

## Size and shape analysis of two close Cyprinidae species (*Garra variabilis*-*Garra rufa*) by geometric morphometric methods

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### Abstract

In this study, the shape and size differences between 30 samples of *Garra rufa* and *Garra variabilis* species captured in Tigris River were investigated by using geometric morphometric methods. According to the results of this study, there is no difference between these two species in terms of size (CS); however, they are quite different from each other in terms of shape and this difference is significant (CVA/MANOVA; Pillai tr.=0.99 and  $p$ -value=0.0016, Shape procrustes ANOVA ; F=16.40,  $p$ -value=<0.0001). These differences are thought to be caused by feeding habits and habitat structure.

**Keywords:** Tigris River, Southeast anatolia, Geometric morphometri, Cyprinidae, *Garra*

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## Introduction

Cyprinidae family, which includes *Garra* species, is one of the richest and most important families in terms of number of fish species and they have spread to different parts of the world. The majority of bony fish in Turkey belongs to this family and is commonly found in freshwater resources. This family is represented by about 1,500 species in the world and there are 30 strains and 70 species living in Turkey. *Garra* species in this family is particularly prevalent in the southern region of the country and represented by 2 species (Kuru, 1971, 1979; Geldiay and Balık, 1996; Durand *et al.*, 2002). In Tigris and Euphrates River systems, *G. rufa* (Heckel, 1843) and *G. variabilis* (Heckel, 1843) species can be found (Kuru, 1975; Kelle, 1978).

According to Geldiay and Balık, (2002) and Kuru, (1986), *G. rufa* has a flat nose tip, two pairs of mustache, well-developed suckers and they have spread around Iran, Iraq and Tigris and Euphrates River systems. They can be found in Adana, Antakya and Southeastern Anatolia Region even though its origin is Mesopotamia. On the other hand, *G. variabilis* has a pointy nose, a pair of mustache, underdeveloped suckers and they have spread around Syria, Iraq, and Anatolia. Its origin is Mesopotamia, but live in Tigris River and spread over Southeast Anatolia in Turkey.

Coad (2010) and Kaya (2012) stated that *G. rufa* lives in moderate and fast rivers that are rock and graveled and

they have mostly benthic characters ; however, *G. variabilis* live in more flowing rivers compared to *G. rufa* and they have a pelagic character.

Ergene and Çavas, (2001) reported that diploid chromosome number of *G. rufa* species living in Eastern Mediterranean is  $2n=44$  and Kılıç-Demirok (2000) reported that diploid chromosome number of *G. rufa obtuse* species living in Tigris River is also  $2n=44$ , respectively. According to Karahan, (2007), this number was reported as  $2N=50$  ( $NF=86$ ) for *G. rufa* samples in Mersin,  $2N=46$  ( $NF=80$ ) for *G. rufa* samples in Hatay,  $2N=50$  ( $NF=92$ ) for *G. rufa* samples in Sivas,  $2N=102$  ( $NF=162$ ,  $NF=160$ ) for *G. rufa* samples in Mardin, respectively.

Karahan, (2007) stated that *G. rufa* and *G. variabilis* species living in Mersin, Hatay, Kahramanmaraş and Sivas have many differences in terms of metric and meristic characteristics. Çiçek (2009) reported that there are significant morphometric differences between *G. rufa* and *G. variabilis* species in Tigris-Euphrates systems. In addition, both Karahan (2007) and Çiçek (2009) suggested that the variations between locations of *G. rufa* are quite high and there are many differences between them in terms of morphometric and meristic characteristics.

In this study, it was aimed to investigate the shape and size (centroid size) differences between *G. rufa* and *G. variabilis* species by comparing them with morphometric methods and

present additional geometric morphometric characters that cannot be detected by linear morphometry methods. In addition, the differences found by linear morphometry methods were also analyzed to see whether they have caused by size differences.

