Landmark-Based Morphological Differences Among the Exotic *Rhinogobius lindbergi* and Its Two Sympatric Gobies (Actinopterygii: Perciformes: Gobiidae) in Sefid River, in the Southern Caspian Sea Basin

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A B S T R A C T

Introduced species may cause harm to native fish populations, which to design any conservative program to control the exotics an identification key is necessary. To find the morphological differences among the exotic *Rhinogobius lindbergi* and its sympatric congeners including the endemic *Ponticla iranicus* and the native *Ponticla gorlap*, a 15-landmark morphometric system was used to examine 90 specimens in Sefid River, in the Southern Caspian Sea basin. Univariate analysis of variance showed significant differences among the means of the three groups for 79 out of 105 standardized morphometric measurements. Principal component analysis (*PCA*) and canonical variates analysis (*CVA*) confirmed the statistically significant difference among these species. The *CVA* scatter plot showed that the 90 studied specimens grouped into three distinct areas with a degree of overlap between *P. iranicus* and *P. gorlap*. Clustering based on Euclidean distances among the groups of centroids using an UPGMA indicated segregation of the three species into two distinct clusters: *P. iranicus* and *P. gorlap* in one group and *R. lindbergi* in the other group. The exotic *Rhinogobius* can be distinguished from the sympatric gobies in Sefid River by short snout (vs. longer), deep body (vs. shallow), deeper head, stout body, and smaller ventral disc.

Keywords: Gobiidae, truss network system, geometric morphometric, landmark, Iran

How to Cite

Introduction

Invasive species are alien (non-native) organisms that have been introduced into an area outside of their natural range, establishing self-sustaining populations and spreading beyond their initial point of introduction, with deleterious impacts on the environment, economy and human health (Lymbery et al. 2014). Biological invasions are now considered a major environmental issue of public concern (Gozlan et al. 2010).

In Iran, there is an exotic goby, *Rhinogobius lindbergi*, which is reported from the Hari River basin (Coad and Abdoli 2000), Anzali Wetland in the

Caspian Sea basin (Abdoli et al. 2000), the Zarrineh River (Zarrinehrud) in Urmia Lake basin (Eagderi and Moradi 2017), the Namak Lake basin (Eagderi et al. 2017), and the Tigris River basin (Sadeghi et al. 2018, Mousavi-Sabet et al. 2019). However, among the above-mentioned basins, the Caspian Sea basin is the only water basin which has native gobies, too. The genus *Ponticola*, with five species is the native genus which its members live in tributaries of the Caspian Sea basin (in Iranian portion). *Ponticola gorlap* (Iljin 1949), *P. syrman* (Nordmann 1840), *P. ratan* (Nordmann 1840), *P. cyrius* (Kessler 1874) and *P. iranicus* Vasil'eva, Mousavi-Sabet and Vasil'ev,

2015 (Vasil'eva et al. 1993; Miller 2003; Medvedev et al. 2013; Vasil'eva et al. 2015) are reported from the basin. Normally, the fishes of the family Gobiidae are usually less important commercially, therefore knowledge on their identification and biodiversity is far from being complete (Miller 2004; Neilson and Stepien 2009; Bogutskaya et al. 2013). Regarding problems in Gobiids identification, separating the exotic and native individuals/populations is not easy. Therefore, to design any conservative program, a practical identification key is strongly necessary. Preparing any key to identify fish species needs powerful analytical manners which the traditional and modern morphological analyzing methods are normally used for fish populations/species. As mentioned above, 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 too (Turan 2004; Yamamoto et al. 2006; Pollar et al. 2007). In addition to traditional method, in recent years, truss network system is increasingly used for morphometric measurements with the purpose of species and/or stock differentiation (Turan 2004; AnvariFar et al. 2011; Mousavi-Sabet et al. 2012; Mousavi-Sabet and Anvarifar 2013; Khataminejad et al. 2013; Kohestan-

Eskandari et al. 2013, 2014; Heidari et al. 2014). Also, geometric morphometrics (*GM*), a quantitative approach to analysis shape, is widely applied to compare and determine shape variations of biological structures (Adams et al. 2004). Despite traditional approaches, in *GM*, data is obtained from the coordinates of landmark points (Adams et al. 2004; Rohlf 2005), which are morphological points of specimens that are of biological interest (Rohlf 2005).

The present study aimed to clarify the morphometric differences among the exotic *R. lindbergi* and its sympatric endemic *P. iranicus* and native *P. gorlap* in Sefid River, in the southern Caspian Sea basin.

