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Research Article

Phenotypic diversity in Hari garra, *Garra rossica* (Nikol'skii, 1900) populations (Cyprinidae) in Iranian river systems

Mazaher Zamani-Faradonbe, Yazdan Keivany*, Salar Dorafshan

Department of Natural Resources (Fisheries Division), Isfahan University of Technology, Isfahan, 84156-83111, Iran

*email: keivany@cc.iut.ac.ir

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Abstract

Since identification of species and populations of fish are essential in biodiversity, conservation and research on their biological characteristics, variation of body shape of different populations of *Garra rossica* from the southern and eastern Iran was investigated by traditional (meristic and morphometric characters) and geometric morphometric methods. A total of 113 specimens of *G. rossica* from four rivers in four basins were caught by electrofishing (May, 2018) and transferred to the laboratory for further analysis. Left sides of the specimens were photographed, then appropriate 13 landmark-points were selected and digitalized in tpsDig2 to extract body shape data. The obtained landmark configurations were superimposed by the General Procrustes Analysis to remove non-shape variations. Finally, Principle Component Analysis, Canonical Variate Analysis and Cluster Analysis were conducted in PAST and MorphoJ. Analysis of 12 meristic and 19 morphometric traits showed that populations differ significantly in 10 meristic and 15 morphometric traits ($p<0.05$). Also, analyzes of the geometric morphometrics shows that the studied groups are distinguished in the shape and size of head, body height, length of the caudal peduncle, and position of the dorsal fin. This study shows that different populations inhabiting different rivers have distinct body shapes.

Keywords: Body shape, Geometry, south and east basins, Canonical Analysis.

Introduction

Fish, due to their biological capacities, are able to adapt to different environmental conditions (Nacua *et al*. 2010). Therefore, the body shape of fish not only reflects their genetic characteristics during the evolutionary process, but also reflects their habitat conditions (Guill *et al*. 2003). Due to the separation of habitats, the members of a fish species form different populations and thus, during the evolutionary processes, undergo changes in the body shape associated with the habitat conditions and differentiate from other populations of their species (Wooton 1991).

Identification of species and populations of fish are essential in biodiversity conservation and research on their biological characteristics such as growth, mortality, fecundity, nutritional relationships and life cycle (Ibanez *et al*. 2007). Body shape characteristics such as morphometric and meristic traits, body biometrics and the form of otolith, are widely used to identify species and populations of fish (Ihssen *et al*. 1981, Cardin 2000, Poulet *et al*. 2005).

In traditional methods, the identification of species and even populations is based on measurable and countable characteristics (Murta 2000, Swain and Foote 1999). Recently genetic methods are also used, but these methods, in addition to being costly, are not readily available in the field. Another method is Landmark-base geometric morphometric that uses the landmark coordinates as body shape data to analyze the shape variations of samples

(Benítez *et al*. 2012). This method can examine the body shape differences in different biological groups with greater accuracy and can be used for purposes such as studying the impact of habitat characteristics on body forms, flexibility responses, species identification, and fish stock assessment studies (Demandt and Bergek 2009).

The genus *Garra* is found throughout southwest Asia and from Africa to Southeast Asia. There are about 73 species (Coad 2017) and more than 10 species are recognized in Iranian inland waters and one of them is *Garra rossica* (Nikol'skii, 1900) (Keivany *et al*. 2016 and Esmaeili *et al*. 2017). The characteristics of this fish include a scaleless head, one to two pairs of short barbels, and a weakly developed mental disc. This species is known from Hormuz, JazMurian, Makran, Mashkid, Sistan, Bajestan, Harirrud and Kavir basins.

Considering the wide distribution of this species in Iranian rivers, it is questioned how the body shape characteristics of this species differ in different basins and how these populations can be distinguished from one another and if it can be considered as a pattern of morphological differentiation due to evolution or morphological variability. Therefore, the present study was carried out in order to evaluate the body shape differences of four populations of the species. The results of this study can help to understand the evolutionary process of this species in and among its distribution ranges.

Material and methods

A total of 113 specimens of *G. rossica* from four rivers were caught (May, 2018) using an electrofishing device and after anesthetization in 1% clove oil solution, they were fixed in 10% neutralized formaldehyde and transferred to the laboratory (Table 1 and Figure 1).