Geometric morphometric (Rohlf and Bookstein, 1990; Bookstein, 1991) is represented by set of helpful methods to study the variation of the form (size and shape) among different groups, finding a profitable application in animal morphometry research (Rohlf, 1998; Fadda and Corti, 2001; Klingenberg *et al.*, 2002; Zelditch *et al.*, 2004; Adams *et al.*, 2004 and 2013) Geometric morphometrics has some advantages over traditional methods of analyzing biological shapes. (1) The use of landmarks anchors the descriptions of shape differences and the explanations for those shape differences in specific regions of the organism. (2) This approach provides independent descriptions of size and shape. (3) The shape differences can be visualized easily by deformation grids. (Rohlf, 2000a and 2000b; Slice, 2001; Rohlf, 2003; and Klingenberg, 2013).

## Materials and methods

In this study 15 *G. rufa* from Tigris river - Hasankeyf and 15 *G. variabilis* adult samples from Tigris River-Bismil was examined by geometric morphometric methods (Fig. 1). The samples was collected in April and July 2013.

GM process is as follows :

Data collecting; images of lateral of samples were digitized by Pentax x 70 digital camera under the same conditions (high, angle and resolution). Digitizing of landmarks; 14 landmarks was collected by TpsDig2 version 2.17 software (Rohlf, 2013a) (Fig. 1).

Superimposition; the obtained landmark configurations were superimposed to remove variation of location, orientation and scale (non-shape variations) by the GPA (General Procrustes Analysis) (Rohlf and Bookstein, 1990) using TpsRelw ver. 1.53 (Rohlf, 2013b). Also, Procrustes Analysis was done by each software itself.

Analysis ; shape and size differences were analysed by MorphoJ ver. 1.06d (Klingenberg, 2011) and PAST ( Hammer *et al.*, 2001).

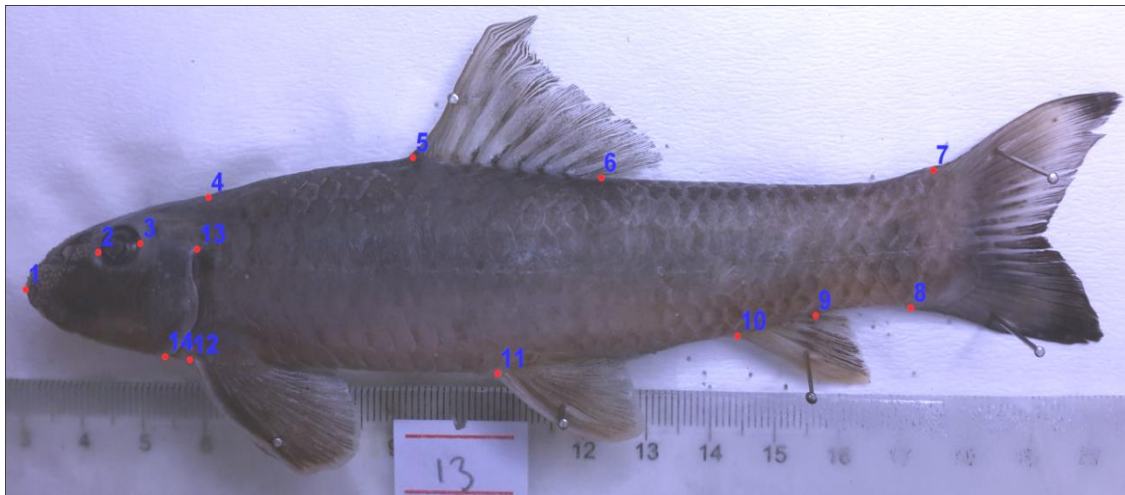


Figure 1: The localities of landmarks (Photo: *Garra rufa*, Hasankeyf).

