DOI:10.31196/huvfd.786577

A Distinction of Some Cyprinid Species from Tigris River Basin According to Scales by Geometric Morphometric Methods

Serbest BİLİCİ[1,a,*]

[1]Istanbul Aydin University Bil College Diyarbakır, Turkey
[a]ORCID: 0000-0002-3787-4452

Geliş Tarihi: 28.08.2020 **Kabul Tarihi: 09.12.2020**

Abstract: In this study, a total of 86 (31♀♀, 55♂♂) cyprinid specimens belonging to the species, *Luciobarbus mystaceus*, *Arabibarbus grypus*, *Luciobarbus esocinus* and *Carasobarbus luteus,* were collected from the Tigris River near Cizre town. The size (as centroid size) and shape of scale separately were analyzed by 2D geometric morphometric methods. The size and shape of species were different but not for sex, according to ANOVA. Mahalonobis length of CVA results shows that only the difference between *Luciobarbus esocinus* and *Carasobarbus luteus* was not significant. DFA results based on T^2 all species scale shape differences were significant except *Luciobarbus esocinus* and *Carasobarbus luteus.*
Keywords: *Cyprinidae, Geometric, Landmark, Morphometric, Scale, Shape, Turkey.*

Dicle Nehir Sistemindeki Bazı Cyprinid Türlerinin Pullarından Geometrik Morfometrik Yöntemle Ayırt Edilmesi

Özet: Bu çalışmada, Cizre ilçesi yakınlarındaki Dicle Nehri'nden *Luciobarbus mystaceus, Arabibarbus grypus, Luciobarbus esocinus* ve *Carasobarbus luteus* türlerine ait toplam 86 (31♀♀, 55♂♂) cyprinid örneği toplanmış ve büyüklüğü (centroid olarak) boyut ve pul şekli ayrı ayrı 2D geometrik morfometrik yöntemlerle analiz edilmiştir. Türlerin boyutu ve şekli farklıydı, ancak ANOVA'ya göre cinsiyet için farklı değildi. CVA sonuçlarının Mahalonobis mesafesine göre, sadece *Luciobarbus esocinus* ve *Carasobarbus luteus* arasındaki farkın anlamlı olmadığını göstermektedir. *Luciobarbus esocinus* ve *Carasobarbus luteus* dışında T2'ye dayalı DFA sonuçları tüm türlerin pul şekil farklılıkları önemliydi.
Anahtar Kelimeler: Balık pulu, Cyprinidae, Geometrik, Belirteç, Morfometrik, Şekil, Türkiye.

Introduction

Cyprinidae is the largest family of freshwater fishes and shows an extensive geographic distribution from North America (northern Canada to southern Mexico) to Africa and Eurasia (Nelson, 2006). Approximately 15% of freshwater fishes in Turkey belong to the Cyprinidae (59 species) (Çiçek et al., 2020; Kuru et al. 2014). The subject of this study, *Luciobarbus mystaceus, Luciobarbus esocinus, Carasobarbus luteus* and *Arabibarbus grypus* are species belonging to the Cyprinidae and are distributed in the Tigris and Euphrates water systems (Beckman, 1962; Coad, 1996; Karaman, 1971; Kuru, 1979).

Fish scale is a useful tool for defining fish in genus or species levels and also for identifying fish in studies of fish phylogeny, sexual dimorphism, age determination, and habitats affecting development (Esmaeili et al., 2007; Esmaeili and Gholami, 2011; Ibáñez et al., 2007, 2016; Jawad, 2005; Jawad and Al-Jufaili, 2007; Miranda and Escala, 2000; Poulet et al., 2005). Although fish scales were considered to be an essential value in the classification of fish, the aspect of the scale has proven to be inefficient at least at the species level, the use of fish scales as the age index besides the use of fish for the life history has been determined (Van Oosten, 1957). It was stated that the external structure of the fish and the models of fish scales were useful in establishing phylogenetic relations (Van Oosten, 1957). Recently, scanning electron microscopic studies have revealed a detailed shape design and the shape of Teleostei scales, renewing the interest in using the scales' surface structure for taxonomic purposes (De Lamater and Courtenay, 1974). It has been suggested that ecological characteristics could be of great importance for identifying groups within the genus *Barbus* (Economidis, 1989; Tsigenopoulos and Berrebi, 2000). Different growth characteristics of fish populations concerning various external factors, seasonal or habitat variability, availability of nutritional resources and therefore scales with the initial contact with the environment indicate an important phenotypic feature about all these factors (Şerban and Grigoraş, 2018).

