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Research Article

Morphological variation of Iranian Goby (*Ponticola iranicus*) in the Anzali Wetland drainage

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Abstract
This study was conducted to compare the morphological characteristics of *Ponticola iranicus* populations from the Anzali Wetland drainage (wetland and it's two draining rivers, namely Siahdarvishan and Pasikhan, Southern Caspian Sea basin). For this purpose, five specimens of *P. iranicus* from the Pasikhan River, 10 from Siah-Darvishan River and 8 from the Anzali Wetland were collected. After anaesthesia, fish were fixed into 10% buffered formalin and transferred to the laboratory for further study. A total of 22 morphometric traits were measured using a digital calliper. The data, after removing size, were analyzed for normality and the significant data were selected and used for multivariate analysis, including Principle component analysis (PCA), Canonical variate analysis (CVA) and Non-parametric multivariate analysis of variance (NPMANOVA). The results showed a significant difference in inter-orbital distance, minimum width of the caudal peduncle and eye diameter between the studied populations ($P<0.05$). Besides, the Anzali wetland population was discriminated from others due to morphological characteristics. The observed morphological changes in P. *iranicus* can be as a result of their habitat adaptation such as current type and feeding.

Keywords: Canonical variate analysis, Iranian Goby, morphometric, principle component analysis.

Introduction
A study on fishes in the aquatic ecosystem is important in terms of biology, ecology and water resources management (Nelson *et al*. 2016). Morphology is an important biological character in fishes because it affects their survival, reproduction, and feeding (Guill *et al*. 2003). Body shape plays an important role in quantity description, morphological comparison (Zelditch *et al*. 2004), and biodiversity and systematic studies (Adams *et al*. 2004). Gobiids include 15 genera and 42 species in Iranian inland waters. The genus *Ponticola* comprises six species and they can be distinguished by having an acute lateral lobe, dentary with several large conical teeth on its rear part, well-developed anterior pelvic membrane and maxilla with an expanded posterior end. Iranian goby, *Ponticola iranicus*, is found in the southern Caspian Sea basin (Esmaeili *et al*. 2018) inhabiting mostly in the mountainous area (Vasileva *et al*. 2015, Coad 2019).
Based on the previous studies, *P. cyrius* was reported from the Anzali Wetland drainage (Ahnelt and Holcik 1996). But recently, Vasileva *et al*. (2015) described *P. iranicus* from the Sefid river drainage including Shahre Bijar, Totkabon and Gisum rivers, Guilan Province, which probably is those previously reported as *P. cyrius*, however, it needs to be confirmed. Little information is available regarding morphological features of Gobiids in the southern Caspian Sea basin and it is expected morphological changes resulted from different habitats that is a common phenomenon in aquatic organisms (Monteiro *et al*. 2003).

Therefore this study aimed to investigate the morphological diversity of Iranian goby in the Anzali Wetland drainage including Wetland, and Siahdarvishan and Pasikhan rivers.

Material and methods

During 2018, five specimens from Pasikhan River (37°10′45.9″N, 49°28′51″E),, 8 from Anzali Wetland (37°24′56″N, 49°24′32.4″E) and 10 from Siahdarvishan River (37°16′31.6″N, 49°22′28.7″E) were collected using electrofishing device. The specimens were anesthetized in a 1% clove oil solution and then fixed into 10% buffered formalin and transferred to the laboratory for further studies. Then, 22 morphometric characters were measured using a digital calliper with an accuracy of 0.01 mm (Table 1). To eliminate size from data, they were standardized based on Elliot *et al*. (1995) using the following formula:

Madj=M(LS/L0)b

Where M is original measurements, Madj = size-adjusted measurements, LS = overall mean of standard length (SL) for all samples, L0 = standard length of the fish and b = slope of the regression of logM on logL0 of all samples. The efficiency of size adjustment transformations was assessed by testing the significance of the correlation between a transformed variable and SL. Lacking significance correlation indicates the complete elimination of size data. The normality of data was tested using Kolmogorov–Smirnov. The normal data were analyzed using One-Way ANOVA and non-normal data by Kruskal-Wallis. Principal component analysis (PCA) was used to analysis the morphology data. PCA summarizes the variances of the measured variables in a smaller number of principal components, and these new components are linear combinations of the main variables that show body shape variations across the samples. To understand the pattern morphological difference between populations, canonical variate analysis (CVA) was applied and non-parametric multivariate analysis of variance (NPMANOVA) was used to assess significant differences between populations. All analysis were performed using PAST v 2.1, SPSS19 and Excel 2013 software.