Traditional morphometric

A total of 12 meristic characters including number of lateral line scales (L.L), scales above L.L, bellow L.L to ventral fin, bellow L.L to anal fin, predorsal scales, circumpeduncle scales, dorsal fin, anal fin, pectoral fin, ventral fin soft rays, caudal fin rays and number of barbels were counted on left side of the specimens. Also, 19 morphometric characters were measured on left side of the specimens using digital caliber (0.1 centimeter, Figure 2). Among these traits, total, fork and standard lengths were used in further analysis without modification, three traits including snout length, eye diameter and cheek length were used in next analysis as a ratio of head length and other measurements as a ratio of standard length (Cicek *et al*. 2016). Kolmogorov–Smirnov test was used to test the normality, then nonparametric data were analyzed by Kruskal–Wallis analysis and parametric data by ANOVA in SPSS-19 software.

Table 1. Sampling locations of *G. rossica* specimens in Iranian Basin.

	Basin	River	Number	
1	Bushehr	FirouzAbad	14	28°51'15.8"N, 52°30'59.1"E
2	Makran	Sarbaz	35	26°34'50.0"N, 61°12'44.8"E
3	Sistan	Nehbandan	54	31°24'32.6"N, 60°42'00.3"E
4	Harirrud	Torogh	10	36°10'15.6"N, 59°30'43.8"E

Geometric morphometric

Using a Canon camera (12 MP resolutions), left side of specimens were photographed, then to extract body shape data, appropriate 13 landmark-points were selected (Figure 3) and were digitized with tpsDig2 software (ver. 2.10) (Rohlf 2010). The obtained landmark configurations were superimposed by the GPA (General Procrustes Analysis) to remove non-shape variations (like location, orientation and scale) (Rohlf and Bookstein 1990), finally Principle Component Analysis, Canonical Variate Analysis and Cluster Analysis were conducted by PAST (Hammer *et al*. 2001) and MorphoJ ver. 1.06d (Klingenberg 2011).

