Discrimination of *Capoeta trutta* (Heckel, 1843) and *Capoeta umbla* (Heckel, 1843) from scales by Geometric Morphometric Methods

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Abstract

In this study, a totally 103 (53♀♀, 50♂♂) *C. trutta* and 82 (67♀♀, 15♂♂) *C. umbla* samples from the Tigris River between Ilısı Dam and Cizre town were collected and ages of scales taken from front and upper section of line lateral of dorsal fins of fishes were determined and one scale from each fish sample photographed by an Olympus digital camera with Canon SX7 model binocular under the same conditions with 40X magnification and then six landmarks were taken by tpsDig. After GPA (General Procrustes Analysis) performed, ANOVA (Analysis of Variance), PCA (Principal Component Analysis), CVA (Canonical Variance Anlysis) and DFA (Discriminant Function Analysis) analysis were conducted by MorphoJ and PAST. In CV (Canonical Variance) Analysis, the permutation p-value of the mahalanobis and procrustes distance between the two species (p<.0001) shows that the difference is quite significant. As a result of the reclassification done by the analysis, we see that 79% of *C. umbla* and 77% of *C. trutta* are classified correctly.

Keywords: Cyprinidae, *Capoeta trutta*, *Capoeta umbla*, Sclae, Discrimination, Geometric morfometry.

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Introduction

The genus of *Capoeta* is distributed geographically in Central Asia, South China, North India, Afghanistan, Turkestan, Georgia, Armenia, Azerbaijan, Uzbekistan (Aral Lake), Middle East and Anatolia around ten species, 5 species and 6 subspecies live in Turkey (Banarescu, 1991; Geldiay and Balık 1999; Coad, 2010). The species of *Capoeta trutta* and *Capoeta umbla* commonly found in the Tigris river basin and generally live in the same environment.

Dartay and Gül (2013) examined Height-weight relationship of *C. umbla* and *C. trutta* species caught from Keban Dam Lake and expressed in *C. trutta* samples, height average and condition coefficient were higher than *C. umbla*, height and weight are highly correlated with each other. Çiçek (2009) compared the samples of *C. trutta* and *C. umbla* were collected from Tigris and Euphrates fresh water systems by meridional and mophological angles. While the measurements in the head region differ from those in terms of meridian and morphometric such as Line lateral scale number, gill rakers, they also reported that there were significant differences in other body parts, such as body length, body depth, and distance between fins out of the head region. Dağlı and Erdemli (2011) taken measurements from morphometric properties and reported that there were statistically significant differences between the two morphometric ratios in their studies.

Demirok and Ünlü (2001) reported that in the karyological analysis of the samples living in the Tigris River these two species indicated the same number of diploid chromosomes (2n) but the total number of chromosome arms (NF) was different.

Turan (2008), compared species and subspecies in molecular comparison of Capoeta specimens collected from Anatolia whether they are appropriate or not according to the conventional classification. According to this, he revealed *C. trutta* and C. *umbla* species brought from different localities show clustering closer to each other according to other species of Capoeta. However, Bektaş *et al*. (2017) emerges results in their moleculer studies *C. umbla* is in small-scaled Capoeta clade, *C. trutta* is in spotted Capoeta clade, and the dissociation times of these two clads to be about mid-miocene.

Analysis on scales by geometric morphometric methods has been reported to be a very useful and reliable tool to distinguish between difficult-to-distinguish genus, species, geographic variants, and local populations, effects of habitat on scale morphology and showing age and seasonal variation. Moreover, contrary to other methods, it has been stated that this method is more economical and easier, harmless and allowing samples to be inspected and monitored because the samples can be released again, and it is possible to obtain many samples from the populations (Bayley, 1973; Richard and Esteves, 1997; Poulet *et al*. 2005; Ibanez *et al*. 2007, 2009 and 2012;

Staszyn *et al*. 2012; Çiçek *et al*. 2016; Bilici *et al*. 2016; Avigliano *et al*., 2017). Teimori (2016), has come to the conclusion that the scale morphology can be used to identify and distinguish morphologically similar species.