Table1. Measured morphometric characters of the studied *Ponticola iranicus* populations

Row	Character	Row	Character
1	Total length (TL)	12	Length of the second dorsal fin base (DFBL)
2	Standard length(SL)	13	Height of the second dorsal fin (DFH)
3	Maximum body depth (Max BD)	14	Length of pectoral fin (PFL)
4	minimum depth of caudal peduncle(Min CD	15	Length of ventral disc (VFL)
5	Predorsal distance (PrDL)	16	Post orbital distance (PsO)
6	Horizontal diameter of eye (ED)	17	Width of caudal peduncle at the anal fin (CPWA)
7	Interorbital distance (IOL)	18	Minimum width of caudal peduncle (Min CPW)
8	Head length (HL)	19	Length of anal fin base (ABL)
9	Head depth at nape (HD N)	20	Head width (HW)
10	Preanal distance (PrAL)	21	Width of upper lip (WUL)
11	Pre orbital distance (Pro)	22	Caudal peduncle Length (CPL)

Results

The results showed the normality of all morphometric data except inter-orbital distance and upper lip width. Tables 2 and 3 show normal and non-normal traits using Kruskal–Wallis and One-Way ANOVA analysis, respectively. The results also showed a significant difference between the studied populations in the inter-orbital distance, minimum width of caudal peduncle and eye diameter ($P<0.05$). PCA and CVA analysis were performed using traits showing significant differences. Two first components in PCA were higher than the Jolliffe line, selected as main components (PC1 = 72.74 and PC2 = 26.25). Both PCA and CVA showed an overlap between the Pasikhan and Siahdarvishan populations, whereas the Anzali population was separated from them (Figs. 1, 2). NPMONOVA showed a significant difference between Pasikhan and Siahdarvishan populations ($P<0.05$) (Fig. 4).

Table 2. Values (mean ± SD), range of characteristics and results of Kruskal- wallis analysis in *Ponticola iranicus* populations in Anzali wetland basin (in mm).

characters	Pasikhan	Anzali wetland	Siahdarvishan	P
IOL	6.81±0.36	6.69±0.76	5.72±0.69	0.02
WUL	1.94±3.38	2.32±6.02	2.48±2.01	0.10

Table 3. Values (mean ± SD) and results of analysis of one-way ANOVA variance and Duncan grouping of morphometric characteristics in analysis in *Ponticola iranicus* populations in Anzali wetland basin (in mm).

