant differences in the morphometric characters if any, between males and females of Caspian vimba.

Data analysis. Size dependent variation was corrected by adapting an allometric method as suggested by Elliott *et al*. [35]:

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

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 from both the groups. The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length [36].

Univariate Analysis of Variance (ANOVA) was performed for each morphometric character to evaluate the significant difference among the locations [37]. In the present study linear discriminant function analyses (DFA), principal component analysis (PCA) and cluster analysis (CA) were employed to discriminate the five populations. Principal component analysis helps in Morphometric data reduction [38] in decreasing the redundancy among the variables [29, 32] and to extract a number of independent variables for population differentiation. The Wilks' lambda was used to compare the difference among all 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 actual error rates of the classification functions. As a complement to discriminant analysis, morphometric distances among the individuals of five groups were inferred to Cluster analysis [38] by adopting the Euclidean distance as a measure of dissimilarity and the UPGMA (Unweighed Pair Group Method with Arithmetical average) method as the clustering algorithm [39].

Statistical analyses for morphometric data were performed using the SPSS version 16 software package, Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) [40] and Excel (Microsoft office, 2010).

RESULTS

Descriptive data for the sex ratio, range (Minimum-Maximum), mean and standard deviation (SD) of length and weight in 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. Differences ($P<0.05$) among the five populations of the Caspian vimba in Astara, Bandar-Anzali, Tonekabon, Sari and Bandar-Torkaman in the southern Caspian Sea basin were observed for 24 out of 25 morphometric characters and 5 out of 10 meristic counts (Table 2). Since in meristic counts dorsal and anal fin spines were monomorph therefore these traits were not used in subsequent analyses. The ANOVA for differences in morphometric characters between female and male Caspian vimba (Table 3) revealed effective morphologic characters on sexual dimorphism ($p<0.05$) in 4 of the 25 that including dorsal fin base, pectoral fin length, anal fin base and predorsal distance, however these traits were not related to sex. Hence, the data for both sexes were pooled for all subsequent analyses.

To examine the suitability of the data for principal component analysis, Bartlett's Test of sphericity were performed and the Bartlett's Test of Sphericity is significant ($P\leq0.01$). In order to determine which morphometric measurement made most effectively differentiates among the populations, the contributions of variables to principal components (PC) were examined. PCA of 25 morphometric measurements extracted three factors with eigenvalues >1, explaining 77.95% of the variance. Also, PCA of 8 meristic counts extracted four factor with eigenvalues >1, explaining 64.39% of the variance (Table 4). The first principal component (PC1) accounted for 69.32% and 19.32% of the variation and the second principal component (PC2) for 4.48% and 16.89% in morphometric and meristic characters, respectively (Table 4). The most significant loadings on PC1 in morphometric traits were all morphometric characters except 4 traits that including eye diameter, postorbital distance, dorsal fin length, and anal fin length, and in meristic counts were anterior and outer Gill rakers. Also, the most significant loadings on PC2 in morphometric traits were eye diameter and in meristic counts were vertebrae. Visual examination of plots of PC1 and PC2 scores revealed that in morphometric characters the specimens grouped into five distinct areas. Also, in meristic counts visual examination of plots of PC1 and PC2 scores, specimens grouped into three areas including Bandar-Anzali, Tonekabon, Sari and two others stations have high overlap with each other (Fig. 3). In this analysis the characteristics with an eigenvalues exceeding 1 were included and others discarded. It is worth mentioning out here that factor loading greater than 0.30 are considered significant, 0.40 are considered more important and 0.50 or greater are considered very significant [41]. For parsimony, in this study only those factors with loadings above 0.7 were considered significant.