Geometric morphometrics (GMMs) is a strong taxonomy tool and its systematic has notable statistical power and deals directly with the Cartesian coordinates of landmarks, rather than with traditional distance, angle, or ratio measurements (Bookstein 1999; Klingenberg, 2011). It is useful to reveal even small morphological variations, which often are invisible by traditional morphometric (Zelditsch et al., 2004).

Analysis on scales by geometric morphometric methods has been reported to be a handy and reliable tool to distinguish between challenging to distinguish genus, species, geographic variants, local populations, effects of habitat on scale morphology, and showing age in addition to 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. Fish scales are extremely suitable materials to be used for 2D geometric morphometric methods, the scales may vary depending on age, gender, and season. Also, scales can be used to determine the source of differences and variations in fish size and shape (Avigliano et al., 2017; Bilici et al., 2016; Poulet et al., 2005; Çicek et al., 2016; Ibáñez et al., 2007, 2009 and 2012; Staszyn et al., 2012)

This study aims to evaluate whether the landmark-based, geometric, morphometric approach to define fish scale morphology is useful in distinguishing species belonging to the same family such as *Luciobarbus mystaceus*, *Arabibarbus grypus*, *Luciobarbus esocinus* and *Carasobarbus luteus*.

Material and Methods

In this study, specimens of *Luciobarbus myctaceus* (n=58), *Arabibarbus grypus* (n=18), *Luciobarbus esocinus* (n=5) and *Carasobarbus luteus* (n=5) belonging to the *Cyprinidae* were obtained by local fisheries from the Tigris River (Figure 1).

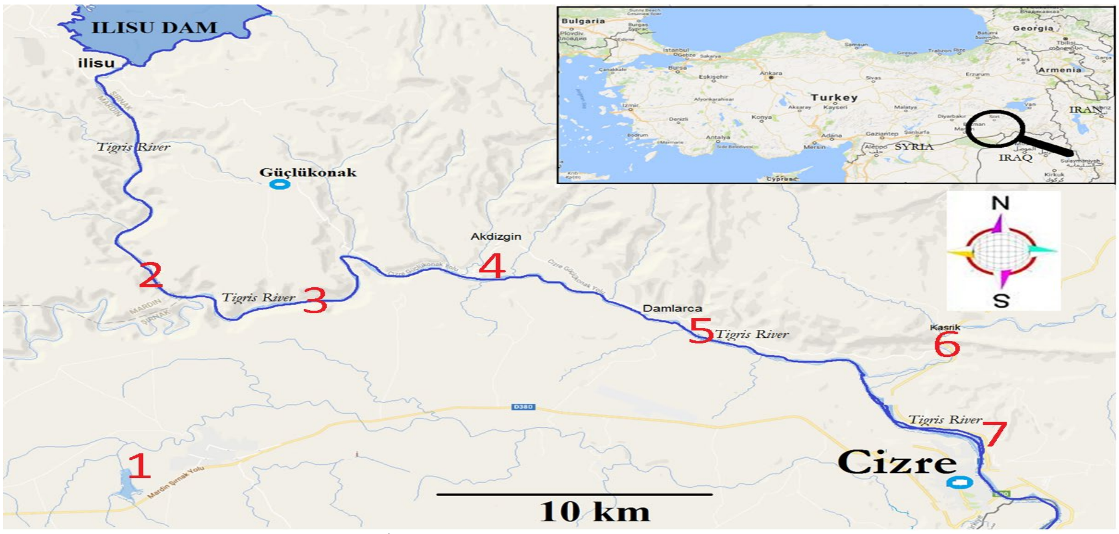

Figure 1. Sample localities (1-Dirsekli Pond (İdil), 2-Tigris River (Güçlükonak), 3-Tigris River (Güçlükonak), 4-Tigris River (Akdizgin), 5-Tigris River (Damlarca), 6-Kasrik Stream, 7-Tigris River (Cizre).