Material and Methods
Fish sampling

The specimens were collected on August 2015 to May 2017 in Sefid River (37º01.153′N, 49º37.985′E) from the southwestern Caspian Sea basin, Iran (Figure 1). Fishes were collected by electrofishing. The sampled fishes were photographed and then fixed in 10% formaldehyde at the sampling site and transported to the Laboratory for further studies. Identification was verified base on Vasil'eva et al. 1993, Miller 2003 and Vasil'eva et al. 2015 in the laboratory.
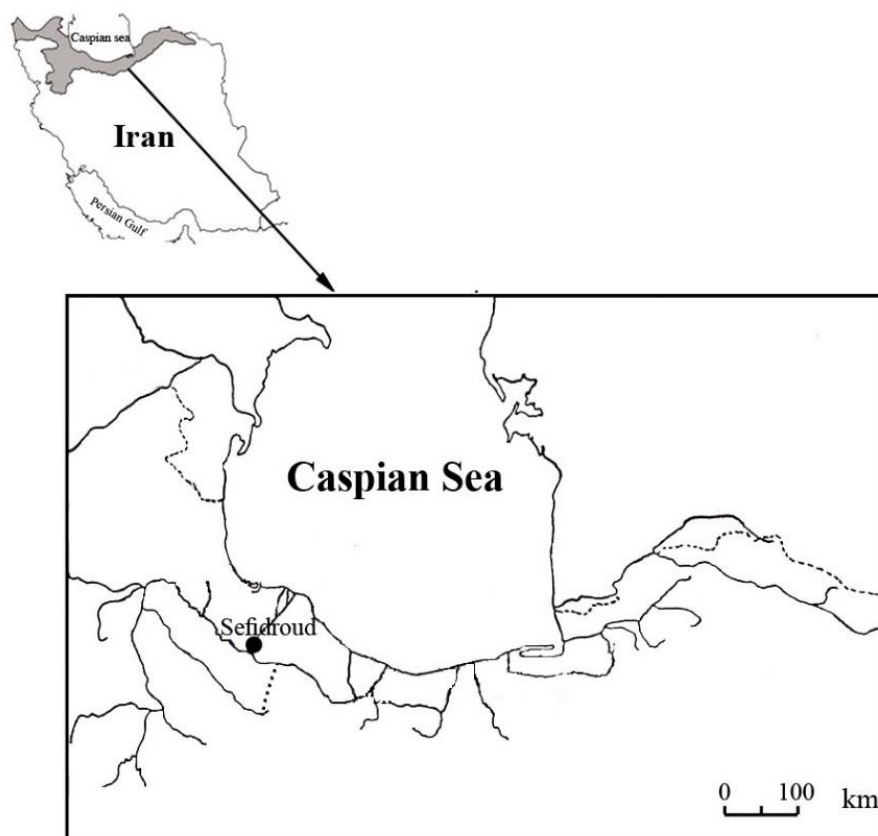

Figure 1. Map of the Iranian part of the southern Caspian Sea basin and location of Sefid River from the southern Caspian Sea basin.

Laboratory work

In the laboratory, Standard Length (±1.0 mm) and body weight (±0.001 g) were recorded for each specimen. For specimens 105 distance measurements between 15 landmarks on the lateral side and 78 distance measurements were surveyed using the truss network system according to Bookstein (1991) with minor modifications for these species (Figure 2). Also, for specimens 44 and 28 distance measurements between 10 and 8 landmarks on ventral and dorsal views, respectively, were surveyed for investigation under and up sides shape variation. The fishes were placed on a white board with dorsal and anal fins held erect by pins. The left body profile of each fish was photographed with a 300-dpi, 32-bit color digital camera (Cybershot DSC-F505; Sony, Japan). Images were saved in jpg format and analyzed with TPSdig to coordinates of landmarks. All measurements transformed into linear distances by computer for subsequent analysis. After image capture, the fish was dissected to identify the sex of the specimen 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 male and female specimens.
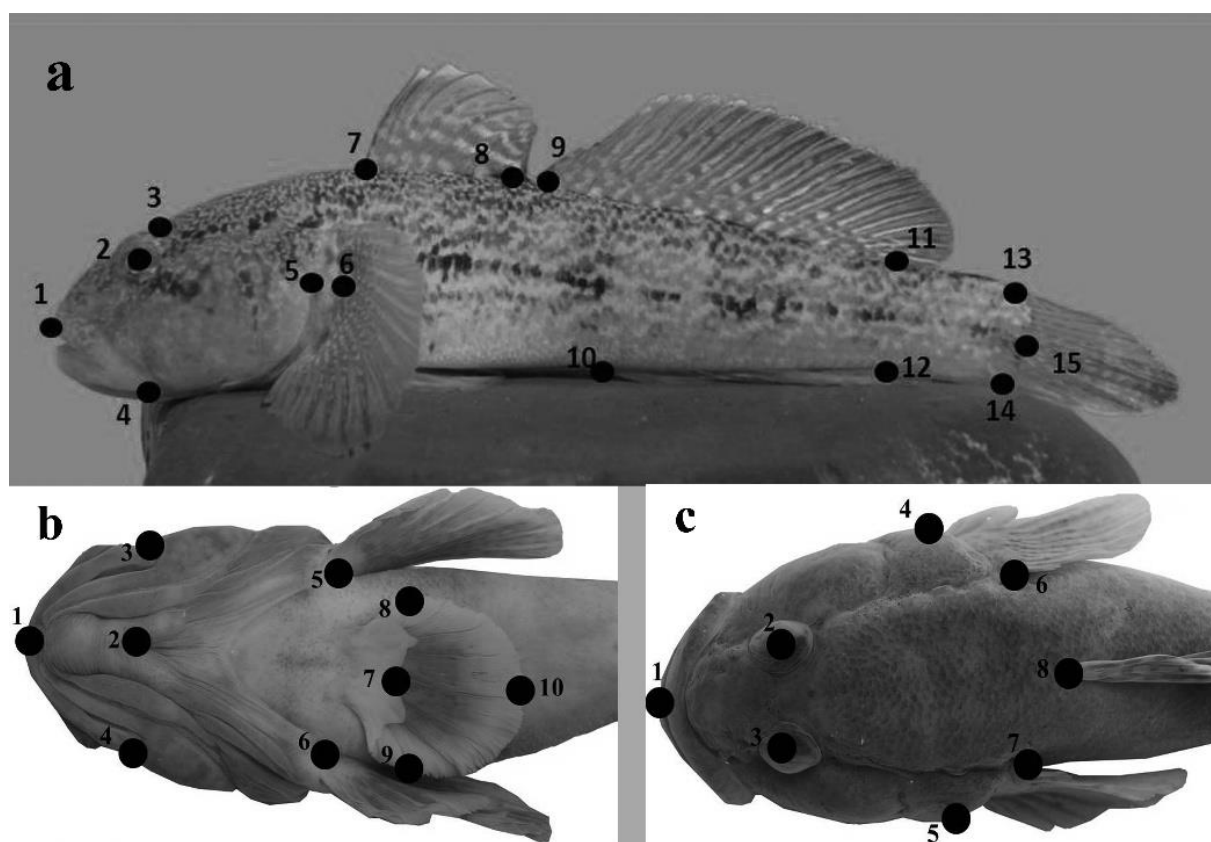