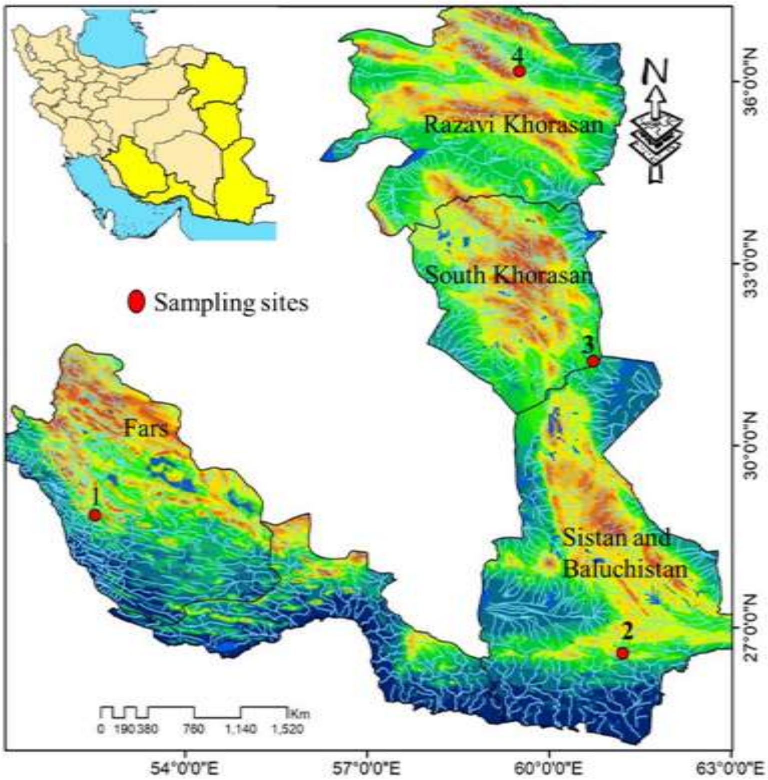

Figure 1. Sampling locations of the *G. rossica* specimens in Iranian Basin

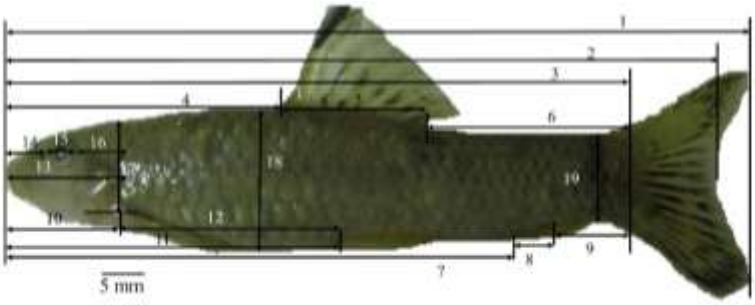

Figure 2. The points of measurements on left side of *G. rossica*. 1: Total length, 2: Fork length, 3: Standard length, 4: Predorsal fin length, 5: Dorsal fin base length, 6: post dorsal fin length, 7: Preanal fin length, 8: Anal fin base length, 9: post anal fin length, 10: Prepectoral fin length, 11: Prepelvic fin length, 12: pectoral-pelvic distance length, 13: Head length, 14: Snout length, 15: Eye diameter, 16: Cheek length, 17: Head depth, 18: Body height, 19: Caudal peduncle depth.

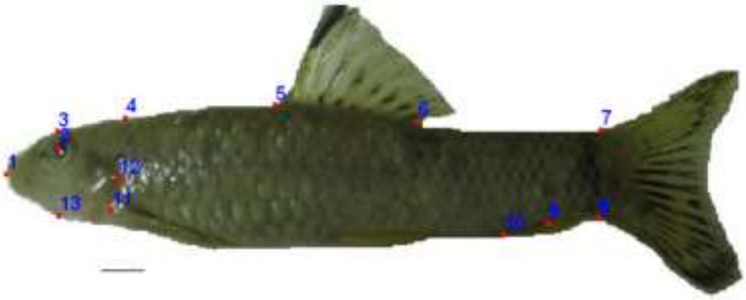

Figure 3. The location of 13 landmarks on left side of *G. rossica* specimens; (1) the anterior-most point on the head; (2) the center of eye; (3) the dorsal margin of head vertical to the center of eye; (4) the junction of the head and trunk; (5) the anterior edge of dorsal fin base; (6) the posterior edge of dorsal fin base; (7) the upper edge of caudal fin base; (8) the lower edge of caudal fin base; (9) the posterior edge of anal fin base; (10) the anterior edge of anal fin base; (11) the anterior edge of pectoral fin base; (12) the posterior end of head; (13) the ventral of margin of head vertical to the center of eye (Zamani Faradonbe *et al.* 2015).

Results

Traditional morphometric

Based on normality test, all meristic characters were nonparametric, so were analyzed with Kruskal–Wallis (Table 2) and showed that studied populations were different in all meristic character ($p<0.05$) except in anal and ventral fin soft rays; whereas all morphometric traits were parametric and the results of ANOVA analysis showed differences in all of them ($p<0.05$), except in postdorsal, preanal and post anal fin lengths (Table **3**).

Table 2. Mean ± SD and Kruskal–Wallis analysis results for nonparametric meristic characters in *G. rossica* populations (p>0.05).

	Meristic characters	FirouzAba	Sarbaz	Nehbanda	Torogh	p
1	Lateral line scales (L.L)	32.93±1.27	35.27±0.76	36.28±1.04	36.00±1.22	0.00
2	Scales above L.L	3.86±0.36	4.91±0.38	6.00±0.69	6.00±1.00	0.00
3	Scales bellow L.L to ventral fin	3.36±0.50	3.91±0.53	5.48±0.43	4.60±0.89	0.00
4	Scales bellow L.L to anal fin	3.00±0.00	3.61±0.50	4.88±0.43	4.80±0.45	0.00
5	Soft rays of dorsal fin	7.07±0.27	7.67±0.48	6.96±0.20	7.20±0.45	0.00
6	Soft rays of anal fin	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	1.00
7	Caudal fin rays	18.71±0.61	19.00±0.00	18.96±0.20	17.00±0.00	0.00
8	Soft rays of pectoral fin	12.71±0.91	14.45±1.39	12.96±1.40	13.80±0.84	0.00
9	Soft rays of ventral fin	8.14±0.86	8.42±0.87	7.92±1.06	8.60±0.55	0.18
10	Barbels number	2.00±0.00	1.21±0.42	1.00±0.00	1.00±0.00	0.00
11	Scale on Predorsal fin	10.08±2.92	9.75±1.05	-	14.00±1.00	0.01
12	Scales on circamucaudal	14.14±0.66	15.76±0.97	18.56±1.60	18.60±1.14	0.00