Since the fish included in this study are commercial fish caught by fishermen, ethics committee approval is not required. The scales were taken from the front and upper sections of the lateral lines of dorsal fins of fishes and age. They were determined and photographed by an Olympus digital camera with Canon SX 7 model binocular under the same conditions. Then, six landmarks (Figure 2) were collected by tpsDig ver. 2.32 (Rohlf, 2016) software and Procrustes analysis were performed. After separating the shape and size of the samples, ANOVA, PCA, CVA/MANOVA, and DFA analyses were performed by using Morpho J1. 06 d (Klingenberg, 2011) and PAST 3.11 (Hammer *et al.*, 2001) programs.

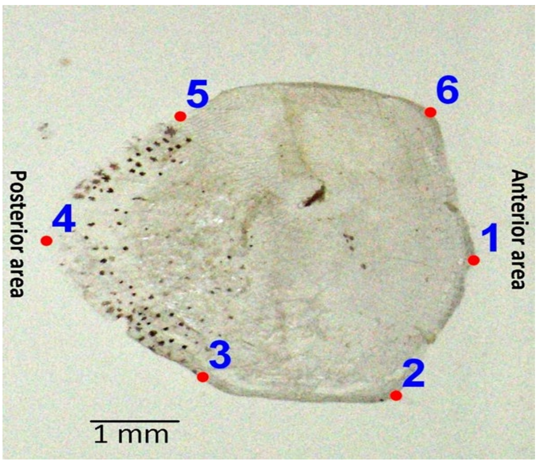

Figure 2. Landmark definitions used in the fish scales.

Results

Arabibarbus grypus and *Carasobarbus luteus* have scales that are very large and close to each other in width and length. Since the length of the *Luciobarbus esocinus* and *Luciobarbus mystaceus* scales are greater than their width, they don’t have a circular shape.

In all species, significant differences were found between species in terms of both size (CS) and shape, but differences between gender were found to be insignificant (Table 1, Figure 3).

Table 1. Procurstes ANOVA results for species and gender

		F	p(parm)
Species	CS	64,14	<.0001
	Shape	3,90	<.0001
Gender	CS	4,67	0,0336
	Shape	0,57	0,8046

(p(parm): parametric p value, **Pillai tr.:** Pillai trece, (**F:** Gooddal F value).

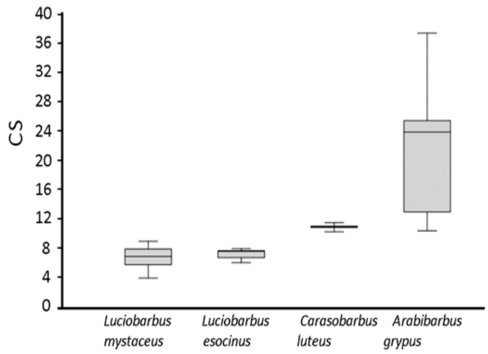

Figure 3. The Box plot of CS of species (The short horizontal lines: Min. and Max., the box down and up bounder: 25 and 75 percentile, the long horizontal line: Average).

In the Basic Component Analysis (PCA) according to species, the first three components account for 65.4% of the total variation. The first three components in PCA explain 65.3% of the total variation according to gender (Figure 4).

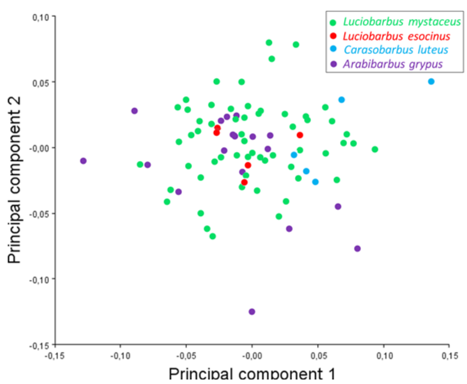

Figure 4. Principal component analysis (PCA) scatter plot.