Figure 2. Locations of the landmarks on lateral (a), ventral (b) and dorsal (c) sides of the goby.

Data analysis

As variation should be attributed 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:

$$Madj = M (L_s / L_0)^b$$

where M is the original measurement, $Madj$ the size adjusted measurement, L_0 the standard length of the fish, L_s the overall mean of the 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 the significance of the correlation between transformed variables and standard length (Mustafic et al. 2008).

Univariate analysis of variance (ANOVA) was performed for each morphometric character to evaluate the significant difference among the fish specimens (Bookstein 1991). Principal component analysis (*PCA*) and canonical variates analysis (*CVA*) were employed to discriminate these fishes from studied rivers. Statistical analyses for morphometric data were performed using the SPSS version 16 software package, past ver. 1.36, MorphoJ and Excel (Microsoft Office 2010).

Results

Descriptive data were examined for the mean and standard deviation (*SD*) of length and weight in case

of sampled specimens (Table 1). Although it is well known that the female and male specimens of the gobies have some morphological differences (Mousavi-Sabet et al. 2012; Vasil′eva et al. 2015), but the interaction between morphometric measurements used in this study by truss network system and sexes uses ANOVA analysis was not significant (P > 0.05), demonstrating

a negligible effect of sex on observed variations; hence, the data for both sexes were pooled for all subsequent analyses. There was no significant correlation between any of the transformed measured morphometric variables and standard length (P > 0.05), indicating that size or allometric signature on the basic morphological data was accounted.

Table 1. Descriptive data [mean ± SD standard length (mm) and body weight (g)] of goby specimens.

Species	n	Standard length (mm)		Body weight (g)	
		Min–max	Mean ± SD	Min–Max	Mean ± SD
R. lindbergi	30	35.71-112.87	70.55±21.85	1.15-34.90	10.25±9.30
P. iranicus	30	53.33-139.92	74.23±23.64	3.77-51.61	12.86±11.85
P. gorlap	30	20.80-111.95	55.30±20.80	1.00-33.05	5.81±8.11

Table 2. The results of ANOVA for morphometric measurements of goby specimens in Sefid River from the southern Caspian Sea basin.