Table 3. Mean ± SD and ANOVA analysis result for morphometric characters in *G. rossica* populations (Data that showed are unmodified)

	Morphometric characters	FirouzAbad	Sarbaz	Nehbandan	Torogh
1	Total length	58.25±6.92^{a}	69.73±10.53^{b}	43.60±9.34^{c}	78.69±19.88^{d}
2	Fork length	53.30±6.28^{a}	63.26±9.32^{b}	40.64±8.83^{c}	74.13±18.44^{d}
3	Standard length	47.57±5.64^{a}	56.90±8.90^{a}	35.02±7.72^{a}	64.67±17.31^{a}
4	Predorsal fin length	23.60±2.54^{ab}	27.60±4.26^{a}	18.64±3.85^{c}	33.50±9.97^{bc}
5	Dorsal fin base length	8.36±1.40^{a}	8.75±1.74^{a}	4.27±0.99^{a}	6.90±2.08^{a}
6	Post dorsal fin length	17.31±5.42	20.63±4.06	12.29±3.35	23.19±5.87
7	Preanal fin length	36.72±4.53	44.67±6.49	27.64±5.93	49.94±12.82
8	Anal fin base length	4.76±1.04	5.76±1.25	3.12±0.75	6.05±2.00
9	Post anal fin length	5.57±1.07	6.25±1.68	4.02±1.13	6.93±1.94
10	Prepectoral fin length	11.21±2.07^{ab}	12.25±2.14^{a}	8.73±1.35^{b}	13.78±4.05^{a}
11	Prepelvic fin length	26.16±3.16^{a}	31.93±4.55^{ab}	20.53±4.17^{b}	36.66±10.56^{ab}
12	Pectoral-pelvic distance length	15.08±2.78^{a}	19.54±3.01^{ab}	11.91±3.05^{ab}	23.43±6.43^{b}
13	Head length	11.81±1.85^{ab}	12.87±1.83^{a}	9.16±1.57^{b}	14.86±4.64^{a}
14	Snout length	3.85±1.21^{b}	4.27±0.63^{b}	2.19±0.52^{a}	4.87±1.74^{b}
15	Eye diameter	2.57±0.41^{a}	3.14±0.39^{b}	2.20±0.53^{ab}	3.51±1.15^{ab}
16	Cheek length	5.34±0.83^{b}	5.44±0.95^{a}	4.77±0.84^{c}	6.93±1.78^{b}
17	Head height	7.97±1.02^{b}	8.83±1.55^{ab}	5.72±1.07^{b}	6.69±2.73^{a}
18	Body height	12.86±1.62^{c}	14.26±2.93^{b}	9.07±1.67^{bc}	14.95±4.70^{a}
19	Caudal peduncle height	6.26±0.69^{a}	7.14±1.30^{a}	5.01±1.06^{b}	9.71±2.05^{b}

Note: a, b: Duncan grouping in ANOVA ($p<0.05$), n.s: no significant ($p > 0.05$).

Geometric morphometric

The results of principle component analysis (PCA) with landmark-points data showed that the three first PCs accounts for 74.08 % of variances (49.02, 17.19 and 7.87 % for PC1, PC2 and PC3, respectively) (Figure 4). In Figure 5 grouping of studied populations were showed along first two axes (PC1 & PC2), while all populations are distinct from each other and well grouped. Investigations of deformation grid along the PC1 and PC2 showed that the populations along PC1 (positive side) have smaller head, lower body height and the populations along PC2 (positive side) have smaller head, dorsal fin in anterior position and longer caudal peduncle length.

