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Variation in the shape and size of the scale of the Tigris bream (*Acanthobrama marmid*, Heckel, 1843) from the Tigris River, Türkiye attributed to Seasonality, Age and Sex: A geometric morphometric study

Variación Estacional, por Sexo y Edad de las características de las escamas de la brema del Tigris (*Acanthobrama marmid*, Heckel, 1843) del rio Tigris, Turquia: un estudio morfométrico geométrico

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ABSTRACT

In this study, the Tigris bream *Acanthobrama marmid* individuals (44 females and 31 males) were captured from the Tigris River. The scale size (as centroid size) and shape were analyzed separately using 2–dimensional geometric morphometric methods. Procrustes ANOVA revealed significant differences in scales size between sexes, while no difference in shape was observed. Groups based on season and age showed significant differences in both size and shape. Female individuals had larger scale sizes than males, with the scales of the Autumn group being larger than those of the Spring and Summer groups. Scale size also increased with age groups. PCA analysis showed variation in the first five components when examined by age, season, and gender. CVA and DFA results indicated significant differences in shape between different age groups and seasonal groups, but no significant differences between sexes were observed.

Key words: Leuciscidae; geometric; landmark; morphometric; scale; shape; Türkiye

RESUMEN

En este estudio, se capturaron individuos de la brema del Tigris *Acanthobrama marmid* (44 hembras y 31 machos) del río Tigris. El tamaño y la forma de las escamas se analizaron por separado utilizando métodos morfométricos geométricos bidimensionales. El análisis de la ANOVA de Procrustes reveló diferencias significativas en el tamaño de las escamas entre los géneros, mientras que no se observaron diferencias en la forma. Los grupos basados en la temporada y la edad mostraron diferencias significativas tanto en tamaño como en forma. Los individuos hembra tenían tamaños de escamas más grandes que los machos, siendo las escamas del grupo de otoño más grandes que las de los grupos de primavera y verano. El tamaño de las escamas también aumentó con los grupos de edad. El análisis de PCA mostró variación en los primeros cinco componentes al examinar por edad, temporada y género. Los resultados de CVA y DFA indicaron diferencias significativas en forma entre diferentes grupos de edad y grupos estacionales, pero no se observaron diferencias significativas entre géneros.

Palabras clave: Leuciscidae; geométrico; punto de referencia; morfométrico; escama; Turquía

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INTRODUCTION

Acanthobrama marmid Heckel, 1843 is a member of the Leuciscidae family and is found in the Tigris–Euphrates River system, Kuveyk and Asi Rivers, and likely in Amik Lake and Bardan Stream near Tarsus [1, 2, 3]. According to Küçük *et al*. [4], *Acanthobrama marmid* is only distributed in the Tigris–Euphrates system, while the populations in Asi, Seyhan, and Berdan River (Tarsus) are identified as *Achantobrama orontis*.

This species is characterized by its compressed body structure and humped back structure on the back of the head, which is especially evident in large individuals. It does not have whiskers and has small scales. A fleshy keel is located between the base of the pelvic fins and the ventral fin. Additionally, it possesses a thick, spine–like, and smooth terminal unbranched dorsal fin ray, as well as a long anal fin (15–22 branches) [2, 3]. Its fins are orange–red in color (FIG. 1). It is a benthopelagic species, typically found in shallow, slow–moving waters with sandy or muddy bottoms. *Acanthobrama marmid* plays a vital role in the ecosystem as a prey species for larger predatory fish and an important component of the food web. In rural areas, the local population consumes it [5].

Scales are structures embedded in the epidermal layer of the fish's body, which are also used in species identification. Ctenoid and cycloid scales are particularly used as identification tools in systematic studies. Compared to molecular techniques, scales are cost–effective, non–destructive, convenient to use, and can serve as suitable bony structures for species identification due to their resistance to digestion by predators' digestive systems [6, 7]. Scale morphology is used in taxonomy and classification studies and has been evaluated for ontogenetic analyses [8] and morphology [9, 10, 11, 12]. The morphological and morphometric characteristics of scales are one of the methods used in the identification and differentiation of fish species and populations [13, 14, 15, 16, 17]. Biological characteristics and age determination studies of *Acanthobrama marmid* are available [18, 19, 20, 21, 22, 23].