The Wilks' lambda tests of DFA indicated significant differences in morphometric and meristic characters of the five populations. In this test, four function in morphometric and meristic characters were highly significant ($P\leq0.01$) (Table 5). The linear discriminant analysis in morphometric gave an average PCC was 98.3%. Medium classification success rates were obtained for Astara (97.44%), Bandar-Anzali (97.83%), Tonekabon (96.05), Sari (100%) and Bandar-Torkaman (100%) that indicating a high correct classification of specimens into their original populations (Table 6). The discriminant analysis in meristic traits, the average of PCC was 55.4% for morphometric characters. The proportion of individuals correctly classified into their original groups were Astara (53.85%), Bandar-Anzali (84.78%), Tonekabon (60.53), Sari (57.75%) and Bandar-Torkaman (21.82%) that indicating a moderate rate of correct classification of individuals into their original populations (Table 6). In both of morphometric and meristic the cross-validation testing procedure were exactly the same as PCC results. Figure 4 indicates the coordinates of five populations in the two first axes of DFA. In this analysis there was a high degree of separation among Caspian vimba specimens in the southern Caspian Sea basin. The morphometric measurements that used in this analysis included standard length, fork length, total length, head length, snout length, eye diameter, dorsal fin length, pectoral fin length, pelvic fin length, pectoral-ventral distance, dorsal fin base, anal fin base, predorsal distance, caudal lower lobe length, preanal distance and the meristic counts were lateral line scales, scales rows between lateral line and dorsal fin origin, scales rows between lateral line and anal fin origin, dorsal fin branched rays, anal fin branched rays.

Clustering analysis based on Euclidean distances among the groups of centroids using an UPGMA in morphometric characters resulted two main clusters Astara and Bandar-Anzali in one group and Tonekabon, Sari and Bandar-Torkaman in other group. The results of this analysis demonstrated close stations are close together, although they are far apart geographically. Also, Clustering analysis based on Euclidean distances among the groups of centroids using an UPGMA in meristic traits populations were clustered in two distinct clads, the first one consists of Astara and Sari and second clad includes Bandar-Anzali, Tonekabon and Bandar-Torkaman populations.

DISCUSSION

The aim of the study was to investigate the hypothesis population differentiation among Caspian vimba populations using traditional method. Some related morphological studies have been recently done in the region on the native fishes [42-45]. Our study results demonstrate that each sampling site represents independent population in each area and there is significant phenotypic variation among the five studied populations and Western, southern and eastern populations in the Caspian Sea can be distinguished morphologically, and represent different stocks. The analysis of variance revealed high significant phenotypic variation between populations (24 out of 25 morphometric characters). Rahmani and Abdoli [20] reported in all morphometric characteristics and 4 out of 10 meristic characteristics showed significant differences between populations.

Discriminant Function Analysis could be a useful method to distinguish different stocks of a same species [46]. In the present study achieved high classification of individuals that were correctly classified in to their respective groups by DFA that this segregation was partly confirmed by PCA. Although, there were some ranges of overlapped somewhat in all of the meristic characters examined among groups. Rahmani and Abdoli [20] compared morphometric and meristic characteristics between three groups of Caspian vimba and reported high ranges of overlap in multivariate analysis. This survey indicated the population differentiation that resulted from different multivariate analysis in morphometric were higher than mersitic. Abbasi *et al*. [18] reported in meristic counts between juvenile and adult of migrant Caspian vimba there is no significant differences.

The PCA and DFA showed a morphological segregation of the studied populations based on the 12 common characters total length, fork length, standard length, head length, snout length, dorsal fin base, pectoral fin length, pelvic fin length, anal fin base, predorsal distance, caudal lower lobe length, preanal distance. Gholiev [28] studied on Cypriniformes and Perciformes in the Caspian Sea and stated from north to south and west to east of the Caspian Sea some of the morphometric characters included head length, snout length, interorbital distance, body width, dorsal fin length and anal fin length reduced while other traits included pectoral-ventral distance and caudal lobe length increase and some traits such head length, anal fin base, caudal peduncle depth, dorsal fin length and body width had highest variability. Also, these changes due to different ecological conditions and created different population in this area.

Results of our survey shown four morphometric characters have differences between female and male that revealed sexual dimorphism. Abbasi *et al*. [18] relying on morphometric and meristic factors had expressed which male and female reproductive *Vimba persa* migrant to the Sefidrud River in 16 morphometric characteristics (particularly body height, length dorsal fin, head length, head depth, postorbital distance, pectoral-ventral distance, anal fin length) were different. Hosseini *et al*. [19] reported female and male brood stocks of Caspian vimba were different in 12 morphometric characteristics, especially in body height, dorsal and anal fin lengths.