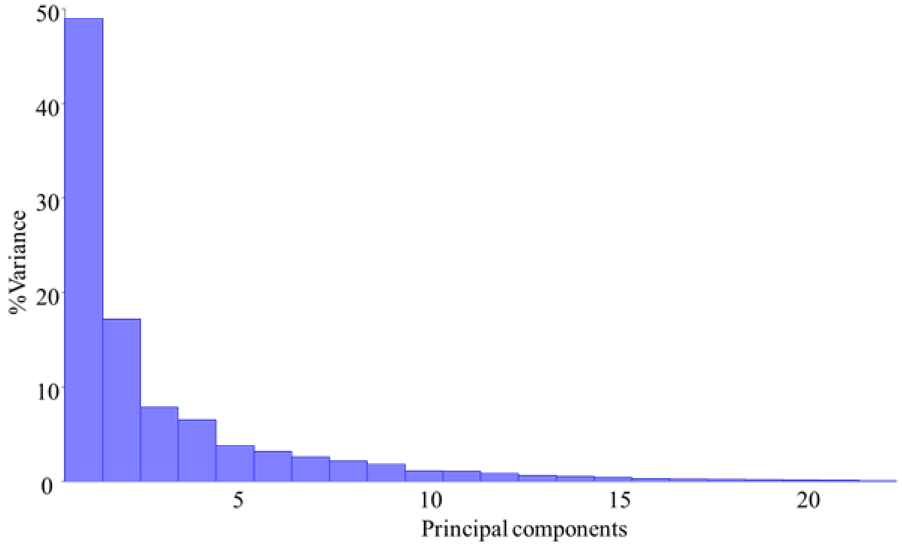

Figure 4. The percentages of total variance for principal components in PCA.

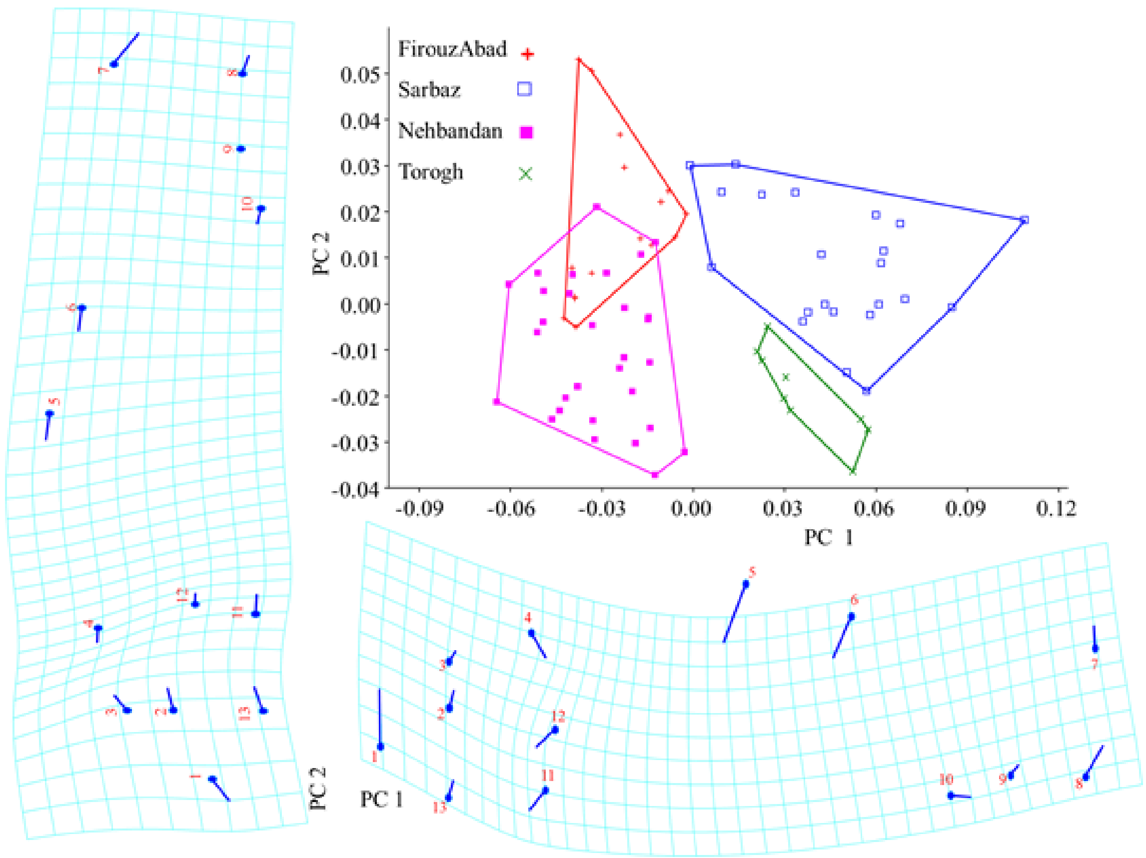

Figure 5. Principal Component Analysis (PCA) of *G. rossica* and TPS-deformation grid along PC1 and PC2.