In the Canonical Variance Analysis (CVA) for species, the first two canonical variances explained 87.5% of the total variance (Figure 4). According to Mahalanobis's length (Mah), the difference between *Luciobarbus esocinus* and *Carasobarbus luteus* is insufficient, but differences in other comparisons are significant (Table 2). According to Procrustes (Proc) distance, *Luciobarbus esocinus* - *Carasobarbus luteus* and *Luciobarbus esocinus* – *Luciobarbus mystaceus*, the difference between the two is not sufficient. Still, the difference for other comparisons is important (Table 2). We did not find any difference between the gender of all species according to Mahalonobis Mahalanobis and Procrustes' distance.

Table 2. CVA results.

Groups	***Luciobarbus esocinus***		***Barbus mystaceus***	
	Mah. D	Proc. D	Mah. D	Proc. D
Barbus mytaceus	1,9405***	0,0394ns		
Carasobarbus luteus	2,6918ns	0,0818***	2,3769**	0,0773**
Arabibarbus grypus	3,0002**	0,0526 ns	2,1918*	0,0497*

Mah. D.: Mahalanobis length, Proc. D.: Procrustes length, *: Permutation p value, *p<0.0001, **p<0.01, ***p<0.05, ns: not significant.

In Discriminant Function Analysis (DFA), the difference according to the parametric (Parm.) and Permutations (Perm.) P values for all species are given in Table 3.

Table 3. DFA results.

Groups	***Luciobarbus esocinus***		***Barbus mystaceus***	
	T^2/ Parm. p	**Perm.p (Proc./ T^2)**	**T^2/ Parm. P**	**Perm.p (Proc./ T^2)**
Luciobarbus mystaceus	19,1381/***	ns/***		
Carasobarbus luteus	46,3131/ns	**/ns	30,2377/**	**/*
Arabibarbus grypus	40,4185/***	ns/***	75,3029/*	*/*

T^2: T-square, Parm. p: Parametric p values, Perm. p: Permutation p value, *p<0.0001, **p<0.01, ***p<0.05, ns: not significant.

The difference according to the parametric (Parm.) and Permutations (Perm.) P values for *Luciobarbus esocinus-Luciobarbus mystaceus and Luciobarbus esocinus-Carasobarbus luteus; Luciobarbus esocinus-Arabibarbus grypus* are significant, whereas, those are insignificant for *Luciobarbus esocinus-Carasobarbus luteus* (Table 3 and Figure 5). The difference of parametric (Parm.) and Permutations (Perm.) P values showed no signification between genders for all species.

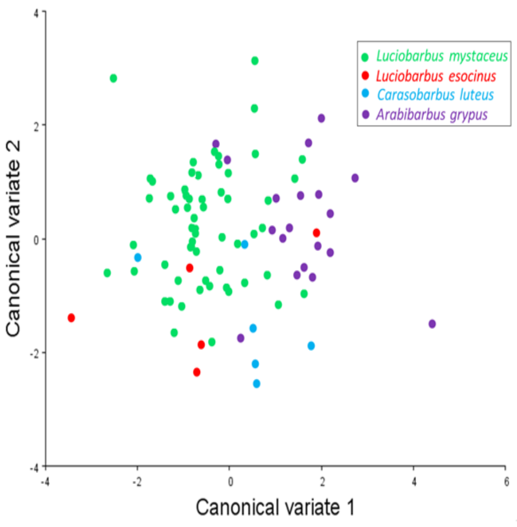

Figure 5. Scatter plot of CVA for species

The difference of shape given by DF analysis of *Arabibarbus grypus*, *Carasobarbus luteus*, *Luciobarbus esocinus* and *Luciobarbus mystaceus* were given in Figure 6.

