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Original Article

Body shape variation of *Garra rufa* (Teleostei, Cyprinidae) populations in the Tigris basin in Iran using geometric morphometric analysis

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Abstract: Geometric morphometric method was used to examine the body shape variations among the six populations of *Garra rufa*, in Iranian part of Tigris basin. A total of 15 landmark-points was used for 170 specimens to hypothesize population differentiation of *G. rufa* in the six rivers and reservoir. In discriminant function analysis, 85.9% of original grouped cases correctly classified. Principal component analysis (PCA) and canonical variates analysis (CVA) confirmed the significant difference between the populations. The results revealed that the studied populations are divided into three clades based on differences in body depth, caudal peduncle length, backward moving of anal fin. Caudal peduncle showed shortening trend in five populations. Narrower body shape was dominated among specimens of four regions. Studies on body shape provide supporting data on fisheries, stock management, and conservative programs.

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Introduction

Geographical isolation and interbreeding are resulted in morphometric variations among populations of a single species (Bookstein, 1991; Torres-Dowdall et al., 2012; Heidari et al., 2013, 2014; Kohestan-Eskandari et al., 2014). Body shape plays an important role in fish locomotion, feeding behavior, and predation reflecting evolutionary adaptation in response to environmental pressures (Webb, 1982; Guill et al., 2003; Mousavi-Sabet and Anvarifar, 2013; Mousavi-Sabet et al., 2018). Therefore, morphological variations of different populations as part of adaptation to their habitats can guarantee the survival of the population (Nacua et al., 2010; Paknejad et al., 2014; Vatandoust et al., 2015). A main purpose of morphometric study is to test hypotheses about the factors affecting body shape. Geometric morphometrics is an approach to study shape using landmark points (Webster et al., 2010*)*. The landmark-based analysis uses various statistical techniques to exclude size, position, and orientation, therefore only shape variables can be extracted (Webster et al., 2010; Adams et al., 2004*)*.

The family of Cyprinidae includes the most diverse taxa distributing in all basins of Iran (Esmaeili et al., 2018). One of the most phylogeographically interesting cyprinid genus is *Garra* distributed in southern, southwestern and northwestern Asia (Esmaeili et al., 2016, 2018; Mousavi-Sabet et al., 2019). The genus *Garra* Hamilton, 1822 with 151 valid species, is one of the most diverse genera of the Labeoninae, and has a widespread distribution ranging from East Asia to Africa (Sayyadzadeh et al., 2015; Mousavi-Sabet and Eagderi, 2016; Froese and Pauly, 2021). In Iran, the genus is found in Tigris, Persis, Hormuz, Makran, Mashkid, and Sistan, Jazmurian, Kerman and Lut basins (Esmaeili et al., 2016).

Garra rufa inhabits harsh ecological conditions in different environment having high ability to tolerance a wide range of environmental factors that results its wide distribution and variation in body shape (Coad, 2018). Therefore, study of population of *G. rufa* could

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Figure 1. Defined landmark points to extract the body shape data. 1. Anterior tip of the premaxilla; 2. Center of the eye; 3. Dorsal edge of the head vertical to the eye center; 4. End of operculum; 5. Nape at the beginning of the scale; 6 and 7. Origin and insertion of the dorsal fin; 7. Upper margin of caudal peduncle; 9. Center of caudal peduncle; 10 Lower margin of caudal peduncle; 11 & 12. Insertion and origin insertion of the anal fin; 13. Origin of the pectoral fin; 14. The lower beginning of gill slit; 15. Ventral edge of the head vertical to the eye center.

well describe its morphometric variation and phenotypic plasticity. Hence, in the present study, we compare the body shape of different populations of *G. rufa* from the Tigris basin using geometric morphometric method.

