A survey on *Luciobarbus mystaceus* (Pallas, 1814) by geometric morphometric methods depend on gender, age and season variations

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Abstract

This study is made to find out the variations of the outer body morphology of *Luciobarbus mystaceus* (Pallas, 1814) belonging Cyprinidae family that lived in Tigris river system between January 2015 and December 2015 with geometric morphometric methods depending on gender, age and season. After being caught with fishnet having different meshes and brought to the laboratory, in total 58 (21♀♀, 37♂♂) *L. mystaceus* samples were photographed from lateral by Sony digital camera. On the basis of photography, 12 landmarks were taken with tpsDig ver. 2.26 and later on the database that contains this landmark coordinates were analyzed by Principal component analysis (PCA), Canonical Variation Analysis) and Distribution Analysis (DA) with MorphoJ ver. 1.06d program. In the CV analysis between age groups; in terms of Mahalanobis distance, VI age group statistically has different forms in comparison with III, IV and V age groups. III-V age groups also statistically have different forms in comparison with other groups except VII and VI age groups. In the seasonal CV analysis, winter group statistically has different forms in comparison with autumn, summer and spring groups in terms of Mahalanobis distance. In CVA Mahalanobis distance between the sexes is 2.0 and the permutation value depending on this; $p<0.0001$. The difference is statistically significant and the distribution between males and females is significant.

Keywords: Cyprinidae, *Barbus*, *Luciobarbus mystaceus*, Variation, Geometric morphometric

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Introduction

The Cyprinidae family is one of the richest and most important fish family members in terms of number of species and has spread to different parts of the world. In the world, this family is represented by about 1500 species, of which 30 genus and 70 species are found in Turkey (Blanc, 1971; Kuru, 1975; Kelle, 1978; Geldiay, 1988; Banarescu, 1990; Winfield ve Nelson, 1991). Members of the Barbinae subfamily form a very small part of the Cyprinidae family. Capoeta, Cyprinion, Barbus and Bertinius species of this subfamily containing the Barbini tribus are distributed in the East Asian periphery, Anatolia, Africa and Europe (Karaman, 1971) Barbus genus is native to East Asia, today dominated in the Old World Cyprinids with more than 800 species scattered throughout Europe, Africa and Asia. It is found in Europe, from Spain to the Black Sea, from the Mediterranean to the Dniepr basin in the north. This monotypic genus has many morphological characters and some species are very similar to each other (Howes, 1987; Banarescu, 1964; 1989). They are the fish species that are hunted intensively and tasty among freshwater fish living in Turkey. Although these species are often preferring flowy, gravelly sandy streams, but they can also seen in stagnant water from time to time. There are seven species and six subspecies of this genus in Turkey's waters (Lindgren,1983; Turan *et al*., 2005).

L. mystaceus is a little known species and very common in the Euphrates and Tigris Rivers (Kuru, 1975). It is also located in Karun and Karkheh Rivers in Iran (Coad, 1979). Along with not being any morphometric and meridional studies of this species, biological properties (Şen *et al*., 2001; Duman, 2002; Dörtbudak *et al*., 2012), heavy metal analysis (Alhas *et al*., 2009), fatty acid analysis; (Konar *et al*., 1999), content of digestive system (Saler *et al.*, 2010) and blood parameters (Yılayaz, 2002) studies had been made in this subject.

Dörtbudak *et al.* (2012) in their study on *L. mystaceus*, they expressed that: age of sexual maturity is between II. and IV., the proportional height and weight gain were highest in the V. age group, absolute height increase is more higher in groups II, III, IV and V, absolute weight gain increases from age III and in this species height increase first, in later ages, the increase in weight was more. Condition factor in females is the lowest in November and in the IV age group, the highest in the March and in the VIII age groups, in males is the lowest in November and in the III age group, the highest in the July and in the VI age groups.

Geometric morphometric method with multivariate statistical applications, offers much more successful tools according to the classical morphometry based on distance, angle and position to capture shape-related information, to test and to visualize them. This method has been used in many studies (Cavalcanti *et al*., 1999; Sara *et al*., 1999, Loy *et al*., 1998 and 2000; Favaloro *et al*., 2003; Ponton *et al*., 2013; Mojekwu and Anumudu, 2015; Çiçek *et al*., 2016) to reveal the effect of ecological factors (of habitat, nutrition, etc.) on shape variations in fishes and has been reported as a very effective and successful method.