In the difference of shape given by DF analysis, in comparison of *Carasobarbus luteus* and *Arabibarbus grypus*; *Carasobarbus luteus* is wider in ventral and dorsal and narrower in anterio-ventral and posterio- dorsal. In comparison of *Luciobarbus esocinus* and *Carasobarbus luteus; Luciobarbus esocinus* is narrower in ventral and dorsal and a larger scale structure in anterio-ventral. In comparison of *Luciobarbus esocinus* and *Arabibarbus grypus; Luciobarbus esocinus* is wider in anterior and anterio-dosal, narrower in posterio-dorsal. In comparison of *Luciobarbus mystaceus* and *Carasobarbus luteus; Luciobarbus mystaceus* is wider in anterior and posterior, narrower scale structure in dorsal and ventral.

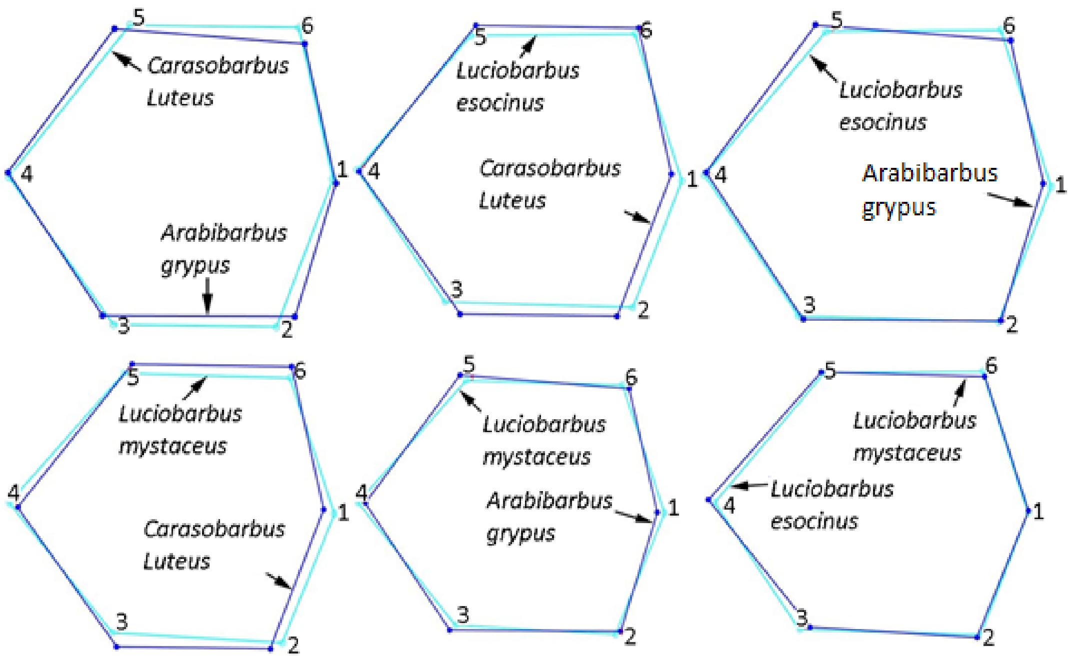

Figure 6. The shape differences between species scale.

Discussion

Scales are a useful taxonomic character used in fish classification. In addition, it is an important tool in revealing the growth, reproduction, and feeding characteristics of fish population dynamics, determining the diet of aquatic predators, or in paleontological analysis (Gupta, 2017). Although the scales used in this study were sampled from the same anatomical region (shoulder area) of the fish, there are some differences in this region in each fish. Also, there is a significant variation throughout the body. A similar situation has been noted in other fish species. In their study on mugilids, Ibanez et al (2007) determined intra-species variations in scales taken from the same region of the fish.

The results show that in the present study, significant differences were found between species in both size (CS) and shape, but the difference between the genders was not found significant in all the species under study. The variation formation seen in elasmoid fish scales provides important information in terms of swimming mode as well as a taxonomic character (Ibanez et al., 2009).

These results show over again that GMMs is so strong tool for analyses size and shape separately to determine the differences and similarity (Bookstein, 1999; Klingenberg, 2011; Zelditsch et al., 2004). As mentioned before fish scales are cut out for 2D geometric morphometric analysis to identify fish in a wide variety of studies (Ibáñez et al. 2007, 2009 and 2012; Poulet et al. 2005; Staszyn et al. 2012).