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 [47]. In the present study, the size effect had been removed successfully by allometric transformation, and the significant differences among the populations are due to the body shape variation when it tested using ANOVA and multivariate analysis. The causes of morphological differences between populations are often quite difficult to explain [48]. It has been suggested that the morphological characteristics of fish are determined by genetic, environment and the interaction between them [48-50]. The environmental factors prevailing during the early development stages, when individual's phenotype is more amenable to environmental influence is of particular importance [50]. The influences of environmental parameters on morphometric characters are well discussed by several authors in the course of fish population segregation [49]. Rahmani and Abdoli [20] reported vimba migratory populations were not separated, but they probably belong to different populations, that might be due to different ecological conditions which results in different immigrants make up each year. Mohamadian *et al*. [21] stated high plasticity in response to differences in environmental conditions made two different populations of Caspian vimba in east and center of the Caspian Sea. Mohamadian *et al*. [23] reported genetic differentiation of Caspian vimba is caused by gene flow and geographic isolation in southern Caspian Sea. It has seems isolation by distance and environmental different conditions such as availability of food items, growth pattern and abiotic characteristics between two stations such as temperature, oxygen, turbidity, and water quality to be the mechanism responsible for population differentiation of Caspian vimba in the southern Caspian Sea basin.

Figure 1. Location of sampling sites including Astara, Bandar-Anzali, Tonekabon, Sari and Bandar-Torkaman in the south Caspian Sea basin.

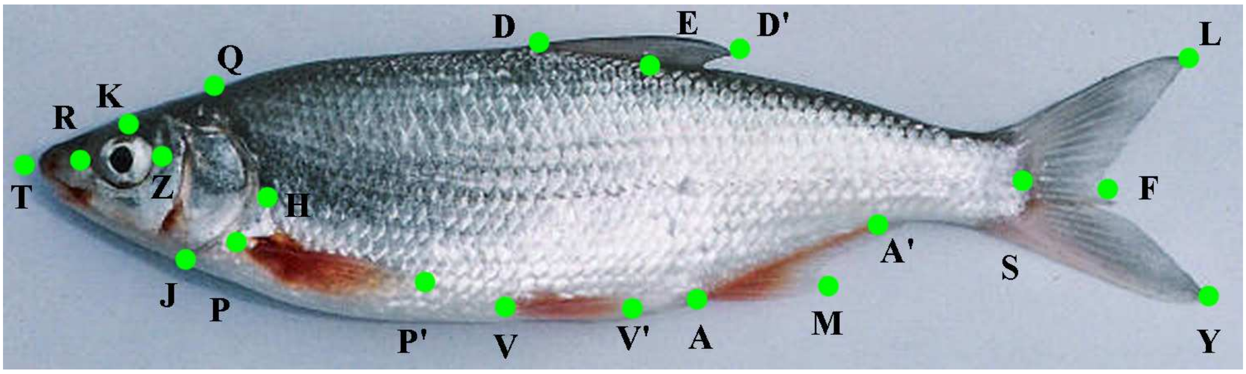

Figure 2. Morphometric characters measured on Caspian vimba samples. Landmarks selected based on studies of S Holcik *et al*. 1989; Samaee *et al*. 2009 and Anvarifar *et al*., 2013. TL: total length, TF: fork length, TS: standard length, TH: head Length, QJ: head depth, TR: snout length (preorbital distance), RZ: eye diameter, KK': interorbital distance, ZH: postorbital distance, D↓: body width, A'↑: caudal peduncle depth, DE: dorsal fin base, DD': dorsal fin length, PP': pectoral fin length, VV': pelvic fin length, PV: pectoral-ventral distance, AW: anal fin length, VA: ventral-anal distance, AA': anal fin base, TD: predorsal distance, ES: postdorsal distance, SL: caudal upper lobe length, SY: caudal lower lobe length, HH': head wide, TA: preanal distance.