Studied populations grouping along CV1 and CV2 in CVA analysis are showed in Figure 6 which revealed a strong grouping (Wilks lambda= 0.00092 and *p*-value = 0.0000000) in four populations of *G. rossica*, so that they are separated from each other. Investigations of deformation grid along the CV1 and CV2 show that the populations along CV1 (positive side) have smaller head, more body height and the populations along PC2 (positive side) have lower body height and lower body height.

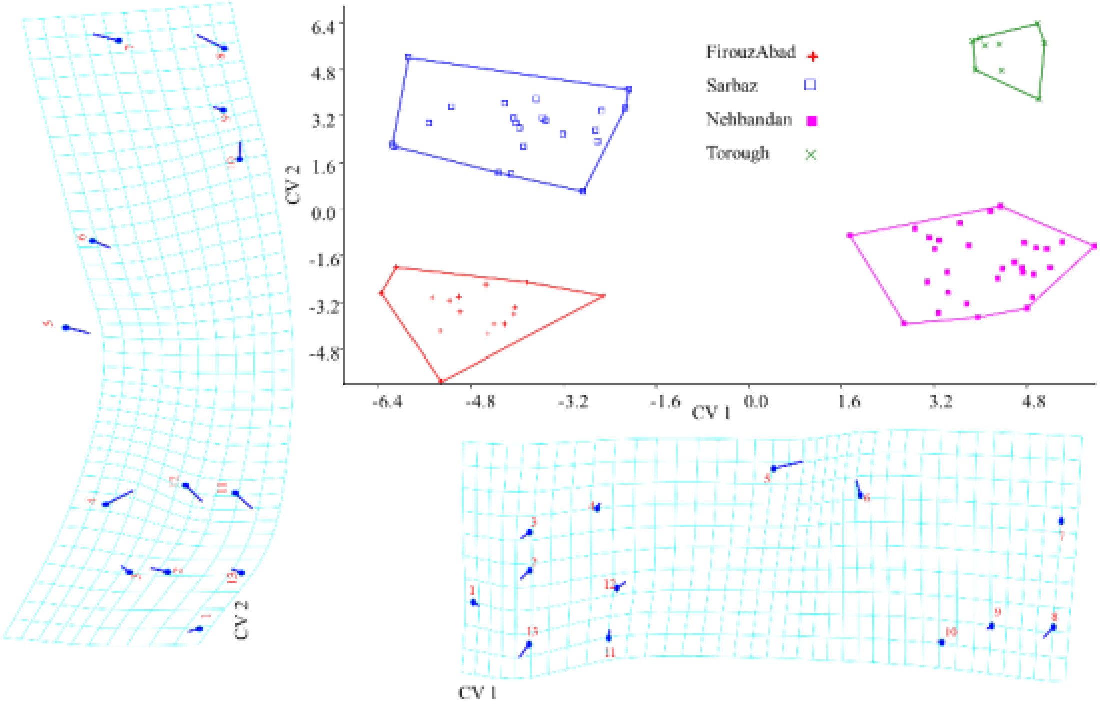

Figure 6. Canonical Variance Analysis (CVA) of *G. rossica* and TPS-deformation grid along the CV1 and CV2.

Results of Mahalanobis distances and Procrustes distances (Table 4 and Table 5) show that there was important differences in body shape of FirouzAbad and Torogh with each other and from the rest sites.

Figure 7 shows the results of cluster analysis of the landmark-points data of *G. rossica* populations and shows that FirouzAbad and Nehbandan population are in the same group and Sarbaz and Torough are in another group.

Table 4. Results of Mahalanobis distances from CVA/MANOVA of *G. rossica* populations

Population	FirouzAbad	Sarbaz	Nehbandan
Sarbaz	6.91		
Nehbandan	9.21	9.42	
Torogh	11.89	9.31	7.73

Table 5. Results of Procrustes distances from CVA/MANOVA of *G. rossica* populations

Population	FirouzAbad	Sarbaz	Nehbandan
Sarbaz	0.079		
Nehbandan	0.044	0.081	
Torogh	0.084	0.043	0.073