In this study, it aimed to contribute information about the species to reveal the variations of external body morphology according to age, sexuality and season by using geometric morphometric methods in *L. mystaceus* species in Tigris river system and to give morphometric information that never made on this species.

Material and Methods

In this study, totally 58 (21♀♀, 37♂♂) *L. mystaceus* species were caught from the Dicle River Güçlükonak location in different seasons between January 2015 to December 2015 and transferred to the laboratory and then gender was determined and their total body length were measured. Twelve landmarks were collected from images by tpsDig ver. 2.26 (Rohlf, 2016) and then the data file that contains landmark coordinates were analyzed with GPA (General Procrustes Analysis), Procrustes ANOVA (Variance Analysis), PCA (Principal Component Analysis), CVA (Canonical Variation Analysis) and DA (Distribution Analysis) by MorphoJ ver. 1.06d (Klingenberg, 2011) and gender, age and seasonal size (CS: Centroid Size) and shape variations were evaluated.

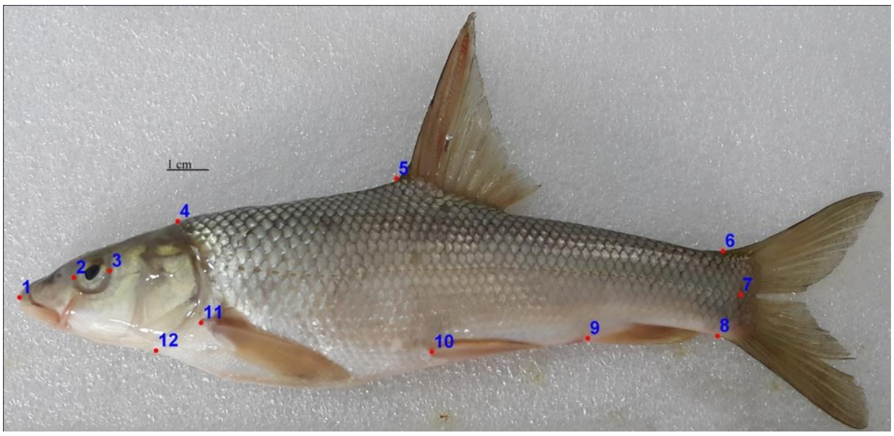

Figure 1: The lateral view of *Luciobarbus mystaceus* and landmark locations used in analysis.

Results

The result of Procrustes ANOVA analysis indicates that there is no size difference (CS) between samples. In the Principal Component Analysis (PCA) first two components explain % 53.3 of total variation (PC1 % 37.6, PC2 % 15.6) and first five components explain % 80 of total variation depending on shape. Nevertheless through PC1 and PC2 axis, no separation was seen (Fig. 2).

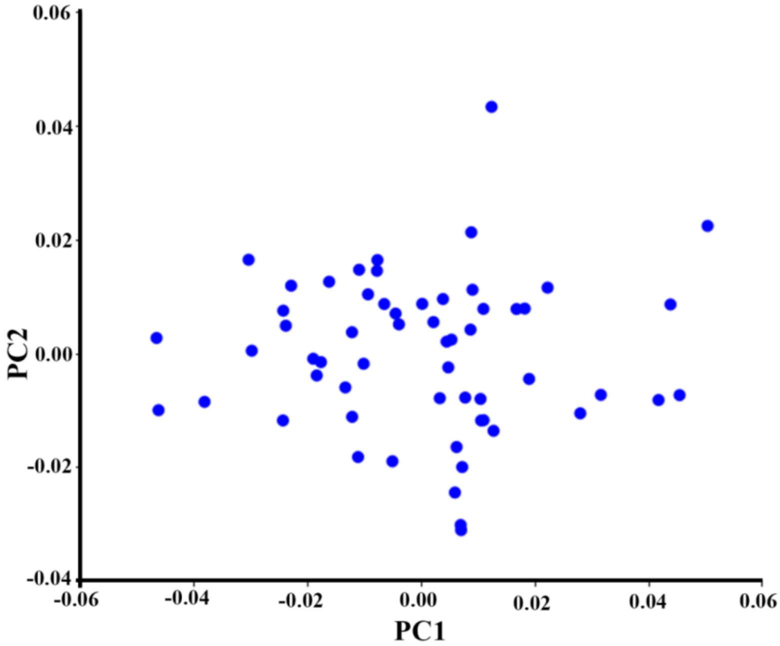

Figure 2: Scatter plot of samples on shape space by Principal Component Analysis.