## Results

### Shape

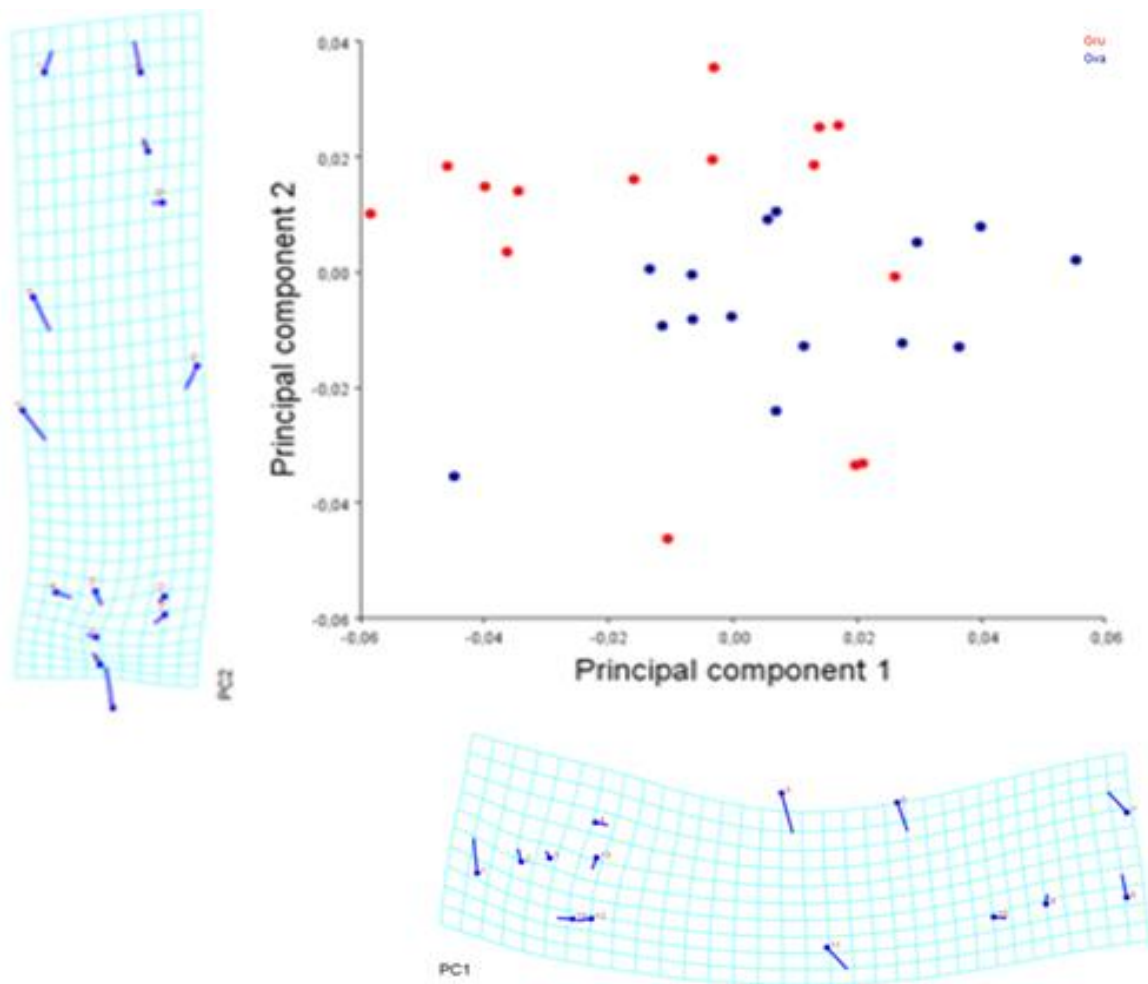
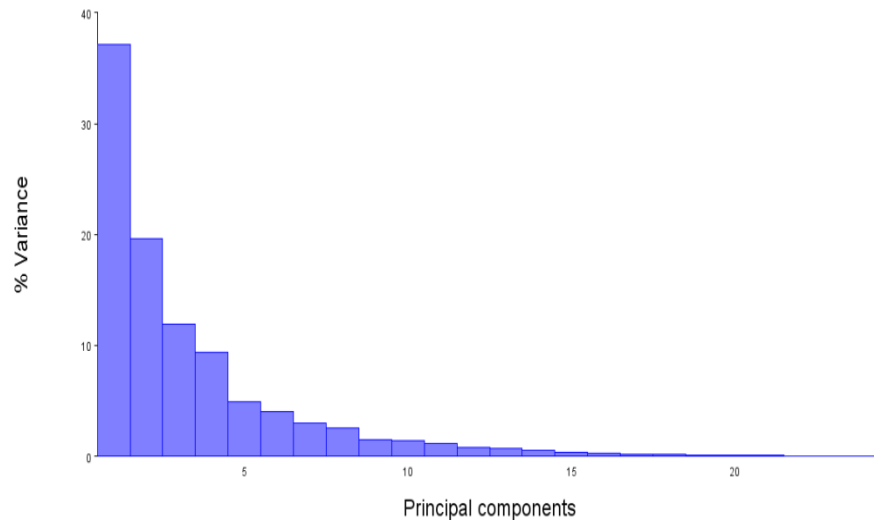