The study utilized geometric morphometric methods to ascertain the distinctive structure of scales attributed to the species and to discern variations among season, age, and male and female individuals.

FIGURE 1. The overall body appearance of *Acanthobrama marmid* (Tigris bream), Dicle River (Photo by E. Ünlü)

MATERIAL AND METHODS

In this study, we collected a total of 75 specimens of *Acanthobrama marmid*, including 44 females and 31 males, from the Tigris River. The localities where the samples were collected are shown in FIG. 2, and the seasonal, sexual, and age distributions of the samples, as well as some water parameters of the locality where they were collected, are given in TABLE I.

The sex of each fish was determined by observing their gonads. Scales from the front and upper sections of the lateral lines of the dorsal fins were taken to determine their age and morphology. The fish scales tissue was cleaned with 5% NaOH for 2 hours, then washed with distilled water, and immersed in 96% ethanol for several minutes to remove any remaining water. Following this the scales were placed between two slides and photographed by an stereo microscope (Olympus SZX7, Tokyo, Japan) and a digital camera (OLYMPUS Camedia C–5060 5.1 MP w/4× Optical Zoom, Tokyo, Japan) under 20× and 40× magnifications. Images were analyzed

FIGURE 2. Map of the study area which samples obtained. Sample localities (1. Tigris River (Güçlükonak–1), 2. Tigris River (Güçlükonak–2), 3. Tigris River (Akdizgin), 4. Tigris River (Damlarca)

TABLE I
Samples distribution and water parameters of the study.

Season	Female	Male	Total
Autumn (November)	12	–	12
Spring (April)	22	16	38
Summer (July)	10	15	25

	Age					
	II	III	IV	V	VI	VII
Sample number	7	23	23	15	5	2

Date	Water temperature (°C)	pH	dissolved oxygen (O_2)	O_2 (%)	Electrical conductivity (EC) $\mu S \cdot cm^{-1}$
26/04/2021	17.7	8.3	9.11	101	306
01/07/2021	24.6	7.86	7.67	97.2	474
04/11/2021	13.5	8.2	8.62	96.8	365

by geometric morphometric procedure [24, 25, 26]. Subsequently, six landmarks (FIG. 3) were digitized using tpsDig ver. 2.32 [27] software, and Procrustes analysis was conducted. Following the separation of shape and size (centroid size=CS) of the samples, Procrustes ANOVA, PCA, CVA/MANOVA, and DFA analyses were performed using Morpho J1.06d [28], R Core Team [29] and Jamovi Ver. 2.4 [30] programs.

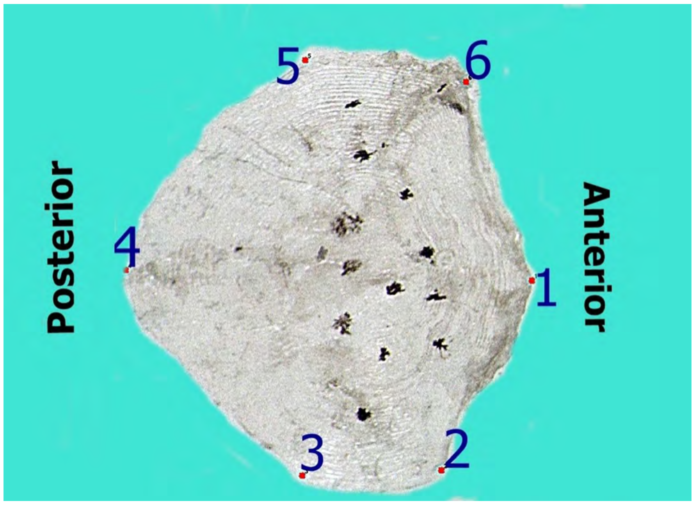

FIGURE 3. Landmark definitions used in the fish scales

RESULTS AND DISCUSSION

When the results of Procrustes ANOVA are examined, a significant difference in size (CS) between sex is found (*P*=0.0051), while no difference in shape is observed. Groups based on season and age are significant in both size and shape (*P*<0001) (TABLE II).