In the canonical variation analysis (CVA) Mahalanobis distance between genders (Female: 21, Male: 37) is 2.0 and the permutation value depending on this, $p<0.0001$. This difference is statistically significant and the separation between males and females is quite clear (Fig. 3).

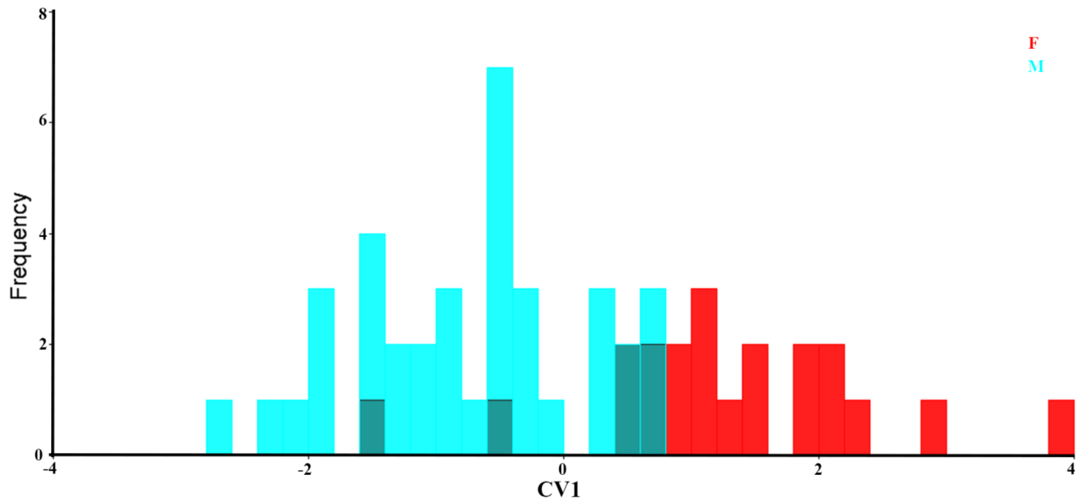

Figure 3: Canonical Variance Analysis Graphic for Sex (F: Female-Red, M:male-Blue).

In the CV analysis between age groups (II: 2, III: 7, IV: 14, V: 15, VI: 17 ve VII: 3); it was found that there is statistically significant shape difference between VI and "III, IV and V" age groups and between III-V age groups at a level of permutation value for Mahalanobis distance $p<0.0001$. Statistically significant shape difference was also found between the other groups except VII and VI age groups at a level of p value $p<0.05$ and $p<0.01$ (Fig. 4 and Table 1).