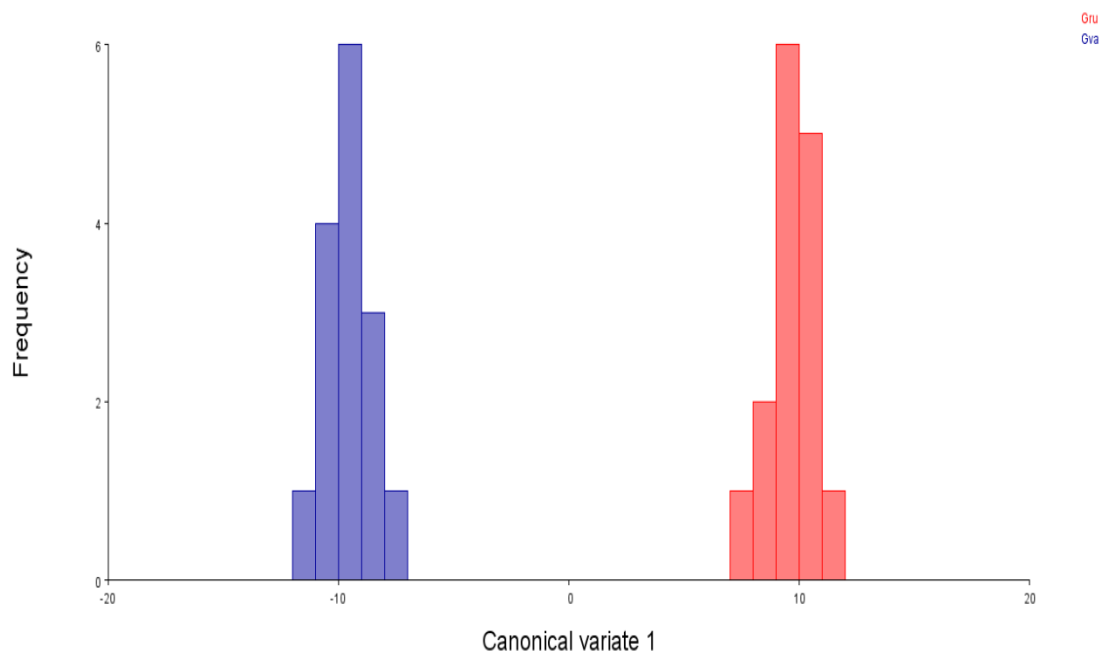
Figure 2: Principal Component Analysis (PCA) of the *Garra rufa* and *Garra variabilis* and tps-deformation grid effect the PC1 and PC2.

PCA based on the data from the lateral shape of samples showed that the first two principal components explain 56.75 % of total variance. The species were ordered along the first principal component moderately in a same range but the species were separated along

second principal component. Shape deformation that effect on PC1 and PC2, mostly found on snout, origin and end of dorsal fin, origin of pelvic fin and caudal peduncle (Fig. 2). The first five PCs explain 82.93 % of total variance (Fig. 3).