TABLE II
Procrustes ANOVA results (F: Goodal's F, CS: Centroid Size)

		F	*P*-value	Pillai tr.	*P*-value
Sex	CS	8.33	**0.0051**		
	Shape	0.91	0.5110	0.13	0.2719
Season	CS	13.97	**< 0.0001**		
	Shape	4.51	**< 0.0001**	0.51	**0.0006**
Age	CS	11.12	**< 0.0001**		
	Shape	2.44	**< 0.0001**	0.83	**0.0101**

In female individuals, scale size is larger than in males, and the scales of the Autumn group are larger than those of the Spring and Summer groups. Scale size increases with age groups (FIGS. 4, 5, 6).

In PCA analysis, when examined by age, PC1 accounts for 29.5%, PC2 for 22.7%, and the first five components explain 85.4% of the total variation. When examined by season, PC1 accounts for 32.9%, PC2 for 19.9%, and the first five components explain 85.3% of the total variation. When examined by sex, PC1 accounts for 36%, PC2 for 20.5%, and the first five components explain 86.2% of the total variation (FIGS. 7, 8, 9).

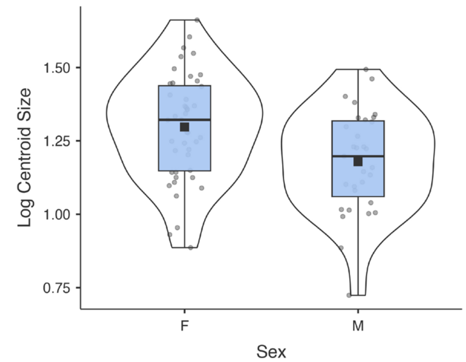

FIGURE 4. Box and Violin plot of CS of scales by sex F: female, M: male

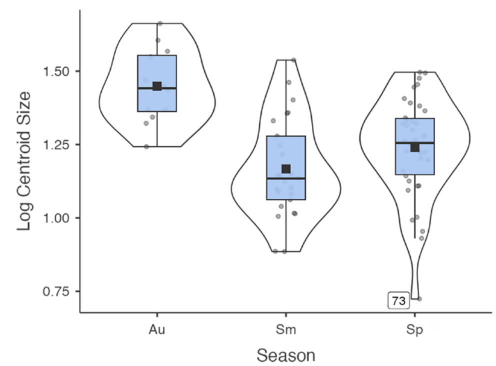

FIGURE 5. Box and Violin plot of CS of scales by season Au: autumn, Sm: summer, Sp: spring

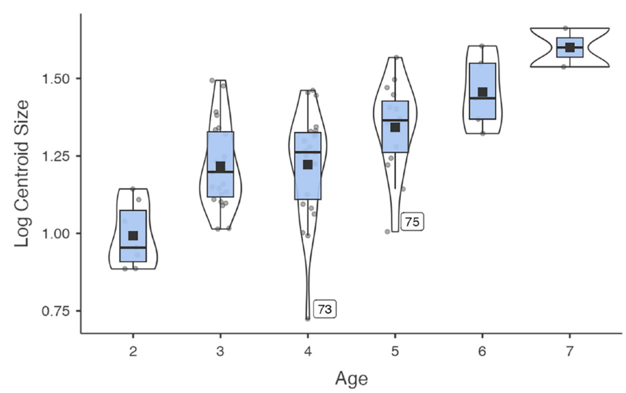

FIGURE 6. Box and Violin plot of CS of scales by age. Numbers represent ages

When looking at the CVA results, the 6-year age group differs significantly from all other groups except the 7-year-old group, while there is no significant difference between the 3-4 and 4-5 age groups and among other groups (TABLE III; FIG. 10). When examining the seasonal groups, there is no significant difference between the Summer and Spring groups, while the difference between Autumn-Summer and Autumn-Spring is significant (TABLE IV; FIG. 11). There is no significant difference between sex (F-M) (TABLE V; FIG.12).