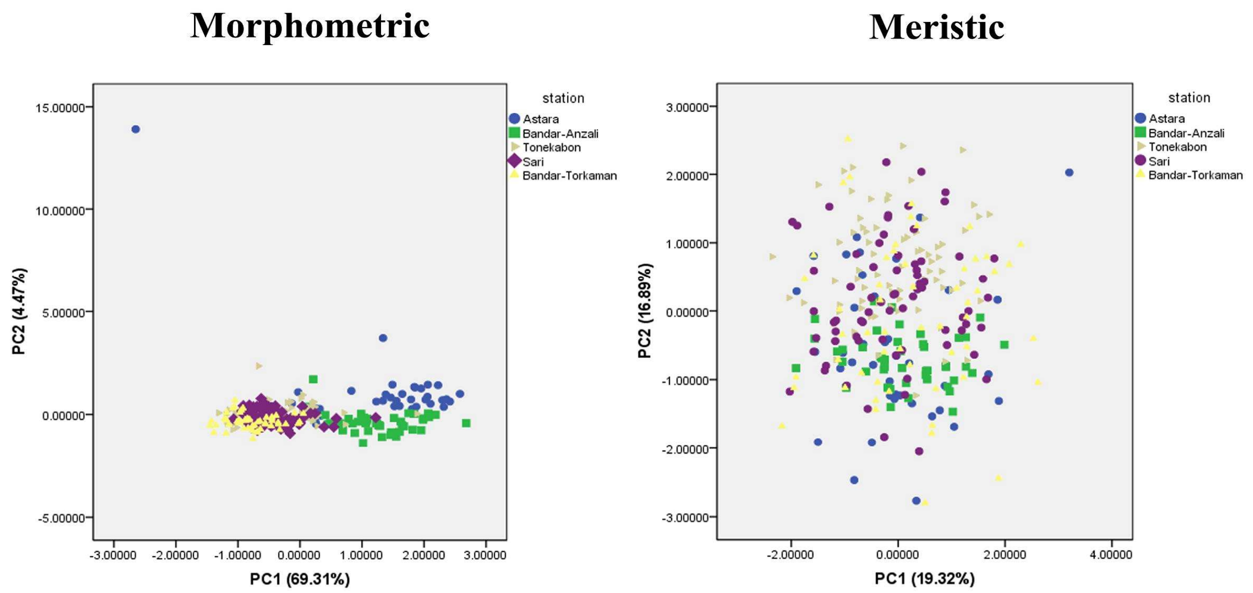

Figure 3. Plot of the factor scores for PC1 and PC2 of all morphometric and meristic measurements for Caspian vimba populations from different sampling sites in the southern Caspian Sea basin.

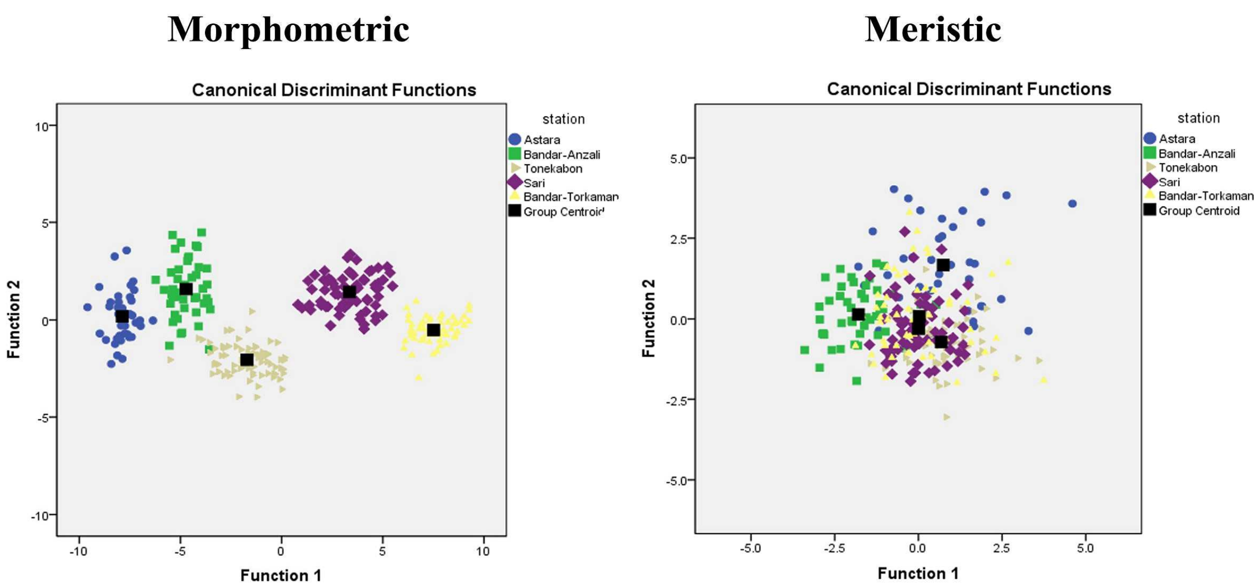

Figure 4. Coordinate Plot for males of Caspian vimba specimens according to the first two discriminant functions for morphometric and meristic data analysis from different sampling sites in southern Caspian Sea basin.