Material and methods

In this study, a totally 103 (53♀♀, 50♂♂) *C. trutta* and 82 (67 ♀♀, 15♂♂) *C. umbla* samples from the Tigris River between Ilısı Dam and Cizre town were collected and ages of scales taken from front and upper section of line lateral of dorsal fins of fishes were determined and just one scale from each fish sample photographed by an Olympus digital camera with Canon SX7 model binocular under the same conditions with 40X magnification and then six landmarks were taken by tpsDig ver. 2.32 (Rohlf, 2016) software (Fig. 1). After GPA (General Procrustes Analysis) performed, ANOVA (Analysis of Variance), PCA (Principal Component Analysis), CVA (Canonical Variance Anlysis) and DFA (Discriminant Function Analysis) analysis were conducted by MorphoJ 1.06d (Klingenberg, 2011) and PAST 3.11 (Hammer *et al*., 2001).

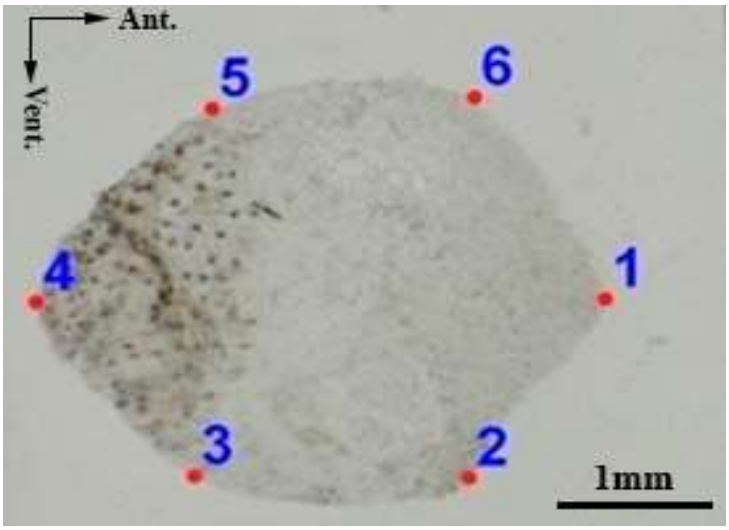

Figure 1: The landmarks location on scale (Photo: *Capoeta umbla* scale, Ant.: Anteriör, Vent: Ventrale).

Results

The difference in terms of scale dimensions (CS:Centroid size) between *C. umbla* and *C. trutta* species is statistically significant (F=8.56, p= 0.0039) and the size of the scale is larger in *C. umbla* species (Fig. 2A). Likewise, there is also a difference in size of scales (CS) among the genders (F=8.07, *p*= 0.0004) and scales were bigger in females (Fig. 2B).