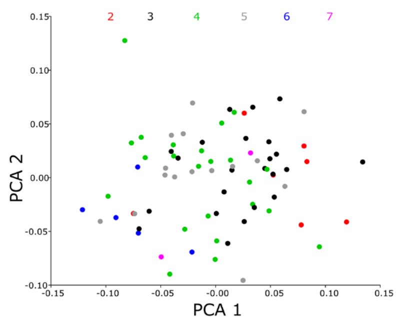

FIGURE 7. Scatter plot of principal component analysis (PCA) showing the distribution of scales by age. Number represent ages

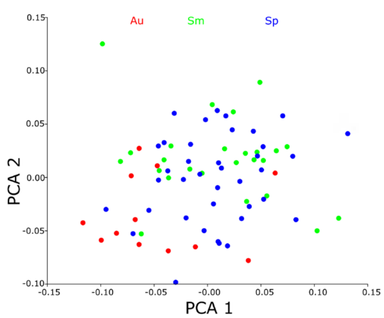

FIGURE 8. Scatter plot of principal component analysis (PCA) showing the distribution of scales by season. Au: autumn, Sm: summer, Sp: spring

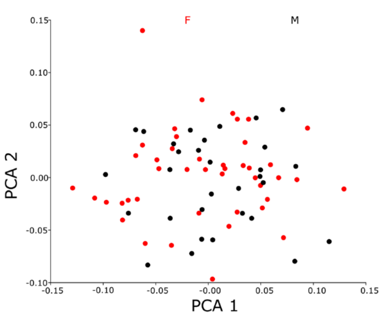

FIGURE 9. Scatter plot of principal component analysis (PCA) showing the distribution of scales by sex. F: female, M: male

TABLE III
CVA result of scales by age

	Age									
	2		3		4		5		6	
	M.Dist / *P*-value	**P.Dist / *P*-value**	**M.Dist / *P*-value**	**P.Dist / *P*-value**	**M.Dist / *P*-value**	**P.Dist / *P*-value**	**M.Dist / *P*-value**	**P.Dist / *P*-value**	**M.Dist / P-value**	**P.Dist/ P-value**
3	1,5243 / **0,0497**	0,0471 / 0,1212								
4	1,8217 / **0,0344**	0,0728 / **0,0133**	0,9992 / 0,1436	0,0381 / 0,0511						
5	2,1384 / **0,0028**	0,0752 / **0,0138**	1,3902 / **0,0084**	0,0408 / 0,0633	1,0575 / 0,3308	0,0213 / 0,8375				
6	3,2478 / **0,0056**	0,1391 / **0,0080**	2,8103 / **0,0001**	0,1132 / **0,0001**	2,1309 / **0,0204**	0,0813 / **0,0067**	2,1597 / **0,0358**	0,0810 / **0,0125**		
7	2,5964 / 0,3006	0,0818 / 0,3589	2,5553 / 0,1412	0,0687 / 0,3034	2,1714 / 0,5779	0,0526 / 0,8124	1,9358 / 0,7225	0,0559 / 0,7136	2,7091 / 0,5497	0,0866 / 0,3733

M.Dist: Mahalanobis distance, P.Dist: Procrustes distance, *P*–value: value of permutation test

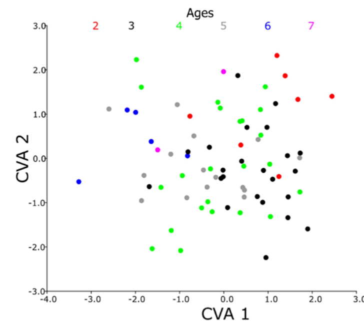

FIGURE 10. CVA plot of scales by age. Number represent ages

TABLE IV
CVA result of scales by season

	Autumn		**Summer**	
	M.Dist / *P*–value	**P.Dist / *P*–value**	**M.Dist / *P*–value**	**P.Dist / *P*–value**
Summer	2.4640 / < **0.0001**	0.0877 / **0.0001**		
Spring	2.2869 / < **0.0001**	0.0768 / **0.0001**	0.6419 / 0.6112	0.0213 / 0.4966