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. However, the researchers should be aware that the scales may vary depending on age, gender, and seasons.

References

Avigliano E, Domanico, A Sánchez, S Volpedo AV, 2017: Otolith elemental fingerprint and scale and otolith morphometry in *Prochilodus lineatus* provide identification of natal nurseries. *Fish. Res.* 2017, 186 (1), 1-10.

Beckman WC, 1962: The Freshwater Fishes of Syria and their General Biology and Management, First Edition. *FAO Fish. Bio. Tec.*, Roma, Italy.

Bilici S, Ünlü E, Cicek T, Satici Ö, 2016: The reproductive biology of *Carasobarbus luteus* and *Capoeta trutta* in the Tigris River, Turkey. *Cybium*, 40(2), 147-153.

Bookstein FL, 1999: Linear methods for nonlinear maps: Procrustes fits, thin-plate splines, and the biometric analysis of shape variability. In: Brain Warping (AW. Toga, Ed.), pp. 157–181. Academic Press, San Diego.

Cicek T, Kaya A, Bilici S, Ünlü E, 2016: Size and shape analysis of two close Cyprinidae species (*Garra variabilis-Garra rufa*) by geometric morphometric methods. *Survey in Fish Res*, 2(2), 35-44.

Coad BW, 1996: Zoogeography of the fishes of the Tigris-Euphrates basin. *Zool. Middle East*, 13, 71–83.

Çiçek E, Sungur S, Fricke R, 2020: Freshwater lampreys and fishes of Turkey; a revised and updated annotated checklist 2020. *Zootaxa*, 4809 (2), 241-270.

Coad BW, 1996: Zoogeography of the fishes of the Tigris-Euphrates basin. *Zool. Middle East*, 13, 71–83.

De Lamater ED, Courtanay WR, 1973: Studies on scale structure of flatfishes. I. The genus Trinectes, with notes on related forms. Proceedings of the 27th Annual conference of the Southeast Association. *Game and fish communication*, pp. 592-608.

Economidis PS, 1989: Distribution pattern of the genus Barbus (Pisces, Cyprinidae) in the freshwaters of Greece. Extrait des “Travaux du Museum d’Historie naturelle Grigore Antipa, 30, 223-229.

Esmaeili HR, Gholami Z, 2011: Scanning Electron Microscopy of the scale morphology in Cyprinid fish, *Rutilus frisii kutum* Kamenskii, 1901 (Actinopterygii: Cyprinidae). *Iranian Journal of Fish Res*, 2011. 10(1), 155-166.

Esmaeili HR, Hojat AT, Teymouri A, 2007: Scale structure of a cyprinid fish, *Capoeta damascina* (Valenciennes in Cuvier and Valenciennes, 1842) using scanning electron microscope (SEM). *Iran J Sci Technol Trans A Sci*, (31), 255-262.

Gupta, N, 2017: Use of fish scales as a tool for research-A Review. *G- Env Sci and Tech*, 4(6), 46-48.

Hammer Ø, Harper DAT, Ryan PD, 2001: PAST-palaeontological statistics, ver. 1.89. Palaeontol Electron, 4(1), 1-9. Ibañez AL, Cowx, IG, O'Higgins P, 2007: Geometric morphometric analysis of fish scales for identifying genera, species, and local populations within the Mugilidae. *Can J Fish Aquat*, 64(8), 1091-1100.

Ibanez, A.L., Cowx, I.G. and O’Higgins, P., 2007: Geometric morphometric analysis of fish scales for identifying genera, species, and local populations within the Mugilidae. *Can J Fish Aquat*, 64, 1091–1100.

Ibáñez AL, Cowx IG, O'Higgins P, 2009: Variation in elasmoid fish scale patterns is informative with regard to taxon and swimming mode. *Zool Linn Soc*, 155(4), 834-844.

Ibáñez AL, Pacheco-Almanzar E, Cowx IG 2012: Does compensatory growth modify fish scale shape? *Environmental Biology of Fishes*, 94(2), 477-482.