**Figure 3: The percentages of total variance for principal components.**



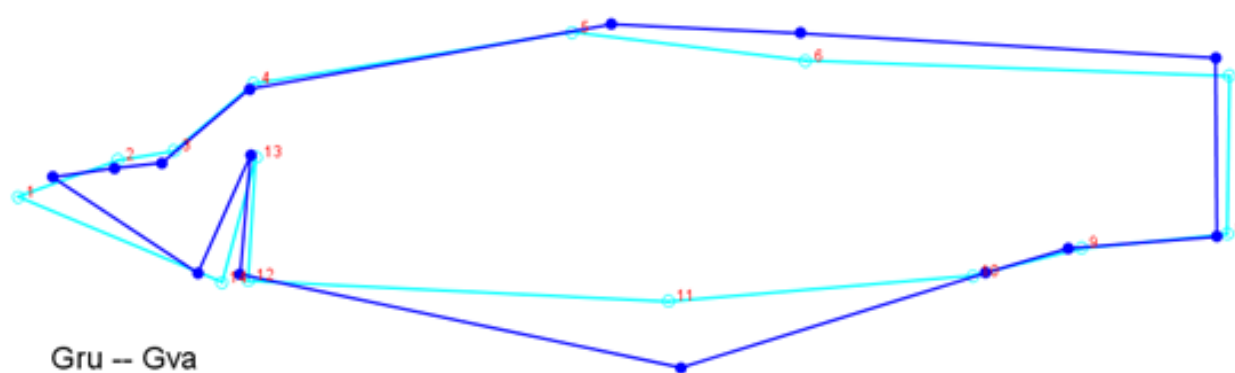
**Figure 4: Canonical variance analysis of *Garra rufa* and *Garra variabilis*.**

The results from CVA/MANOVA (Fig. 4) and Procrustes ANOVA (Table 1.) show that there was different between

the lateral shape of two groups and the differences were significant (Pillai tr.=0.99 and  $p$ -value =0.0016 )

**Table 1: Results of procrustes ANOVA.**

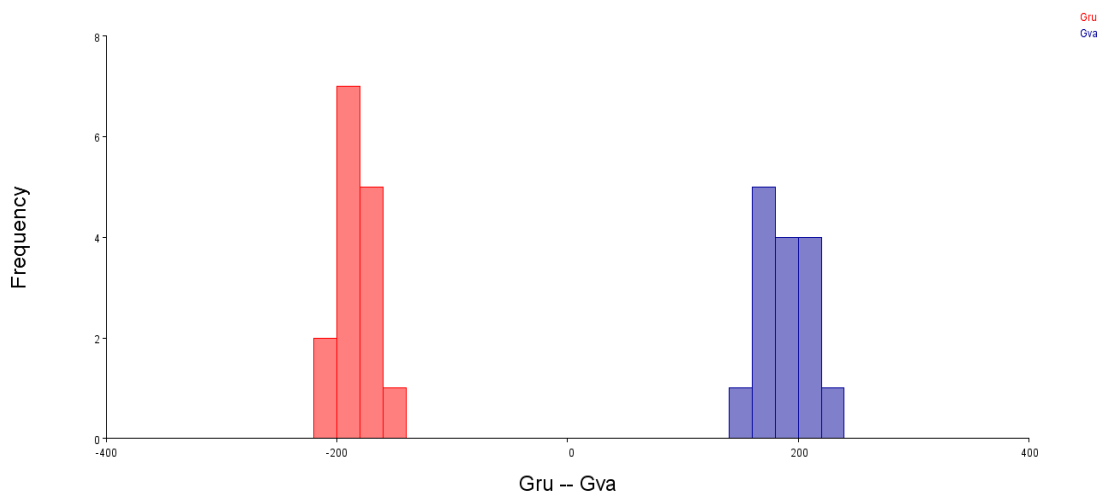
	F	$p$ -value
Size (CS)	0.16	0.6951
Shape	16.40	<.0001



**Figure 5: Shape differences between *Garra rufa* and *Garra variabilis* (Gru : *Garra rufa*, empty circles, Gva : *Garra variabilis*, filled circles).**

According to the shape mean, *G. rufa*'s snout was more forward and downward (LM: 1), the dorsal fin was prolonged and origin of dorsal fin more forward (LM: 5 and 6). The caudal peduncle was more narrow and backward (LM: 7 and 8) and the body depth was less than *G. variabilis* (a vertical line from LM : 5 to 11). The body and caudal peduncle depth were higher, but the snout was backward and upward and dorsal fin more short in *G. variabilis* (Fig. 5).