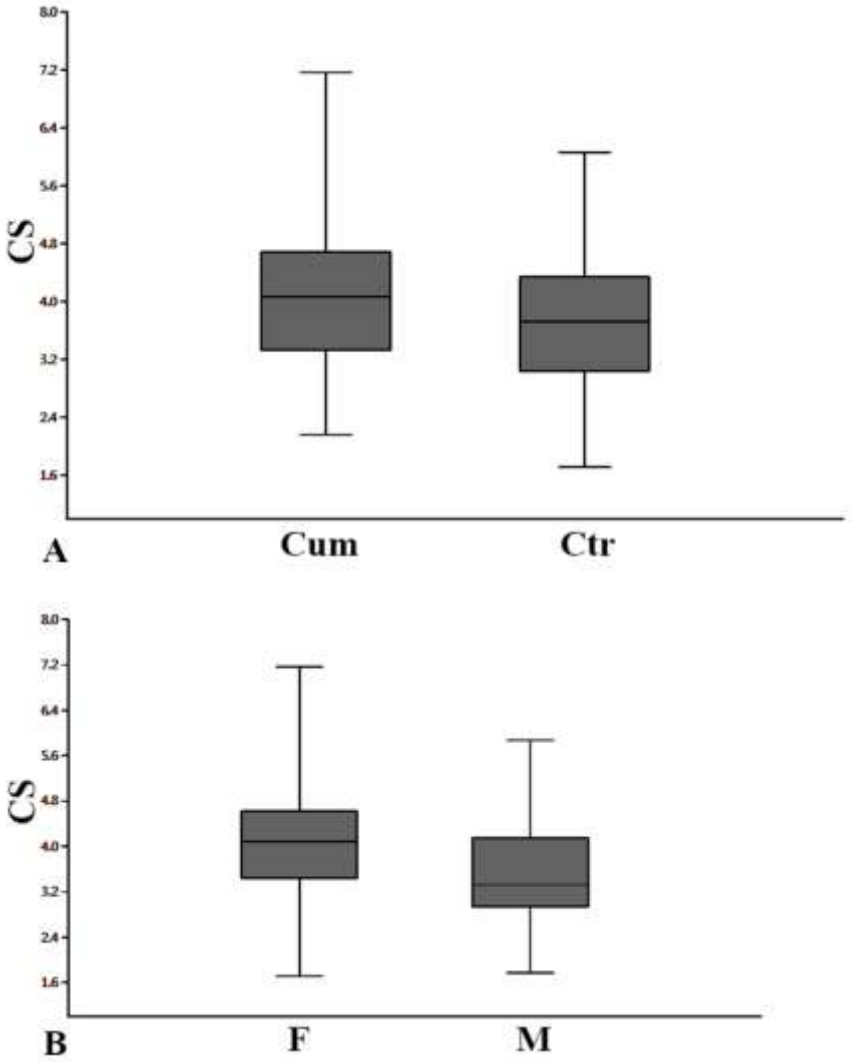

Figure 2: The box plot for CS of Species (A) and Sex (B) (*Capoeta trutta*, Cum: *Capoeta umbla*, F: Female, M: Male, CS: Centroit Size).

Significant differences were found between species in terms of shape according to ANOVA (Variance Analysis) and MANOVA (Multiple Variance Analysis) results (for ANOVA: F=10.44, $p<.0001$ and for MANOVA: pillai tr = 0.40, $p<.0001$). Though there was a difference between genders in terms of scale shape, it is not as high as between species (for ANOVA: F = 1.84, $p= 0.0222$ and for MANOVA: pillai tr = 0.16, $p=0.0190$).

In PC (Principal Components) Analysis for Species, the first two components explain 52.2% of the total variation, but there is no dissociation along the PC1 and PC2 axes (Fig 3A). For genders, the first two components account for 51.1% of the total variation and there is no grouping along the first two axes (Fig. 3B).

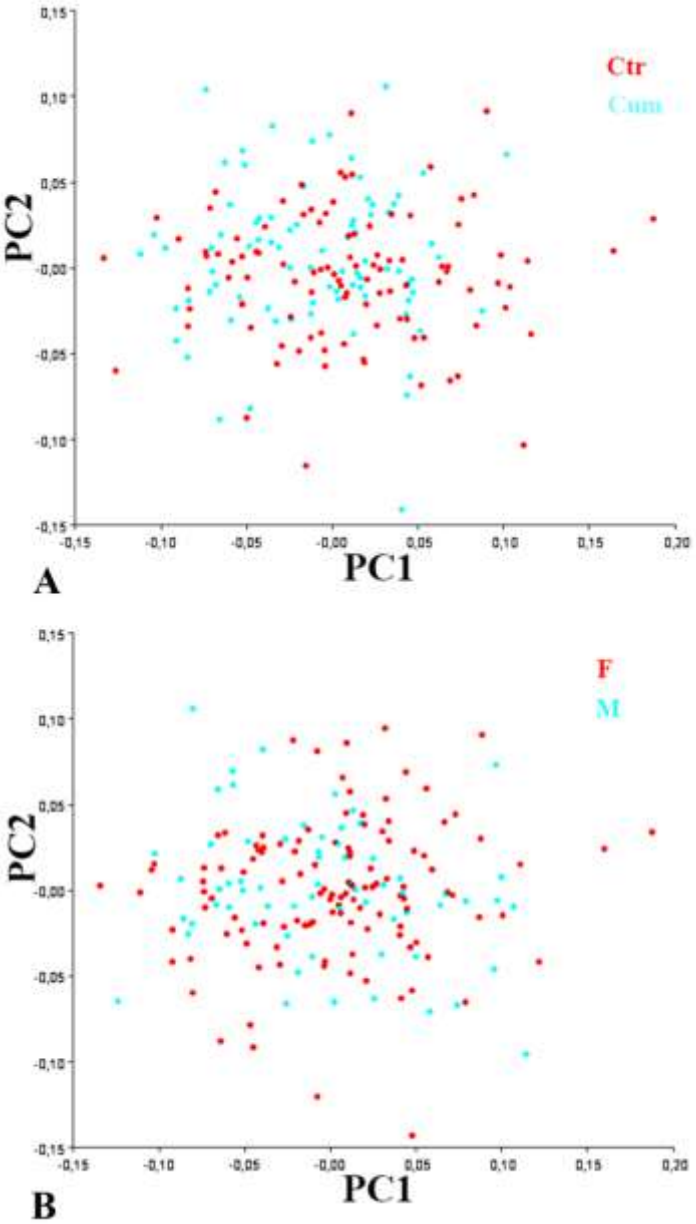

Figure 3: Scatter plot of PCA for Species (A) and Sex (B) (*Capoeta trutta*, Cum: *Capoeta umbla*, F: Female, M: Male).