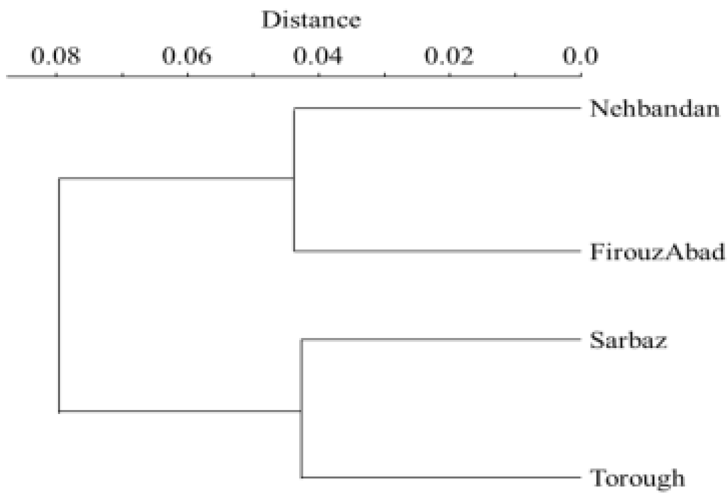

Figure 7. Cluster analyses of *G. rossica*.

Discussion

The results of this study indicate that the body shape of *G. rossica* populations inhabiting different habitats and rivers are different and these differences show the habitat-specific separation in the studied populations. The four studied populations were different in most meristic (11 out of 12 traits) and morphometric traits (13 out of 19 traits).

The main reason for the separation of these populations in different rivers is probably due to the geographical separation of these populations which often leads to a decrease in the gene flow between them (Menken and Raijmann 1996). In other words, genetic differences and also differences in body shape among populations increase with increasing geographical barriers or isolation. On the other hand, the differences could be because of differences in environmental conditions such as temperature, turbidity, depth, velocity and food availability (Zamani-Faradonbe *et al*. 2015). Differences in the body forms of populations from different habitats and even in different species of same habitat, indicate the differences in the type of adaptation of fish to the type of habitat (like riffles or pools) (Tabatabaei *et al*. 2014). Morphological variability and evolutionary progress of body shape of various populations of fish indicate that the habitat conditions along with the geographical separation are the determinant factors that change the phenotypic traits of the fish inhabiting that area.

Askari *et al*. (2014) by examining the effects of seasonal variations (spring and autumn) on the body shape of *G. rufa* populations in Shapour River (Bushehr basin, Fars province, Iran), found that the differences were significant in 11 out of 24 morphometric traits and one of the 10 meristic traits among males between two seasons and in females, the differences were significant in 19 morphometric traits and two meristic traits. The results of their study indicated that changes in morphometric traits are higher in two seasons than in meristic traits, and fish respond to seasonal variations with body shape changes (Askari *et al*. 2014).

Changes and differences in body shapes are important if lead to adaptations to environmental conditions and increases survival rates in aquatic habitats. Such adaptations are related to results of the need for compatibility with hydrodynamic forces to maintain energy during biological behaviors (Vogel 1994, Nacua *et al*. 2010). In this study, population like FirouzAbad river that in PCA and CVA graph was separated from other populations and was in negative side of all axes, showed that the body shape of this populations has bigger head, more body height, dorsal fin in anterior position of body and lower caudal peduncle length compared to other populations;

Cicek *et al*. (2016) showed that the *G. rufa* groups (in Tigris River system of Southeast Turkey) which were close to each other on the similarity graph were geographically far from each other and concluded that those populations had similar habitat specifications. They suggested that different distribution of some populations in canonical discriminant analysis graph might be based on the habitat characters; geographical distance and isolation mechanisms deriving from barrage sets might be the reasons of different distributions (Cicek *et al*. 2016).
Although locality of the groups were different, they usually showed similar distributions. Therefore, we can associate similar habitat conditions with each populations. The phenotypic variability of the populations of a species in various environments is a phenomenon that results from the effects of environmental factors on this generation and previous generations in terms of adaptation and speciation (Adams *et al*. 2004). So these differences in body shapes among populations of *G. rossica* probably reflects differences in habitat conditions and perhaps genetic differences.

Conclusion

Since the purpose of this study was to compare the body shape of different populations of G. rossica inhabiting different rivers and basins, the results showed that studied populations have many differences in many traits of meristic and morphometric characters and in shape and size of head, body, caudal peduncle and dorsal fin position. Although the results of this study are derived from both traditional and geometric morphometric methods, this study cannot easily express the superiority of either of these two methods because these two methods are based on different criteria. Further studies are needed to provide a better understanding of the genetic and other differences in these populations.

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