characters	Pasikhan	Anzali wetland	Siahdarvishan	P
(TL)	65.00±0.00	65.00±0.00	65.00±0.00	-
(SL)	55.87±1.32 [a]	56.25± 3.87 [a]	53.90±2.00 [a]	0.157
(Max BD)	10.44±1.5 [a]	11.87±1.4 [b]	11.28±0.62 [ab]	0.108
(PrDL)	18.95±1.61 [a]	20.07±1.28 [a]	18.53±1.41[a]	0.084
(Min CD)	5.35±0.37[a]	6.44±0.60[b]	5.63±0.44[a]	0.001
(Min CD)	16.6±.061[a]	16.82±1.41[a]	16.42±1.12[a]	0.788
(DFH)	16.01±13.05[a]	13.19±8.48[a]	11.64±1.94[a]	0.334
(ABL)	14.06±1.73	14.22±2.82[a]	13.32±3.28[a]	0.777
(PFL)	13.38±1.19[a]	13.44±2.14[a]	13.85±3.36[a]	0.927
(VFL)	12.12±1.04[a]	12.99±2.39[a]	12.18±3.50[a]	0.797
(CPL)	10.32±1.27[a]	12.26±2.13[a]	11.72±1.51[a]	0.157
(DFBL)	16.25±5.30[a]	16.42±4.39[a]	16.82±0.25[a]	0.952
(CPWA)	4.40±0.80[a]	4.89±0.79[a]	4.58±1.96[a]	0.832
(HL)	15.51±3.76[a]	16.27±4.35[a]	15.17±1.34[a]	0.755
(ED)	3.84±0.58[ab]	4.18±0.90[b]	3.31±0.56[a]	0.042
(PrO)	5.03±0.63[a]	5.07±0.83[a]	4.41±0.82[a]	0.164
(PsO)	8.34±0.58[a]	9.31±0.93[a]	7.92±1.90[a]	0.144
(HD N)	10.22±0.60[a]	11.20±0.71[a]	10.7±1.93[a]	0.483
(PrAL)	23.87±10.69[a]	26.53±7.27[a]	29.12±0.20[a]	0.375
(HW)	13.29±1.84[a]	12.54±1.48[a]	12.47±2.16[a]	0.712

Discussion

It is difficult to explain the causes of morphological differences between populations because the morphological characters are under control and interaction of both environmental and genetic conditions (Sawdin and Foote 1999, Salini *et al*. 2004, Pinheriro *et al*. 2005). During the early life stage of fishes, the same environmental conditions are often led to similar body shape patterns (Pinheriro *et al*. 2005). The results of the present study showed that the populations are different in some characters viz. minimum width of the caudal peduncle, eye diameter, and inter-orbital distance. Tajbakhsh *et al*. (2016) also reported morphological separation of three populations of P. bathybius on the southern coast of the Caspian Sea using a truss network system, which mainly was related to meristic characters. Besides, Heidari *et al*. (2019) reported that two populations of Rhinogobius similis are separated based on their morphological characteristics.

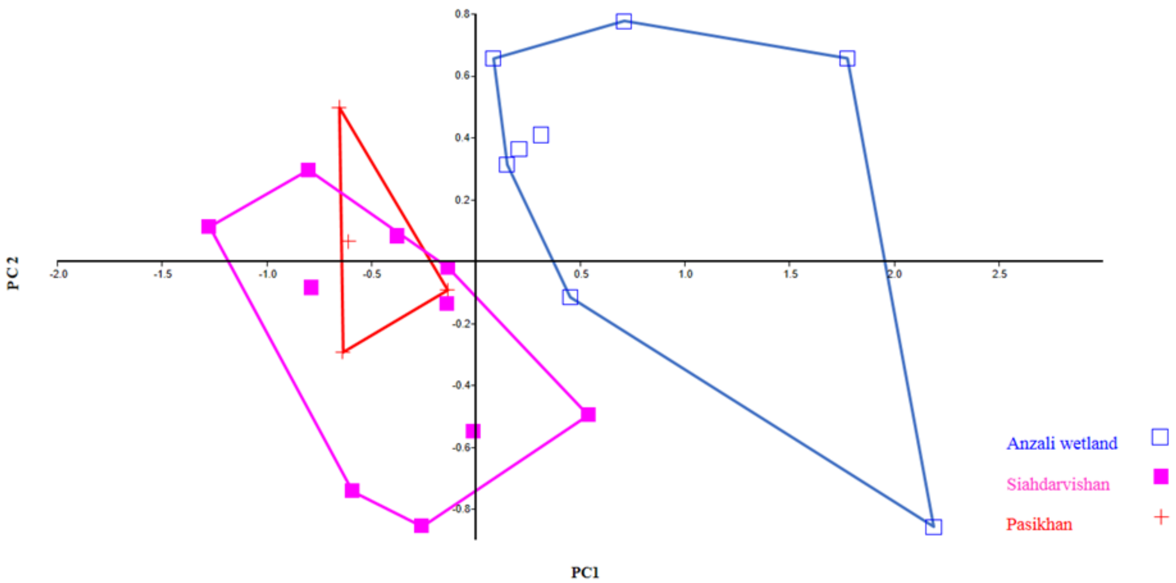

Figure 1. Principal component analysis of the morphometric characteristics of *Ponticola iranicus* populations in Anzali wetland drainage.