Ibáñez AL, Jawad LA, Sadighzadeh Z, 2016: Morphometric variation of fish scales among some species of the family Lutjanidae from Iranian waters. *Cah. Biol. Mar*, 57, 289-295.

Jawad L, Al Jufaili SM, 2007: Scale morphology of greater lizardfish *Saurida tumbil* (Bloch, 1795)(Pisces: Synodontidae). *J. Fish Biol*, 70(4), 1185-1212.

Jawad LA, 2005. Comparative morphology of scales of four teleost fishes from Sudan and Yemen. *J. Nat. Hist.*, 39(28), 2643-2660.

Karaman M, 1971: Süβwasserfische der Türkei. 8.Teil. Revision der Barben Europas, Vorderasiens und Nordafrikas. Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut, 67, 175–254.

Klingenberg CP, 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.*, 11(2), 353-357.

Kuru M, Yerli S, Mangıt F, Ünlü E, Alp A, 2014: Fish biodiversity in inland waters of Turkey. *Acad Doc for Fish Aquacul*, 1(3), 93-120.

Kuru M, 1979: The fresh water fish of South-Eastern Turkey-2 (Euphrates-Tigris Sisteme). *Hac Bull Nat Sci Eng*, 7–8, 105–114.

Miranda R, Escala M, 2000: Morphological and biometric comparison of the scales of the barbels (Barbus Cuvier) of Spain.*J Morphol*, 245 (3), 196-205.

Nelson JS, 2006: Fishes of the world. Fourth Ed. John Wiley and Sons. Inc., Hoboken, New Jersey. 601 p.

Oosten JV, 1957: The skin and scales. *The Physiology of Fishes*, Vol. 1, 207-244.

Poulet N, Reyjol Y, Collier, H, Lek S, 2005: Does fish scale morphology allow the identification of populations at a local scale? A case study for rostrum dace Leuciscus leuciscus burdigalensis in River Viaur (SW France). *Aquat Sci*, 67 (1), 122-127.

Richards, R.A., and Esteves, C. 1997. Use of scale morphology for discriminating wild stocks of Atlantic striped bass. *Trans Am Fish Soc*, 126: 919–925.

Rohlf FJ, 2016: tps Dig, version 2.26. See http://life. bio. sunysb. edu/morph/soft-dataacq. html. Structural and morphometric study of scales in *Petzea rudd*

(Scardinuz racovitzai MÜLLER 1958). *Applied Eco. and Env. Res.*16(5), 6063-6076.

Staszny A, Ferincz A, Weiperth A, Havas E, Urbanyi B, Paulovits G, 2012: Scalemorphometry study to discriminate gibel carp (*Carassius gibelio*) populations in the balaton-catchment (Hungary). *Acta Zool Acad Sci Hung*, 58, 19–27.

Şerban C, Grigoraş G, 2018: Structural and morphometric study of scales in *Petzea rudd* (Scardinuz racovitzai MÜLLER 1958). *Appl Ecol and Eviron Res*, 16 (5), 6063-6076.

Teimori, A, 2016. "Scanning electron microscopy of scale and body morphology as taxonomic characteristics of two closely related cyprinid species of genus Capoeta Valenciennes, 1842 in southern Iran. *Current Science*, (00113891) 111.7.

Tsigenopoulos CS, Berrebi P, 2000: Molecular Phylogeny of North Mediterranean Freshwater Barbus (Genus Barbus: Cyprinidae) Inferred from Cytochrome b Sequences: Biogeographic and Systematic Implications. *Molecular Phylo and Evol*, 14, 165–179.

The skin and scales. *The Physiology of Fishes*, Vol. 1, 207-244.

Zelditsch ML, Swiderski DL, Sheets HD, Fink WL, 2004: Geometric morphometrics for biologists: A primer. San-Diego etc.: *Elsevier Academic Press*,. 443 p.

***Correspondence:** Serbest BİLİCİ
Istanbul Aydin University Bil College Diyarbakır, Kayapınar-Diyarbakır, Turkey.
e-mail: serbestbilici@hotmail.com