Characters	F	P	Characters	F	P	Characters	F	P	Characters	F	P
1-2	11.75	0.00	3-4	2.22	0.09	5-10	1.41	0.24	8-13	9.10	0.00
1-3	15.13	0.00	3-5	0.28	0.84	5-11	2.49	0.06	8-14	8.92	0.00
1-4	6.19	0.00	3-6	2.61	0.05	5-12	4.14	0.00	8-15	12.88	0.00
1-5	11.20	0.00	3-7	4.22	0.00	5-13	9.28	0.00	9-10	23.10	0.00
1-6	9.66	0.00	3-8	3.15	0.03	5-14	4.11	0.00	9-11	4.51	0.00
1-7	17.67	0.00	3-9	3.33	0.02	5-15	8.44	0.00	9-12	4.09	0.00
1-8	18.66	0.00	3-10	5.08	0.00	6-7	1.79	0.15	9-13	7.72	0.00
1-9	5.79	0.00	3-11	5.54	0.00	6-8	2.58	0.07	9-14	6.35	0.00
1-10	9.46	0.00	3-12	13.98	0.00	6-9	4.60	0.00	9-15	9.77	0.00
1-11	11.26	0.00	3-13	8.46	0.00	6-10	0.59	0.62	10-11	0.22	0.88
1-12	23.86	0.00	3-14	11.94	0.00	6-11	1.80	0.15	10-12	8.29	0.00
1-13	7.02	0.00	3-15	8.72	0.00	6-12	0.99	0.40	10-13	7.65	0.00
1-14	8.40	0.00	4-5	10.95	0.00	6-13	14.05	0.00	10-14	2.80	0.04
1-15	5.30	0.00	4-6	9.92	0.00	6-14	7.77	0.00	10-15	9.37	0.00
2-3	8.76	0.00	4-7	4.92	0.00	6-15	12.78	0.00	11-12	16.89	0.00
2-4	3.56	0.02	4-8	4.27	0.00	7-8	0.89	0.45	11-13	10.85	0.00
2-5	4.85	0.00	4-9	1.03	0.38	7-9	7.28	0.00	11-14	4.80	0.00
2-6	6.17	0.00	4-10	4.71	0.00	7-10	13.71	0.00	11-15	19.42	0.00
2-7	9.57	0.00	4-11	6.94	0.00	7-11	2.91	0.04	12-13	16.96	0.00
2-8	4.99	0.00	4-12	13.23	0.00	7-12	1.13	0.34	12-14	9.06	0.00
2-9	2.21	0.09	4-13	0.67	0.57	7-13	10.02	0.00	12-15	18.95	0.00
2-10	4.44	0.00	4-14	0.76	0.52	7-14	11.32	0.00	13-14	1.81	0.15
2-11	3.33	0.02	4-15	0.38	0.77	7-15	20.63	0.00	13-15	1.42	0.24
2-12	20.26	0.00	5-6	14.46	0.00	8-9	2.30	0.08	14-15	7.78	0.00
2-13	7.70	0.00	5-7	1.34	0.26	8-10	19.13	0.00			
2-14	2.12	0.10	5-8	1.48	0.22	8-11	7.15	0.00			
2-15	1.97	0.12	5-9	2.46	0.07	8-12	3.74	0.02			

Statistically significant differences in goby specimens were observed in 79 morphometric characters out of 105 studied. Of these 79 characters, 72 characters were found to be highly significant (P≤0.01) and were used further for multivariate analysis (Table 2). The value of Kaiser-Meier-Olkin coefficient (*KMO*) for the overall matrix is 0.721 for goby specimens and Bartlett's Test of sphericity is significant (P≤0.01). The results of *KMO* and Bartlett's suggest that the sampled data is appropriate to proceed with a factor analysis procedure.

In order to determine which morphometric measurement affected populations differentiates mostly, the contributions of variables to principal components (*PC*) were examined. The *PCA* of 72 morphometric measurements for goby specimens extracted 12 factors with eigenvalues >1, explaining 93.91% of the variance (Table 3). Of these, the first explained 28.37% and the second 16.35% of the variance. The most significant weightings on *PC* I were from 1-5, 1-6, 5-15, 2-6, 2-5, 6-15, 6-9, 4-6, 4-5, 6-13, 6-14, 5-13, 1-14, 5-14, 1-4, 1-13, 1-11, 1-12,

1-2, 5-12, 1-10, 1-8, 1-3, 1-7, 1-9 and on *PC* II were from 1-12, 12-15, 9-12, 12-13, 12-14, 3-12, 2-12, 10-12, 5-12, 4-12 and 3-14.

Canonical variates analysis confirmed the significant difference among the goby specimens.

The scores of the two canonical variables for each river revealed that goby specimens grouped into three species while there was a relativity high degree of overlap between *P. iranicus* and *P. gorlap* (Figure 3).

Table 3. Eigen values, percentage of variance and percentage of the cumulative variance of morphometric measurements for goby specimens in Sefid River from the southern Caspian Sea basin.