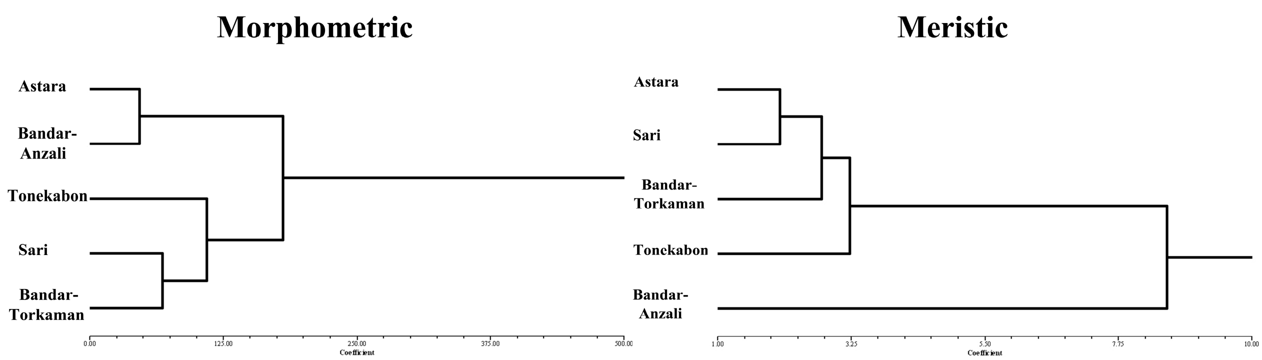

Figure 5. Dendrogram derived from cluster analyses of 25 morphometric and 10 meristic measurements on the basis of Euclidean distance for Caspian vimba populations in the southern Caspian Sea basin.

Table 1. Descriptive data (Mean ± S.D. and Min-Max) of siahkooli from sampling sites including Astara, Bandar-Anzali, Tonekabon, Sari and Bandar-Torkaman in the south Caspian Sea basin.

Station	Sex	N	Min-Max (length mm)	Mean± S.D. (length)	Min-Max (weight gr)	Mean± S.D. (weight)
Astara	Male	32	14.24-191.59	144.85±31.71	26.2-70.02	45.62±10.05
	Female	7	116.69-158.71	140.32±13.81	29.34-68.18	43.53±12.18
Bandar-Anzali	Male	30	142.86-206.27	175.10±15.55	44.44-109.71	68.53±19.02
	Female	16	173.21-217.75	193.97±12.14	45.11-142.91	82.94±23.24
Tonekabon	Male	46	117.67-216.66	146.73±18.54	37.49-178.01	93.69±33.70
	Female	30	106.30-211.24	151.31±22.26	42.56-178.54	97.86±39.16
Sari	Male	39	165.45-244.78	187.59±13.89	61.58-207.40	93.42±24.85
	Female	33	163.08-268.0	200.89±19.44	59.65-278.0	124.40±43.44
Bandar-Torkaman	Male	28	153.24-258.15	198.25±25.06	37.85-183.68	96.14±38.52
	Female	27	147.08-241.31	198.24±20.94	38.20-164.97	95.87±37.54

Table 2. Results of ANOVA of morphometric characters of siahkooli samples between stations sampling sites Astara, Bandar-Anzali, Tonekabon, Sari and Bandar-Torkaman in the south Caspian Sea basin.

Morphological measurements	F value	P value	Morphological measurements	F value	P value	Morphological measurements	F value	P value
TL	70.62	0.00	**DD'**	27.07	0.00	**TA**	132.29	0.00
TF	200.07	0.00	**PP'**	76.18	0.00	**L1**	43.57	0.00
TS	1418.32	0.00	**VV'**	112.67	0.00	**Squ.sup**	8.03	0.00
TH	111.26	0.00	**PV**	103.23	0.00	**Squ.inf**	4.15	0.00
QJ	67.62	0.00	**VA**	84.12	0.00	**D1**	.	.
TR	33.84	0.00	**AM**	23.87	0.00	**D2**	31.00	0.00
RZ	9.43	0.00	**AA'**	53.31	0.00	**A1**	.	.
KK'	71.13	0.00	**TD**	163.63	0.00	**A2**	9.23	0.00
ZH	0.79	0.53	**ES**	128.80	0.00	**Gr1**	1.00	0.41
D↓	159.40	0.00	**SL**	107.02	0.00	**Gr2**	0.94	0.44
A'↑	95.40	0.00	**SY**	93.66	0.00	**Vn**	2.31	0.08
DE	69.43	0.00	**HH'**	89.33	0.00			