M.Dist: Mahalanobis distance, P.Dist: Procrustes distance, *P*–value: value of permutation test

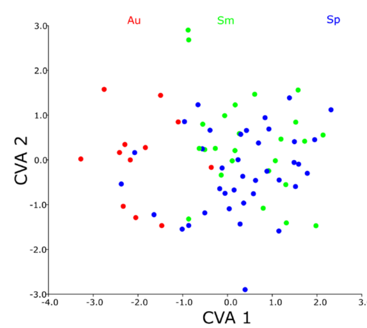

FIGURE 11. CVA plot of scales by Season. Au: autumn, Sm: summer, Sp: spring

TABLE V
CVA Result of scales by gender

	Female	
	M.Dist / *P*–value	**P.Dist / *P*–value**
Male	0,7875 / 0,1833	0,0209 / 0,4603

M.Dist: Mahalanobis distance, P.Dist: Procrustes distance, *P*–value: value of permutation test

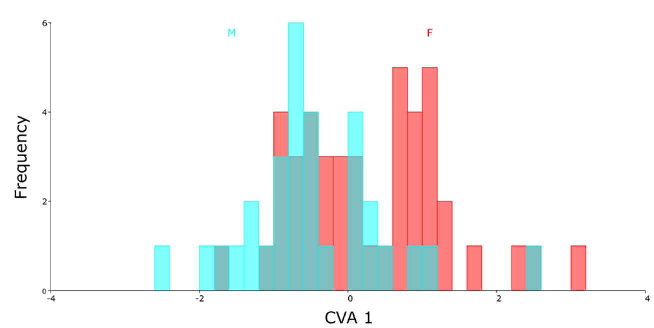

FIGURE 12. CVA plot of scales by sex. F: female, M: male

Upon reviewing the DFA results, significant differences were found between age groups 2–4, 5–6, 3–4, 5–6, and 4–6, while sufficient differences were not observed among other age groups (TABLE VI; FIG. 13). Significant differences were found between the seasonal groups Autumn–Summer and Spring, but there was not enough difference observed between Summer and Spring (TABLE VII; FIG. 14). No significant differences were observed between sexes (TABLE VIII; FIG. 15).

TABLE VI
DFA results f of scales by age

		Age				
		2	**3**	**4**	**5**	**6**
3	T^2	19,4271				
	Param. P	0,1293				
	Perm. P (Proc./T^2)	0,1200 / 0,1300				
4	T^2	18,6414	14,1287			
	Param. P	0,1456	0,1960			
	Perm. P (Proc./T^2)	**0,0140** / 0,1500	**0,0600** / 0,1820			
5	T^2	46,2855	26,5816	9,7766		
	Param. P	**0,0170**	**0,0246**	0,4679		
	Perm. P (Proc./T^2)	**0,0140** / **0,0170**	0,0650 / **0,0210**	0,8200 / 0,4600		
6	T^2	101,0029	43,3982	28,1028	16,1055	
	Param. P	0,1505	0,0065	0,0438	0,3656	
	Perm. P (Proc./T^2)	**0,0070** / 0,1550	< **0.0001** / **0,0060**	**0,0090** / **0,0440**	**0,0140** / 0,3740	
7	T^2	37,6469	20,6773	9,6780	9,0769	8,4726
	Param. P	0,7085	0,1513	0,5808	0,7534	0,8535
	Perm. P (Proc./T^2)	0,3530 / 0,6840	0,3060 / 0,1610	0,8400 / 0,6250	0,7250 / 0,7140	0,3660 / 0,4090