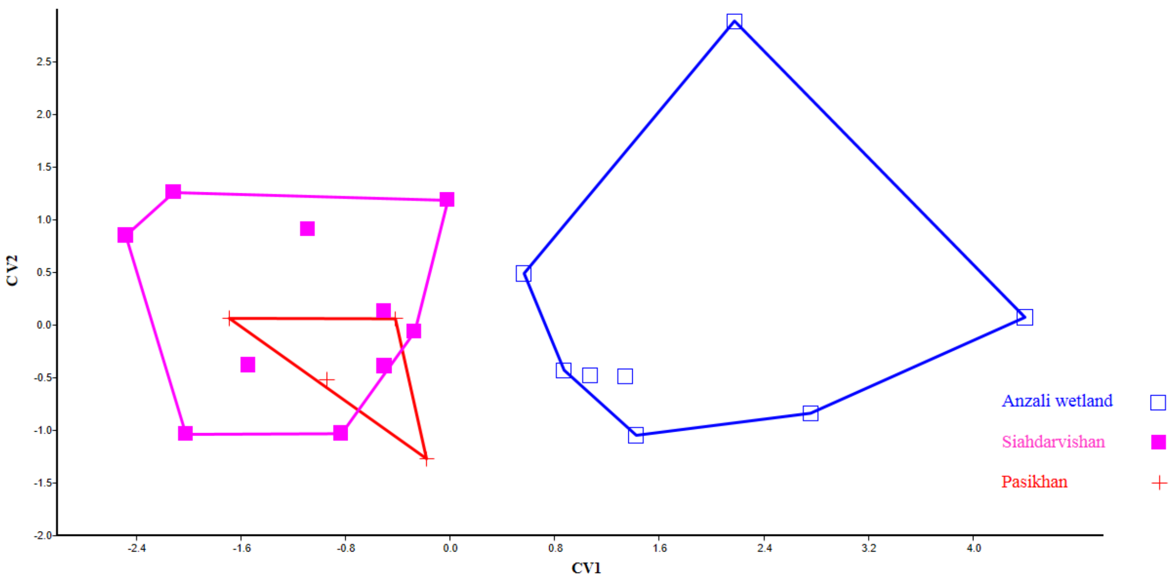

Figure 2. Canonical analysis of the morphometric characteristics of *Ponticola iranicus* populations in Anzali wetland drainage.

Morphometric diversity can be resulted by either phenotypic plasticity due to adaptation to their habitat characters or ecological changes of habitats or their interactions (Annoni *et al.* 1997). Hence, the observed morphological differences in the studied populations of *P. iranicus* can be a result of adaptation to different habitats i.e. river and wetland ecosystems. For instance, a larger eye in Anzali wetland population can be considered as a change for searching food ability in a turbid waterbody. Swain and Holtby (1989) pointed out that differences in size and shape of fish eyes affect by feeding type, life history, the depth which they inhabit and the light condition of their habitats. Therefore, the turbidity conditions and higher depth of wetland have been led to increasing eye diameter in those wetland populations as an adaptation to improve their vision to finding food and avoiding predators.

Besides, the lower width of the caudal peduncle in riverine populations i.e. Siahdarvishan and Pasikhan can be considered as adaptive features to decrease drag in current water (Langerhans and Reznick 2010). Nikmeher *et al*. (2018) showed morphological differences between P. gorlap populations in the southern Caspian Sea basin showing differences related to head and caudal peduncle, which affects especially searching food.

Conclusion

As a conclusion, it can be mentioned that morphological changes resulted from different habitats is a common phenomenon in *P. iranicus* inhabiting the southern Caspian Sea basin and these differences are mostly related to head and caudal peduncle regions.

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