Materials and Methods

A total of 170 specimens of *G. rufa* were collected from six rivers of Iranian part of the Tigris basin, including Godarkhosh (33°30′16″N, 45°54′3″E), Sirvan (35°05'12.3"N 46°05'19.2"E), Chardavol (33°45′N 46°34′E), Leileh (35°03'30.9"N 45°57' 27.7"E), Baneh rivers (36°00'31.1"N 45°54'15.6"E), and Siah-Gav twin lakes (32°52'03.1"N 47°42'03.7"E) using electrofishing device during September 2013 - September 2015. The collected fish were preserved in 10% buffered formalin after anesthesia. Sexual dimorphism does not appear in Genus *Garra*; therefore, sex determination has not been carried out. The left side of each individual (with dorsal and anal fins were fixed by pins) was photographed, then 15 homologous landmark-points were defined and digitized on 2D images using tpsDig2 software version 2.16 to extract body shape data (Rohlf 2004) (Fig. 1).

Generalized Procrustes analysis (GPA) carried out on data to remove non-shape data. Principal Component Analysis (PCA) was used to explain the variance-covariance structure to summarize the variation among the specimens. The Multivariate analysis of variance (MANOVA) and canonical variates analysis (CVA) were used to investigate power of distinction among groups. Mahalonobis distance also calculate to reveal the distance between the studied populations in terms of morphology. All

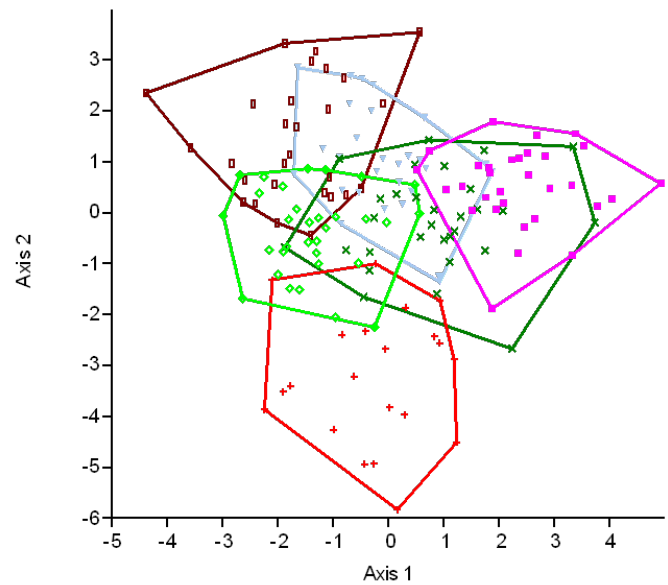

Figure 2. The results of Canonical discrimination analysis (CVA) of the six studied population of the *Garra rufa* in Tigris basin with respect to the first two canonical variables based on body shape extracted from landmarks.

statistical analyzes were done using PAST software (Hammer, 2012) at the 95% confidence limit. Clustering analysis was performed as the Euclidean square distance clustered algorithm (Sneath and Sokal, 1973).

Results

PCA showed 52.81% of shape variations of the first two components derived from the variance-covariance matrix. However, the screen plot in PCA showed, four component situated above the Jullife broken line (Julliffe cut-off=5.996e-05) include 74.67% of variance with significant level (Table 1). The CVA/ MANOVA identified significant differences in body shape among the studied populations of *G. rufa* (P=2.305e-60 Wilkils lambda=2.67). The pairwise

Table 1. Eigenvalue and variance of the first four principle component of six studied populations of the *Garra rufa* in Tigris basin of Iran.

PC	Eigenvalue	% Variance
1	0.000896986	35.16
2	0.000450501	17.659
3	0.000299303	11.732
4	0.000198041	7.7628
total		72.31%

Table 2. Mahalanobis distance analysis for the six studied population of the *Garra rufa* in Tigris basin of Iran.