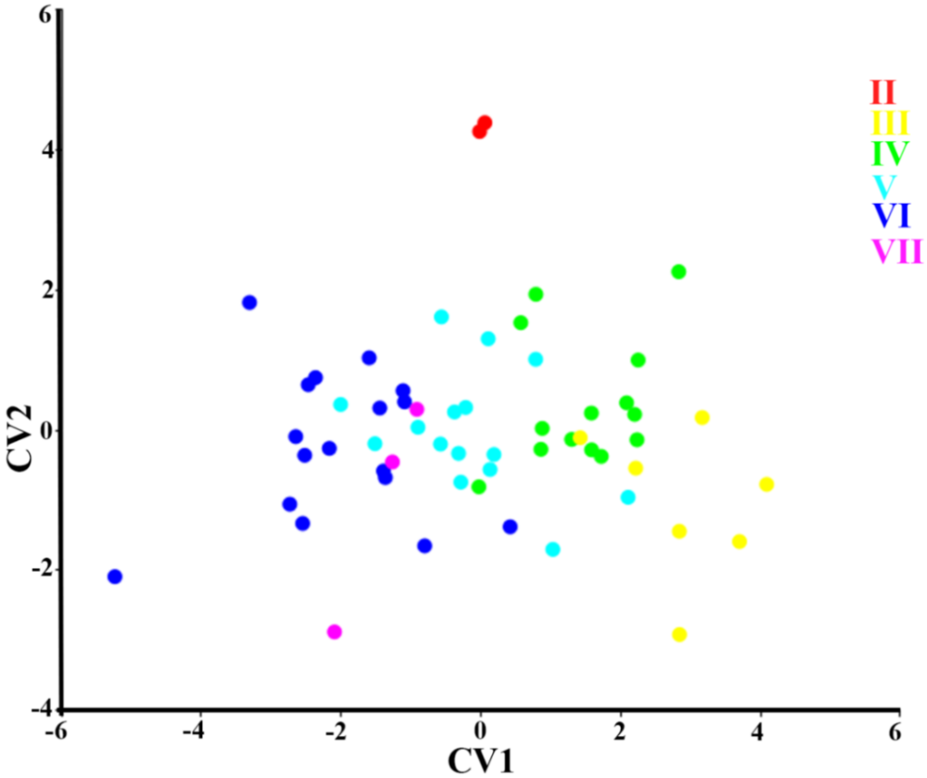

Figure 4: Canonical Variance Analysis Graphic for Ages.

Table 1: Mahalanobis distance and permutation p value between Ages (*$p<0.05$, **$p<0.01$, ***$p<0.0001$, n.s= not significant).

Age Groups	II	III	IV	V	VI
III	6,1*				
IV	4,6**	2,6*			
V	4,7**	3,6***	2,5***		
VI	5,1**	5,0***	3,7***	2,5***	
VII	6,0*	5,2**	4,0**	3,4*	3,0 $^{n.s}$

In the CV analysis between seasonal groups (Au: 14, Sm: 9, Sp: 12 ve Wn: 23); it was found that there is statistically significant shape difference between winter and other seasons, and between autumn-summer groups at a level of permutation value for Mahalanobis distance $p<0.0001$. Statistically significant shape difference was also found between other groups at a level of permutation value $p<0.01$ (Fig. 5, Table 2).

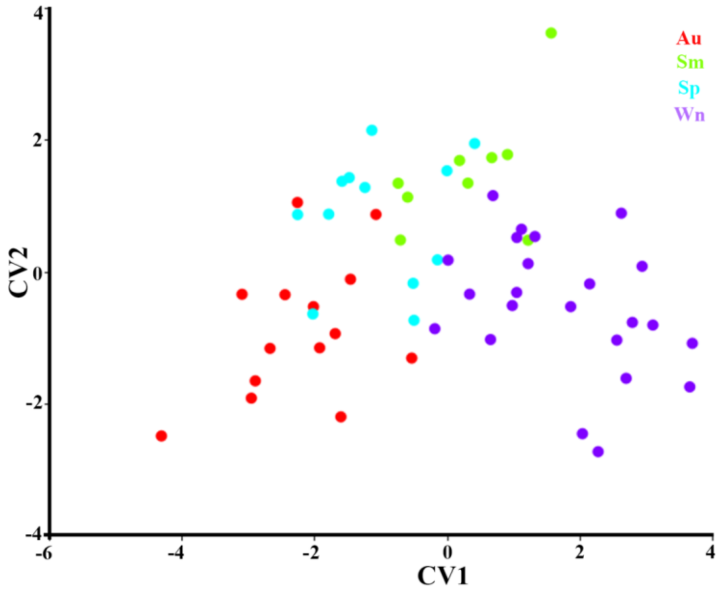

Figure 5: Canonical Variance Analysis Graphic for seasons (Au: Autumn, Sm:Summer, Sp:Spring, Wn:Winter).

Table 2: Mahalanobis distance and permutation p value between seasons (Au: Autumn, Sm:Summer, Sp:Spring, Wn:Winter, *p<0.05, **p<0.01, ***p<0.0001, n.s= not significant).

Season Groups	Au	Sm	Sp
Sm	3,5***		
Sp	2,5**	2,5**	
Wn	4,0***	2,7***	3,2***

In the paired comparisons that distribution function analysis made, no adequately ($p>0.05$) shape difference was found between genders groups. According to the results of parametric p value ($p=0.0256$) and p value ($p=0.0230$) for T^2 between IV-V age groups, permutation p value ($p=0.0230$) for T^2 between IV-VI (Figure 6A and 6B), parametric p value ($p=0,0068$) between Au-Wn (autumn-winter) and permutation p value ($p=0,0090$) for T^2 from the seasonal groups; statistically significant shape difference was found (Fig. 6C).