In CV (Canonical Variance) Analysis, the permutation p-value of the mahalanobis (1.63) and procrustes (0.04) distance between the two species (p<.0001) shows that the difference is quite significant. Although there is a partial overlap in the CVA graph, the separation between the two species is quite extensive (Fig. 4A) In CVA for gender, the permutation p value (p=0.0136) of Mahalanobis distance (0.66) shows the difference, but no difference was found for the Procrustes distance. It is seen that there is much less discrimination between genders on the CVA graph (Fig. 4B).

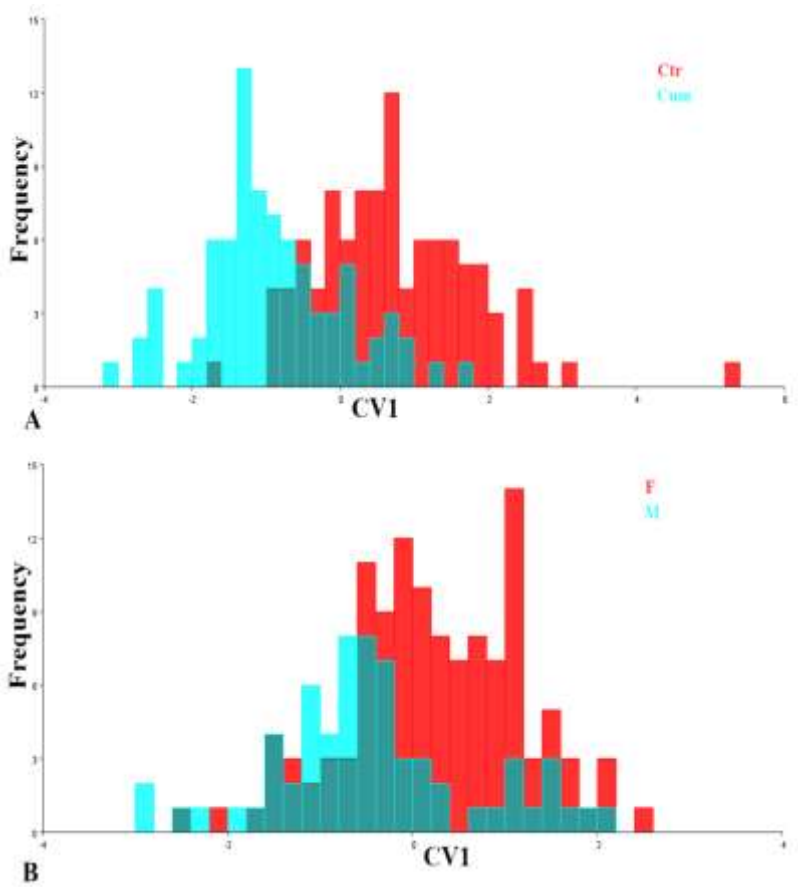

Figure 4: Scatter plot of CVA for Species (A) and Sex (B) (*Capoeta trutta*, Cum: *Capoeta umbla*, F: Female, M: Male).

In the CV analysis for species and sex together, there was no difference between female and male of the same species, the difference in the permutation p value of mahalanobis distance between the female-female, male-male and female-male groups of different species was found to be quite significant (Table 1).

In DF (Discriminant Function) analysis, parametric p values for T^2 (mahalanobis distance) between two species and permutation p values (p<.0001) for distance between mahalanobis and procrustes show that the difference is quite high. As a result of the reclassification done by the analysis, we see that 79% of *C. umbla* and 77% of *C. trutta* are classified with an accuracy. In DF (Discrimination Function) analysis for genders, parametric p values for T^2 (mahalanobis distance) (p=0.0271) and permutation p values (p=0.0250) for mahalanobis distance shows that difference.

Table 1: The Species-Sex groups that significantly different according to Mahalanobis Distance in CVA.