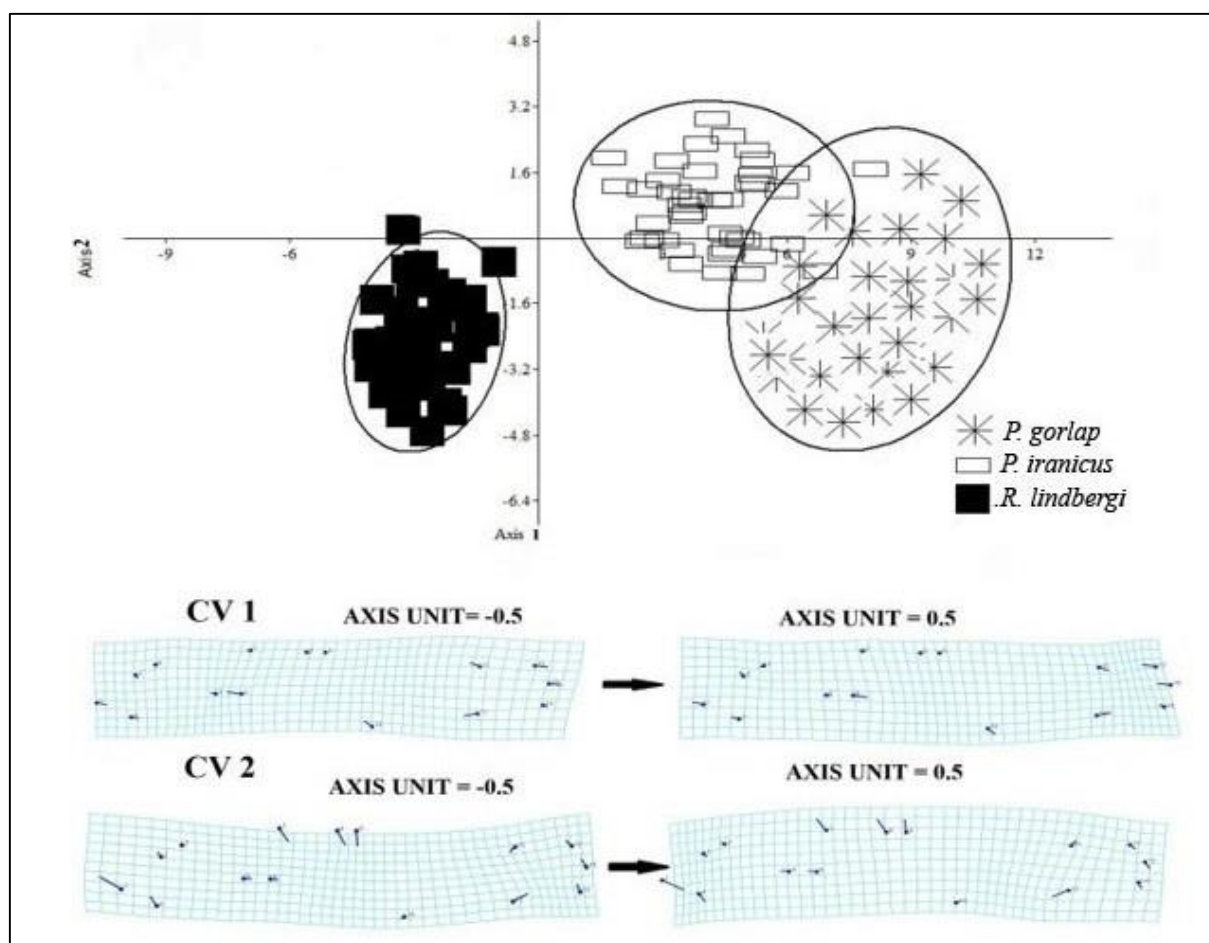| Factor	Eigenvalues	Percentage of variance	Percentage of cumulative variance
1	20.141	28.367	28.367
2	11.606	16.347	44.714
3	8.061	11.354	56.068
4	6.906	9.727	65.795
5	4.550	6.408	72.203
6	4.242	5.974	78.178
7	2.754	3.879	82.056
8	2.447	3.447	85.503
9	2.097	2.953	88.456
10	1.478	2.082	90.538
11	1.350	1.901	92.439
12	1.041	1.466	93.905

Figure 3. Scatterplot of standardized canonical variates functions 1 (CV1) and 2 (CV2) for morphometric characteristics of goby specimens

The Wilks' lambda tests indicated differences between fishes when their morphometric measurements were compared by means of discriminant analysis. In this test