Discriminant Function Analysis (DFA) results show that two groups were well separated and difference between means of Procrustes distance was 0.064, Mahalanobis distance was 19.291 and the difference between *G. rufa* and *G. variabilis* were significant (T-square : 2791.289,  $p$ -value: 0.0016). Discriminant classification grouped each sample in their original group 100 % correctly (Fig. 6 and Table 2).



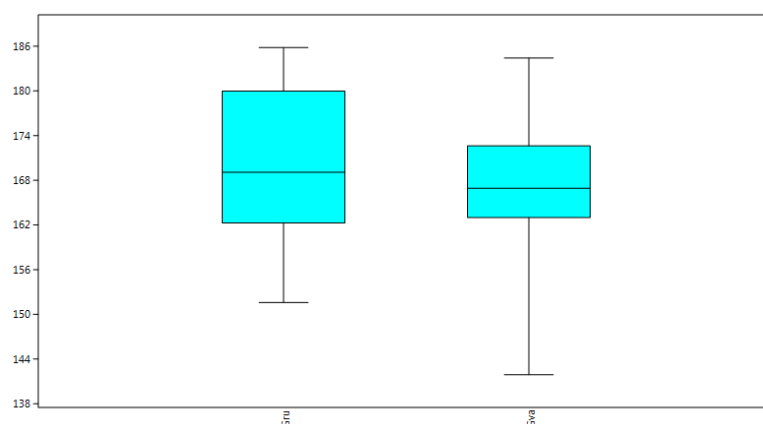
**Figure 6; Discriminant scores of *Garra rufa* and *Garra variabilis*.**

**Table 2: Classification / misclassification tables from discriminant function.**

True Group	Allocated to	
	<i>G. rufa</i>	<i>G. variabilis</i>
<i>G. rufa</i>	15 (100 %)	0
<i>G. variabilis</i>	0	15 (100 %)

*Size*

The differences of size (as Centroid Size) between *G. rufa* and *G. variabilis* were not significant (Fig. 7).



**Figure 7: The Box plot of CS of groups (The short horizontal lines: Min. and Max., the box down and up boundary: 25 and 75 percentile, the long horizontal line: Average. Gru: *Garra rufa*, Gva: *Garra variabilis*).**

## Discussion

According to the results of analyses, the shape differences between these two species are significant and there is high degree of distinction between them. In the categorization performed in accordance with DFA analysis, each sample is in its own group with an accuracy rate of 100 % (Table 2). However, some *G. rufa* samples show very slight similarities with *G. variabilis* samples (Fig. 2). These results are consistent with morphological analyses of Geldiay and Balık (2002), Kuru (1986), Linear morphometric analyses of Karahan (2007) and Çiçek (2009).

Considering shape differences between these two groups (Fig. 5), *G. rufa* has a longer nose, mouth located below, wider horizontal eye diameter, longer dorsal fin ahead, narrower and longer tail and less body height; whereas, *G. variabilis* has shorter nose, mouth is located within dorsal, shorter horizontal eye diameter, the origin of dorsal fin is shorter and located a bit behind, the tail region is shorter and thicker and the body height is quite higher compared to *G. rufa*. These results are consistent with findings of Karahan (2007) and Çiçek (2009). The ventral position of mouth of *G. rufa* agrees with benthic character structure as suggested by Coad (2010). The small head structure and thicker tail structure of *G. variabilis* supports the statement of Coad (2010) who suggested that *G. variabilis* lives in faster flowing rivers.

The size difference (CS) between these two species is not sufficient (Size Procrustes ANOVA;  $F=0.16$ ,  $p$ -value=0.6951) (Fig. 7) and all differences are fully sourced from their shapes.

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