Gruplar	Ctr, F	Ctr, M	Cum, F
Ctr, M	0.74		
Cum, F	1.43*	1.79*	
Cum, M	1.96*	2.38*	1.02

(*p<.0001, *Capoeta trutta*, Cum: *Capoeta umbla*, F: Female, M: Male).

In the DF analysis for species and sex together, similar to CVA analysis there was no difference between female and male of the same species, parametric p values for T^2 (mahalanobis distance) between the female-female, male-male and female-male groups of different species and the permutation p value (p<.0001) of mahalanobis distance shows that the difference is quite high (Table 2).

The scale mean shape difference based on DFA, *C. trutta*'s scales were larger than *C. umbla*'s ones but shorter than at anterior side. However, *C. umbla*'s were larger at posterior side (Fig. 5).

Table 2: The Species-Sex groups that significantly different parametric *p* value for T^2 and permutation *p* value (*p<.0001) for Mahalanobis Distance and T^2 value (Ctr: *Capoeta trutta*, Cum: *Capoeta umbla*, F: Female, M: Male, p(param.): Parametric *p* value, p(perm.) permütatation *p* value, n.s.: not significant).

Gruplar	Ctr, F; T^2/p(param.)/ p(perm.)	Ctr, M; T^2, p(param.), p(perm.)	Cum, F; T^2, p(param.), p(perm.)
Ctr, M	13.4/ n.s./ n.s.		
Cum, F	66.2/*/*	87.9/*/*	
Cum, M	50.5/*/*	78.9/*/*	12.3/n.s./ n.s.

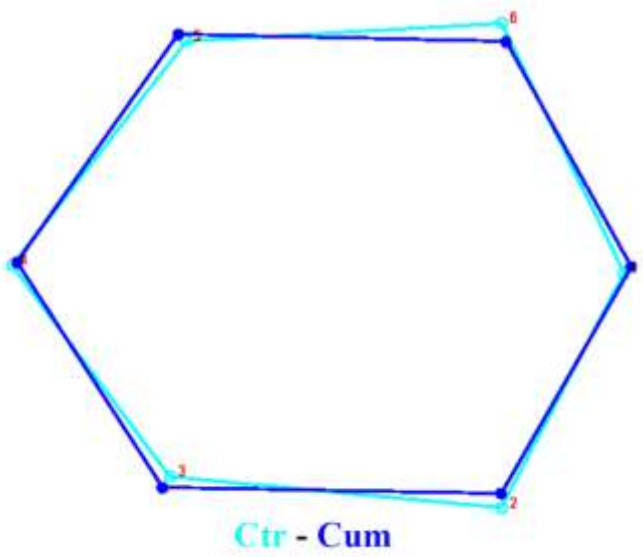

Figure 5: Scale shape differences between *Capoeta trutta* (Ctr=ligt blue) and *Capoeta umbla* (Cum=dark blue).

Discussion

Dartay and Gül (2013) stated that height average and conditional coefficient of *C. trutta* higher than *C. umbla* in their study. However, in this study, it was found that the size of the scale of *C. umbla* (as CS) was higher than that of *C. trutta* (Fig. 2A). This result is important in that it shows that height and weight in these species may not be positively related to scale size. Çiçek (2009) and Dağlı and Erdemli (2011) reported significant differences between these two species in terms of their morphometric and meridian in their study. The results of this study for scales are in line with these works. Çiçek *et al*. (2016) stated that scale size of *C. umbla* is different between genders but there is no difference in shape in their study. In our this study, it was found that the size of the scale is different among the genders (Fig. 2B) but there is no difference in shape between female and male of the same species (Tables 1 and 2). This is quite similar to study of Çiçek *et al*. (2016) in terms of results. Bektaş *et al*. (2017) stated in their studies *C. umbla* is in small-scaled Capoeta clade, *C. trutta* is in spotted Capoeta clade. In our this study, contrary to Bektaş *et al*. (2017) it is seen that *C. trutta* is smaller than *C. umbla* in terms of scale size. In our this study, difference in these two species in terms of size and shape of scales is compatible with the result of that these two species are karyologically different according to studies of Demirok and Ünlü (2001).

As a result, as stated by Richard and Esteves (1997), Poulet *et al*., (2005), Ibanez *et al*. (2007, 2009 and 2012), Staszyn *et al*. (2012) and Teimori (2016), it is seen that geometric morphometric studies made with scales are a very safe and useful method to identify and distinguish morphologically similar taxons that are close to each other.

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