all functions were highly significant ($P \leq 0.01$). The histogram of discriminant functions for pairwise groups in goby specimens is shown in Figure 4.
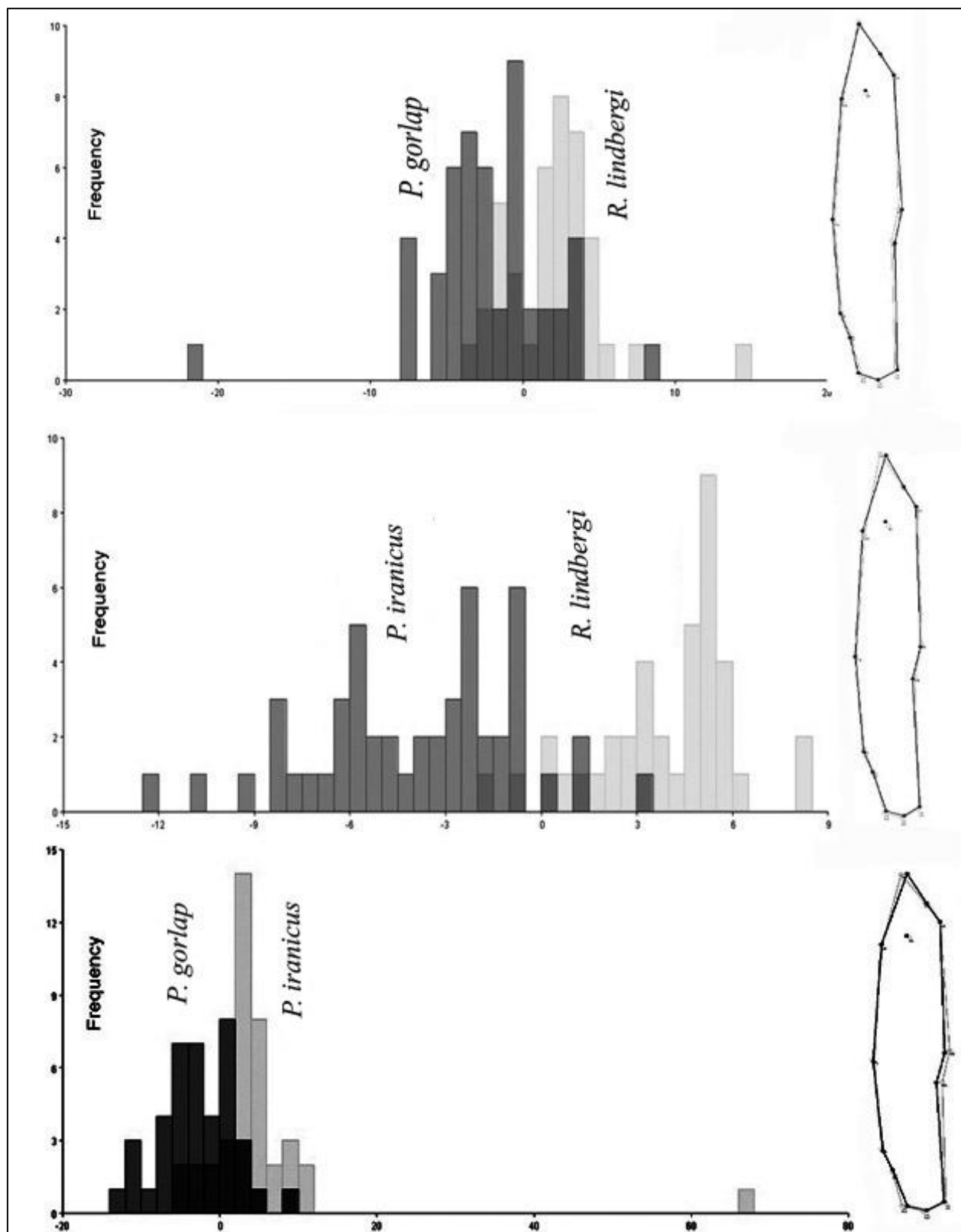
Figure 4. Histogram of discriminate analysis (DA) functions for pair wise competitions' of gobies (left). Shape differences on the extremities of each river (right).

Body shape differences show a longer snout, shallow body and head depths, and elongated body for *P. gorlap* and *P. iranicus*, vs. relatively short snout, high body and head depths and stout body for *R. lindbergi*. Also, underside and upside shape variation analysis show a longer snout and disc for *P. gorlap* and *P. iranicus*, vs. relatively short snout and disc and high body for *R. lindbergi* (Figure 5).

The dendrogram derived from the cluster analysis of Euclidean distances among groups

of centroids showed that the three populations of gobies segregated from each other into two distinct clusters, *P. gorlap* and *P. iranicus* appeared in one cluster while *R. lindbergi* belonged to the other clusters (Figure 6).
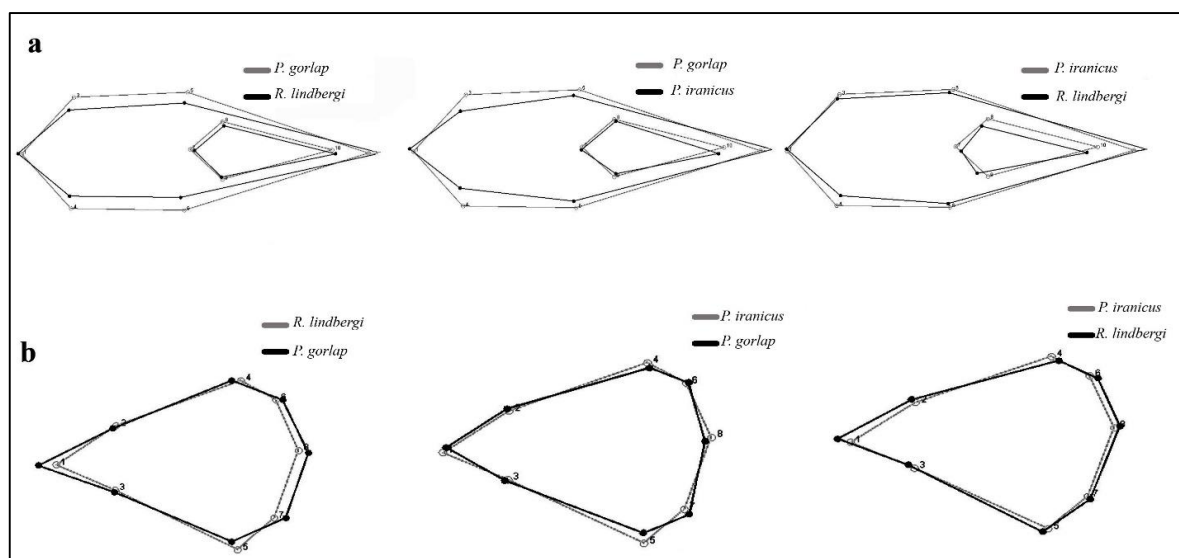