	Godarkhosh	Sirvan	Siagav	Chardavol	Leilerizan	Banerood
Godarkhosh						
Sirvan	3.42					
Siagav	3.63	3.09				
Chardavol	5.02	4.69	3.80			
Leilerizan	3.55	3.60	3.52	4.16		
Banerood	4.48	3.70	3.02	4.90	4.17	

Table 3. Classification matrix showing the number and percentage of individuals that were correctly classified.

	sites	Godarkhosh	Sirvan	Siagav	Chardavol	Leilerizan	Bane	Total
Original (%)	Godarkhosh	89.3	3.6	3.6	0	3.6	0	100
	Sirvan	10.7	78.6	7.1	0	3.6	0	100
	Siagav	3.3	6.7	80	0	3.3	6.7	100
	Chardavol	0	0	4.8	90.5	4.8	0	100
	Leilerizan	0	3.1	6.2	0	90.6	0	100
	Baneh	0	3.2	6.5	3.2	0	87.1	100
Cross-validate (%)								
	Godarkhosh	67.9	14.3	3.6	0	14.3	0	100
	Sirvan	25	53.6	14.3	3.6	3.6	0	100
	Siagav	3.3	16.7	50	0	16.7	13.3	100
	Chardavol	4.8	0	19	66.7	4.8	4.8	100
	Leilerizan	3.1	3.1	6.2	0	84.4	3.1	100
	Baneh	3.2	3.2	6.5	3.2	3.2	80.6	100

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. 85.9% of original grouped cases correctly classified. 67.6% of cross-validated grouped cases correctly classified.

Hotelling's test of the groups showed significant differences in all groups ($P<0.0001$). The CVA plot depicted based on the first two CVs clustered *G. rufa* population into three distinct groups and all population, showed in some extends overlapping (Fig. 2). Mahalonobis distance showed the most distances of the chardavol population from others in terms of the body shape (Table 2).

The body shape changes were latero-dorsal shift of the pectoral fin, shorter caudal peduncle, and smaller head with longer snout in Chardavol population. In Sirvan population, lower body depth and shorter caudal peduncle became highlighted. Population of Baneh can be identified by having lower body depth and longer caudal peduncle, whereas that of Leilerizan showed lower body depth, and shorter caudal peduncle. However, the Godarkhosh population showed ventral position of the snout, shorter caudal peduncle, and deeper body depth. Population of Siah-Gav spring can be identified by the deepest body depth, ventral position of the snout and short caudal peduncle.

Discriminant analysis (DA) revealed 85.9% of original grouped cases correctly classified. 67.6% of cross-validated grouped cases correctly classified (Table 3). The dendrogram derived from cluster analysis of Euclidean square distances showed that six populations of *G. rufa* segregated from each other into