T^2: T–square, Param. P: Parametric *P*–values, Perm. P: Permutation *P*–value, Bolded: significant

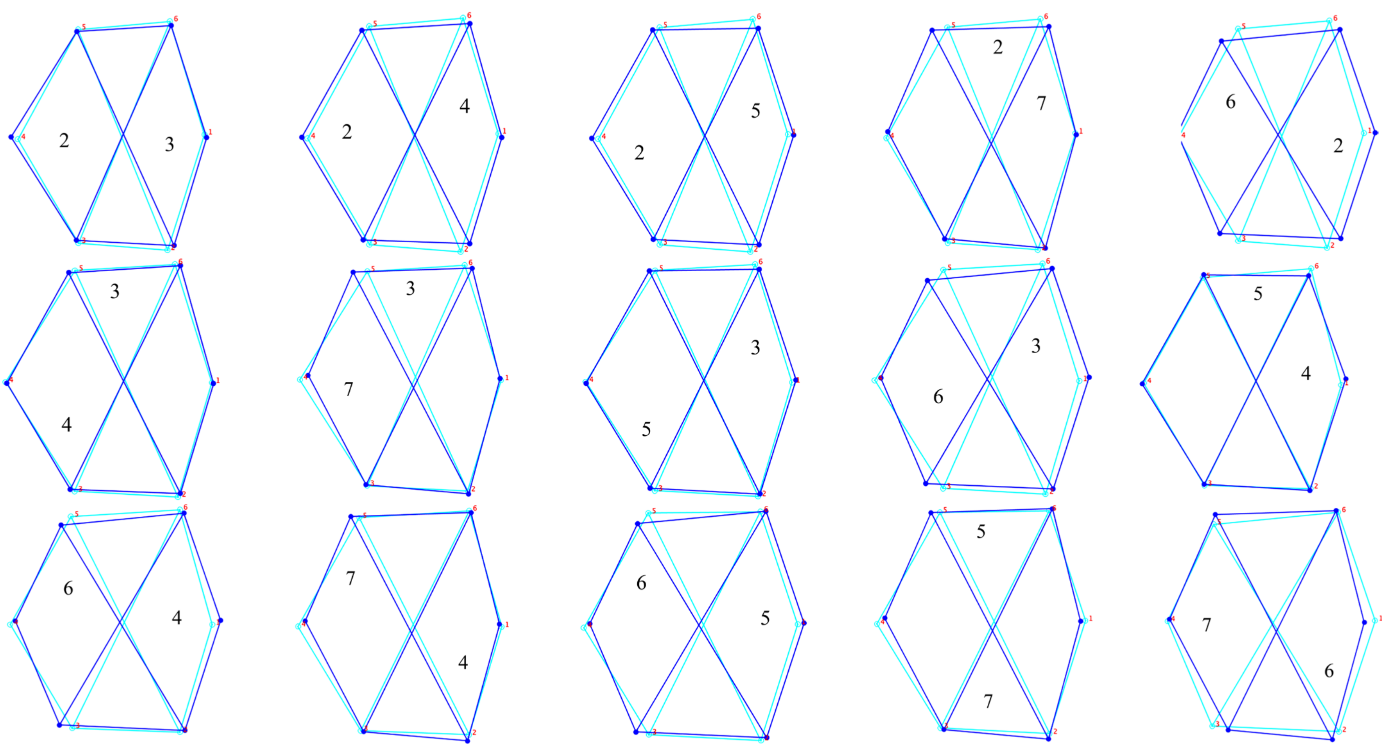

FIGURE 13. Shape differences of scales by age. Numbers represent ages

TABLE VII
DFA result of scales by season

		Autumn	**Summer**
	T2	72,5635	
Summer	Param. P	< **0.0001**	
	Perm. P (Proc./T2)	< **0.0001** / < **0.0001**	
	T^2	50,9476	6,2569
Spring	Param. P	**0,0001**	0,6965
	Perm. P (Proc./T^2)	< **0.0001** / < **0.0001**	0,5020 / 0,6890