Figure 5. Underside and upside shape variation analysis of gobies in Sefid River from the southern Caspian Sea basin. (a) Ventral view (b) dorsal view.
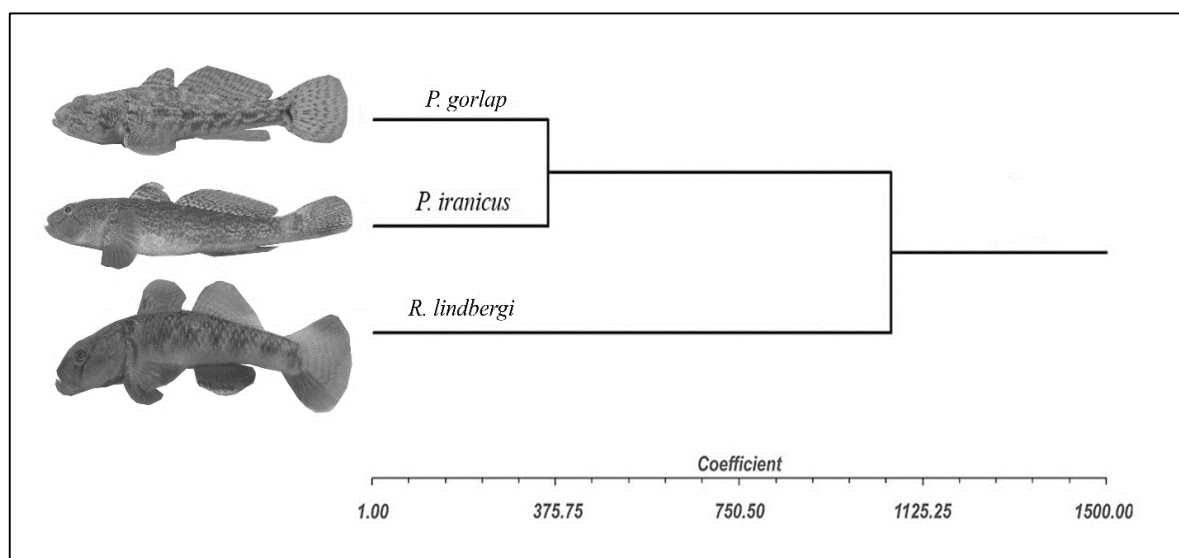

Figure 6. Dendrogram derived from cluster analyses of morphometric measurements on the basis of Euclidean distance for gobies in Sefid River from the Southern Caspian Sea. Mean shape of species in the relation of consensus shape of the species are also represented.

Discussion

The results of the multivariate analysis demonstrated that the three examined species are correctly separated from each other, which the two *Ponticola* (*P. iranicus* and *P. gorlap*) are classified as one clade and the *Rhinogobius* in the other clade. It is well known that both studied genera, *Rhinogobius* and *Ponticola*, are clearly distinct groups morphologically, and as the *Ponticola* are larger species than the *Rhinogobius*, they can be easily distinguished if the *Ponticola* is in its maximum range size. Despite these differences, separating small *Ponticola* specimens in the same size as *Rhinogobius* is not so easy or sometimes is unrecognizable by morphological characters. Therefore, the obtained results of the present study provide helpful identification key to separate them from each other. The results for the ANOVA analysis showed that 79 out of 105 transformed morphometric measurements were significantly different in these groups of Gobies living in the southern Caspian Sea basin, which demonstrates a high phenotypic variation among them. The *PCA* showed morphological segregation of the studied fishes based