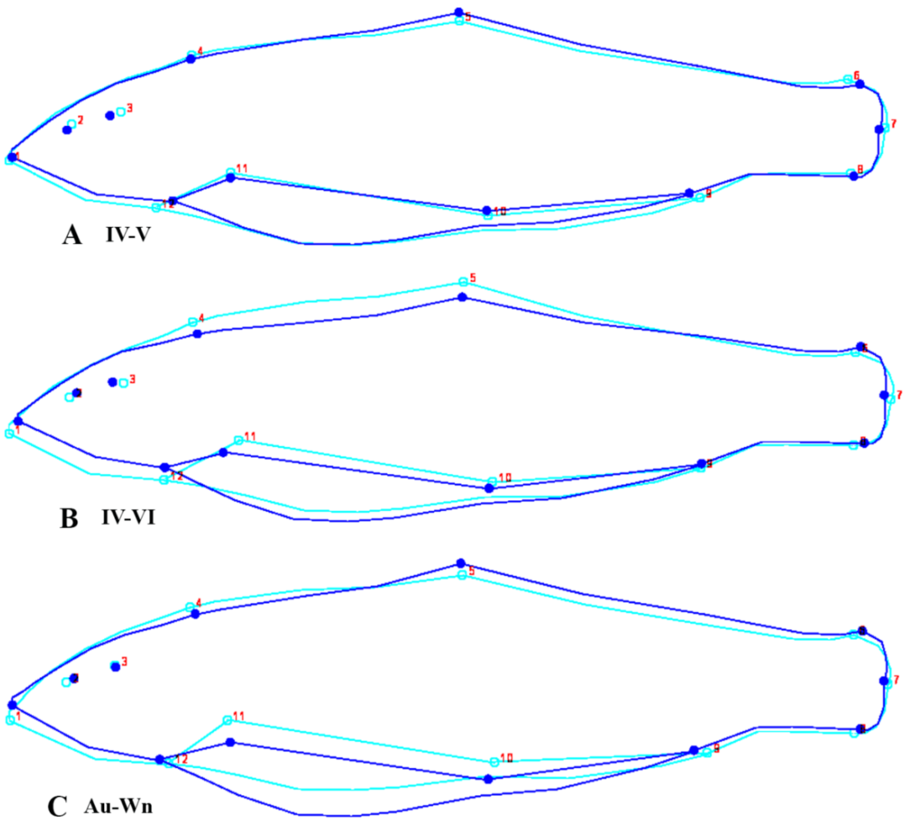

Figure 6: The groups have significant shape differences by Discriminant Function Analysis (A: IV light blue-V dark blue; B: IV light blue-VI dark blue; C: Au=autumn-light blue-Wn=Winter-dark blue).

Discussion

The result of Procrustes ANOVA indicates that there is no difference between sample groups in terms of size (PCS). Principal component analysis distribution indicates that there is no clear difference through PC1 and PC2 axis and first five components explain the major part of the total variation (Fig. 2).

Canonical Variation Analysis indicates the significant differences between gender groups (Fig. 3), seasonal groups (Table 2) and age groups except VI and VII (Table 1) according the permutation p values depending on Mahalanobis distance at various levels (p<0.05, p<0.01 and p<0.0001). Furthermore, III and IV age groups separates from VI and VII age groups through CV1 axis, V age group is in the transition group and II age group separates from all other age groups according to CV2 axis (Fig. 4). It is seen that in the seasonal groups; autumn and winter groups are separated through CV1 axis and other groups (Summer and Spring) are transition group (Fig. 5).

In the shape differences between IV and V age groups that are obtained from discriminant function analysis and have significant differences; it is seen that the change is in dorsal and ventral direction, in a sense body depth increases (Fig. 6A). In the IV and VI age groups; again in dorsal and ventral direction, and also in anterior body (cephalic and between dorsal-pelvic fin) we see that there is a height increase (Fig. 6B). Between the seasonal groups; it is seen that also in form differences between autumn and winter group, the change is mostly in anterio-ventral (between cephalic and pelvic fin) and postrio-dorsal (between dorsal and caudal fin) direction (Fig. 6C). These results are conformed with the results that informed in the study of Dörtbudak *et al*. (2012) about first reproduction age; ages that have rational, absolute and weight increases and the found maximum and minimum months and ages for condition factors given about *L. mystaceus.* Furthermore we see that; with this study like the others, geometric morphometric method is an efficient way to visualize and analyze the variations that are formed by the factors such as age, season and gender.

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