T^2: T–square, Param. P: Parametric *P*–values, Perm. P: Permutation *P*–value, Bolded: significant

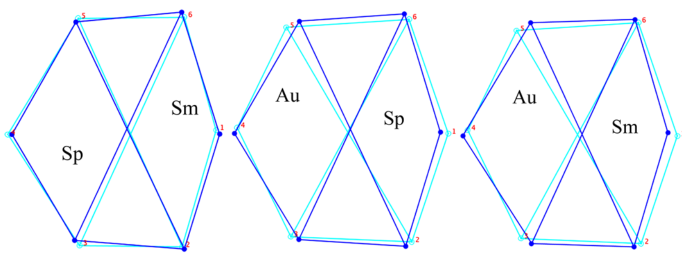

FIGURE 14. Shape differences of scales by season. Au: autumn, Sp: Spring, Sm: summer

TABLE VIII
DFA result of scales by sex

		Female
	T^2	11,2785
Male	Param. p	0,2719
	Perm. P (Proc./T^2)	0,4550 / 0,2470

T^2: T–square, Param. P: Parametric *P*–values, Perm. P: Permutation *P*–value

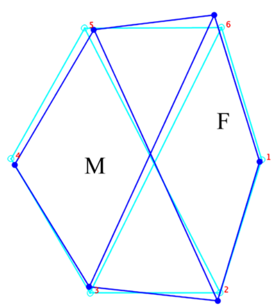

FIGURE 15. Shape differences of scales by sex F: female, M: male

Fish scales contain small growth rings that allow us to determine the age of the fish. These growth rings are typically arranged around a center and are composed of $CaCO_3$ compounds [31, 32]. Variations in these rings occur because fish scales generally grow excessively when feeding is abundant, typically during spring and summer, and slow down or stop altogether when feeding is inadequate, especially during winter [33]. As the structure of annual growth rings in fish scales is influenced by environmental conditions, this type of differentiation can be significant based on the physicochemical parameters of the environment and feeding [34]. In this sense, changes in the shape of fish scales can allow for differentiation in populations [35, 36]. Additionally, inter/intraspecific morphological variability may indicate genetic differences among samples or can respond to environmental conditions within the framework of phenotypic plasticity [37, 38].

Geometric morphometrics is important in fish scales studies because it allows for the quantitative analysis of shape and size variation in a way that traditional morphometrics cannot achieve [38, 39]. This method provides a detailed and comprehensive understanding of the shape and size changes in fish scales, which can be used to address questions related to taxonomy, evolution, and ecology. Additionally, geometric morphometrics allows for the visualization and analysis of complex patterns of shape variation, making it a valuable tool for researchers studying fish scales [12, 40]. Çiçek *et al.* [41] applied geometric morphometric methods successfully on *Capoeta trutta* and *Capoeta umbla* species. In the present study, it was achieved on *Acanthobrama marmid* species at the same success. In the size analysis performed according to sex, it was seen that female samples were larger than males. These results show that fish species can be successfully distinguished by morphometric geometric analysis.

This type of analysis has been used successfully in previous studies. For example, studies on fish scale and otolith morphometry and geometry [13, 34, 41, 42, 43, 44, 45, 46] have yielded important results in this field. In addition, studies examining the relationship between fish size and otolith morphometry [47, 48] were also effective in determining the species.

CONCLUSIONS

Geometric morphometric analyses are highly accurate in species discrimination and detecting diversity, offering a significant advantage in future studies due to their ease, effectiveness, low cost, reliability, and simplicity. Fish scales are essential for species identification, making geometric morphometric analysis a vital tool in future biological research. Procrustes ANOVA showed a significant size difference between sexes but no difference in shape. Significant variations in both size and shape were also found among groups based on season and age. Additionally, PCA, CVA, and DFA analyses revealed distinct patterns in scale size and significant differences within age and seasonal groups, but not between sexes.

Conflict of interest

The authors have no declaration of competing interests.

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