on the characters head shape, pre-dorsal, pre-anal distances, pre-orbital and post-orbital distances, body length, body depth, caudal peduncle depth, caudal peduncle length, dorsal and anal fins origin and caudal fin origin for gobies. Body shape differences for these fishes shows a longer snout, shallow body and head, and elongated body for *P. iranicus* and *P. gorlap*, vs. relatively short snout, deeper body and head and stout body for *R. lindbergi*, for general appearance see Figure 7.
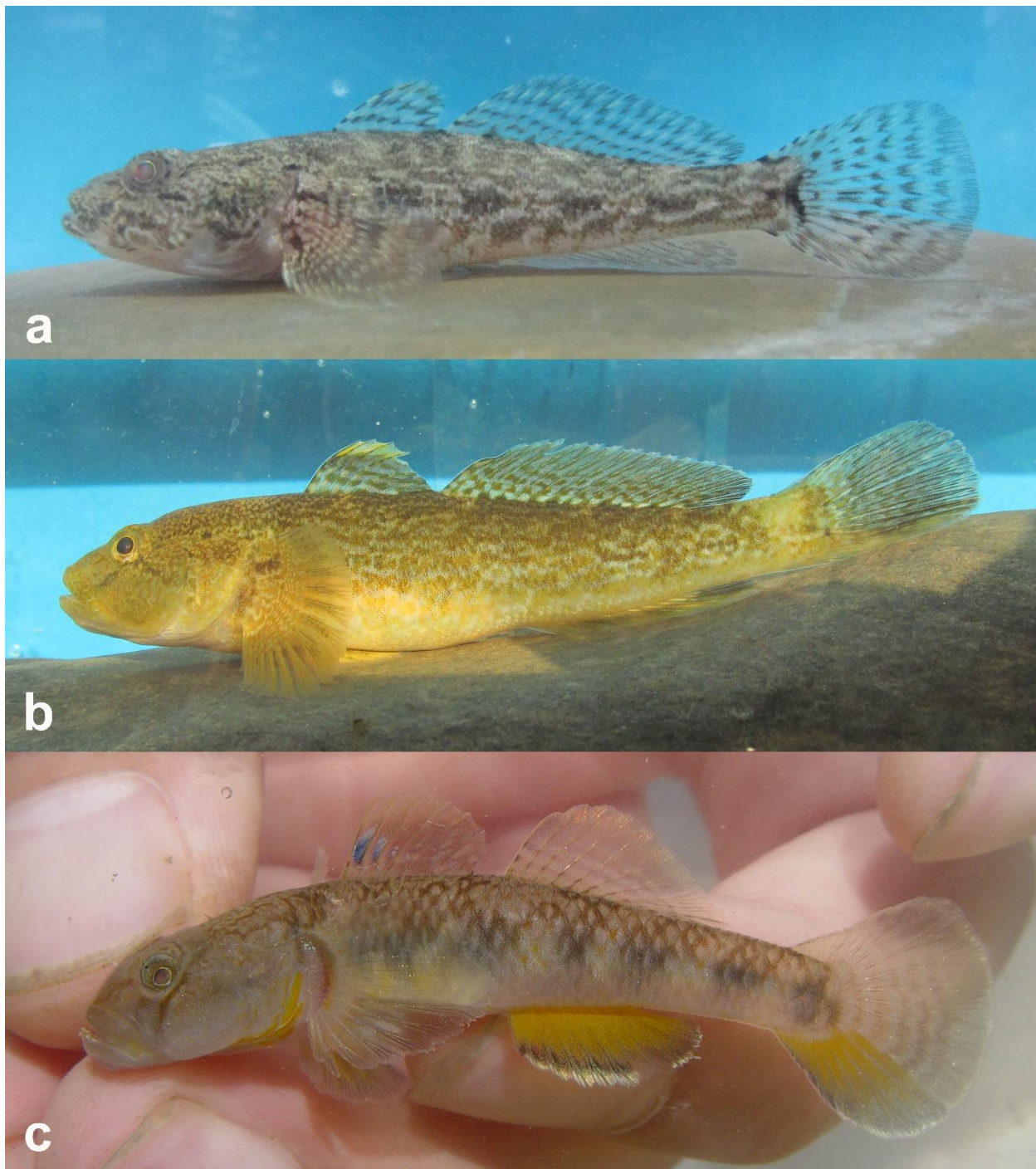

Figure 7. *Ponticola gorlap* (a), *Ponticola iranicus* (b) and *Rhinogobius lindbergi* (c).

CVA 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 (Bookstein 1991; Buj et al. 2008; Torres et al. 2010; AnvariFar et al. 2011; Heidari et al. 2013; Heidari et al. 2014). The results of *CVA* obtained in the present study indicated the relative segregation among three species of gobies in the studied areas. This was confirmed by plotted *CV*1 and *CV*2 scores for each sample that showed 90 specimens grouped into three distinct areas with a degree of overlap between *P. iranicus* and *P. gorlap*. 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 (Bookstein 1991). 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 species are often quite difficult to explain (McLaughlin and Grant 1994; Cadrin and Friedland 1999; Pakkasmaa and Piironen 2000; Cadrin 2000; Vatandoust et al. 2014a; Vatandoust et al. 2014b; Vatandoust et al. 2015; Paknejad et al. 2014). It has been suggested that the morphological characteristics of fish are determined by an interaction between genetic and environmental factors (Pinheiro et al. 2005). The phenotypic variability may not necessarily reflect population differentiation at the molecular level (Turan 2004; Yamamoto et al. 2006; Pollar et al. 2007; Eschmeyer and Fong 2011).

The present findings revealed the potential power of the landmark-based methods for the identification of goby specimens. The present study provides basic information about the differences of *Ponticola* species and the exotic *R. lindbergi* in Sefid River from 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 the aquarium trade.

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