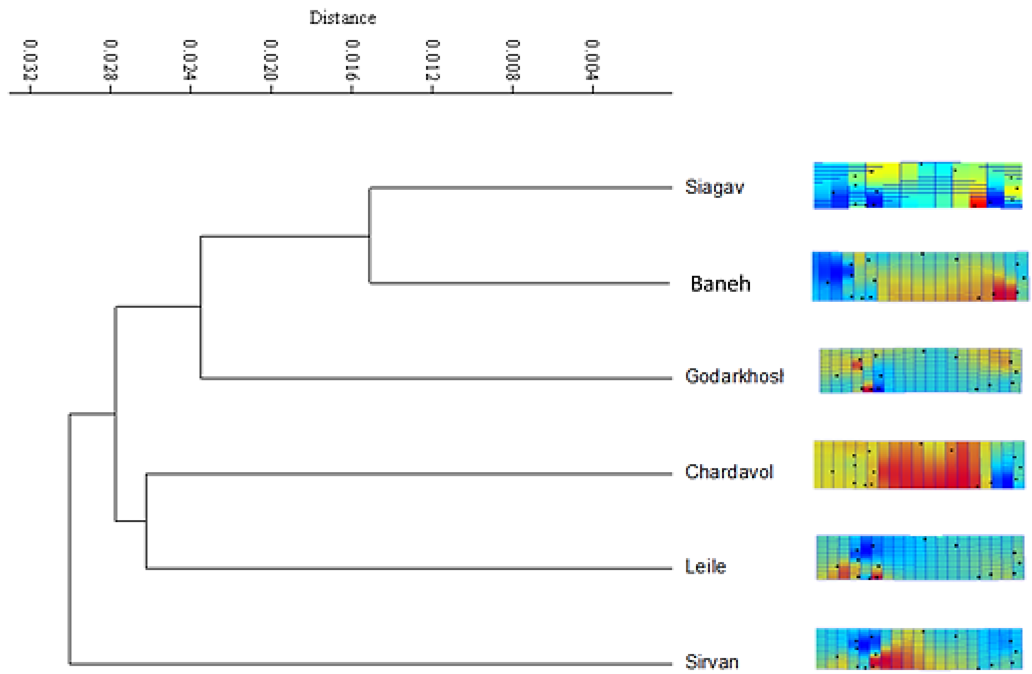

Figure 2. Dendrogram derived from cluster analyses of morphometric variables on the basis of Euclidean distance of the six studied population of the *Garra rufa* in Tigris basin of Iran. The mean shape of species in relation of consensus shape of the *Garra rufa* are represented.

three distinct clusters, *G. rufa* from the Siah-Gav and Baneh appeared in one cluster, along with Godarkhosh population, Leileh and Chardavol formed the second one, and the last one refers to the Sirvan population (Fig. 4).

Discussions

The experimental phenotypic discrepancies among the *G. rufa* populations discovered morphologically separated stocks in the studied populations of Tigris basin in Iran. Phenotypic plasticity can be implied by phenotypic variations among the population specimens. Head and mouth shape variation can be considered as reflective of differences in selection of food items and direction of feeding (Langerhans et al., 2003). A fish with a mouth oriented upward usually feeds in the water column vs from bentic feeding behavior (Andersson et al., 2005). Morphological adaptations in freshwater fishes according to wide variety of physiological and environmental conditions result in genetic divergence and/or phenotypic plasticity (Gatz, 1979; Wainwright et al., 1994; Eklov et al., 2006). Phenotypic plasticity responded to environmental variations refer to niche patterns of resource utilization, behavior, and/or habitat use (Gatz, 1979; Wainwright et al., 1994; Eklov et al., 2006; Langerhans, 2008). Moreover, diet pattern could influence morphology, while particular dietary items induce morphological change within or among populations (Wainwright et al., 1994). However, abiotic factors including food abundance, temperature (Hossain et al. 2010), body shape (Beacham, 1990), amount of food (Currens et al., 1989), and type of food or feeding mode (Pakkasmaa, 2001; Peres-Neto and Magnan, 2004; Proulx and Magnan, 2004) as well as biotic factors affect phenotypic plasticity (He et al., 2013). As mentioned before, in our study Siah-Gav reservoir showed the deepest body depth after Godarkhosh vs. narrower body in other riverine habit. Similarly, Haas et al. (2010) found that deeper-bodied *Cyprinella* (Cyprinidae) are indicative of reservoir compared to riverine habitats. Our study on population of *G. rufa* in Chardavol showed lately higher position of the pectoral fin. It is supposed that lateral positioning of the pectoral fins correlates with the locomotory characteristics of particular species to improve maneuvering (Webb, 1982; Bandyopadhyay et al., 1997).

Overall, studies on some species confirmed that shape differences could be related to trophic ecology (Costa and Cataudella, 2007), regarding local adaptation and ecological radiations (Schluter and McPhail, 1992; Langerhans et al., 2003). Overall, geographical isolation plays key role on producing morphological variation in fish species (Yamamoto et al., 2006). Importance of environmental conditions and biogeographical patterns on morphological differentiation within communities is linked to the zoogeographical history of a region (Hoagstrom and Berry, 2008). Selective pressures influencing individual mechanisms revealed how the process of evolution moves within the population in response to adaptation in their habitat.

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