Table 3. Results of ANOVA for sex dimorphism of morphometric characters in siahkooli samples from sampling sites in the south Caspian Sea basin.

morphometric measurements	F value	P value	morphometric measurements	F value	P value	morphometric measurements	F value	P value
TL	0.01	0.93	**D↓**	0.64	0.43	**AA'**	6.86	0.01
TF	2.05	0.15	**A'↑**	0.94	0.33	**TD**	4.70	0.03
TS	9.76	0.00	**DE**	3.77	0.05	**ES**	1.50	0.22
TH	0.72	0.40	**DD'**	2.96	0.09	**SL**	0.78	0.38
QJ	0.79	0.37	**PP'**	3.90	0.05	**SY**	2.19	0.14
TR	1.13	0.29	**VV'**	2.65	0.10	**HH'**	4.17	0.04
RZ	0.94	0.33	**PV**	1.76	0.19	**TA**	3.65	0.06
KK'	0.24	0.63	**VA**	0.08	0.77			
ZH	0.01	0.93	**AM**	1.39	0.24			

Table 4. Eigenvalues, percentage of variance and percentage of cumulative variance for the two and four principal components in morphometric and meristic of siahkooli specimens in the southern Caspian Sea basin

	Morphomtric			Meristic		
Factor	**Eigenva.**	**Per. of Var.**	**Per. of Cumu. var.**	**Eigenva.**	**Per. of Var.**	**Per. of Cumu. var.**
PC1	17.33	69.32	69.32	1.55	19.32	19.32
PC2	1.12	4.48	73.79	1.35	16.89	36.21
PC3	1.04	4.16	77.95	1.18	14.78	50.99
PC4				1.07	13.40	64.39

Table 5. Result of Wilks' lambda test for verifying difference among populations of siahkooli when morphological measurements are separately compared using discriminant function analysis.

	Test of Functions	Wilks' Lambda	Chi-square	Df	sig
Morphometric	1 through 4	0.00	1606.32	60.00	0.00
	2 through 4	0.08	685.50	42.00	0.00
	3 through 4	0.26	372.33	26.00	0.00
	4	0.57	155.74	12.00	0.00
Meristic	1 through 4	0.31	326.38	20	0.00
	2 through 4	0.54	172.94	12	0.00
	3 through 4	0.83	49.74	6	0.00
	4	0.96	9.60	2	0.00

Table 6. Percentage of specimens classified in each group and after cross validation for morphometric and meristic data of siahkooli populations in south of Caspian Sea basin.

			Predicted Group Membership									
			Morphometric					Meristic				
		Station	Astara	Bandar-A.	Tonekabon	Sari	Bandar-T.	Astara	Bandar-A.	Tonekabon	Sari	Bandar-T.
Cross-validated[a]	**Count**	Astara	38	1	0	0	0	21	3	4	6	5
		Bandar-A.	0	45	1	0	0	1	39	1	1	4
		Tonekabon	1	2	73	0	0	2	1	46	15	12
		Sari	0	0	0	72	0	5	1	13	41	11
		Bandar-T.	0	0	0	0	55	10	15	13	5	12
	%	Astara	97.44	2.56	0.00	0.00	0.00	53.85	7.69	10.26	15.38	12.82
		Bandar-A.	0.00	97.83	2.17	0.00	0.00	2.17	84.78	2.17	2.17	8.70
		Tonekabon	1.32	2.63	96.05	0.00	0.00	2.63	1.32	60.53	19.74	15.79
		Sari	0.00	0.00	0.00	100.00	0.00	7.04	1.41	18.31	57.75	15.49
		Bandar-T.	0.00	0.00	0.00	0.00	100.00	18.18	27.27	23.64	9.09	21.82

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.

CONCLUSION

In conclusion, the present study showed that each sampling site represents independent population. The results can be interesting for management, aquaculture, restocking and conservation programs of this valuable 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.

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