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Morphological variability of otolith organs in three congeneric *Pagellus* species

by

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Abstract

Morphological features of sagittal otoliths in *Pagellus acarne*, *P. bogaraveo*, and *P. erythrinus* samples collected from the Aegean Sea were used to assess the variability both between *Pagellus* species and between otolith pairs employing scanning electron microscopy (SEM) and contour shape analysis. Twenty-one otolith morphological characters were compared in detail using SEM analysis. Twelve of these characters differ between *Pagellus* species and are suitable for distinguishing between the three congeneric *Pagellus* species. Wavelet functions of otolith contour shape were compared for six *Pagellus* groups. The wavelet functions obtained from the contour shape analysis showed high variability in the antero-dorsal, postero-dorsal, and antero-ventral regions of otoliths among the six groups analyzed. The results of principal component analysis (PCA) of the wavelet data provided a clear distinction between the groups under study. Cluster analysis supported the PCA results and basically divided them into two main branches. One of these branches contained only *P. erythrinus*, while *P. acarne* and *P. bogaraveo* were present in the other branch. This is the first study to provide detailed morphological characters and wavelet analysis of left- and right-side otoliths in these three congeneric *Pagellus* species from the Aegean Sea.

Keywords: Otolith organs, morphology, ear structures, interspecific variation, wavelet shape, *Pagellus*

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1. Introduction

Otoliths are one of the most useful bony structures that have found several practical applications in many studies (Özpiçak et al. 2021; Yedier & Bostanci 2022). These structures also provide information on the environmental, biological, and ecological behavior of fish, providing a permanent record of their life history, as well as enabling the identification and discrimination of populations/stocks and species (Tuset et al. 2008). The morphometry, morphology, shape, and chemical composition of otoliths are used in ecological research to identify prey, in fisheries science to distinguish stocks and species, in fisheries management to assess migration patterns, and to describe the morphofunctional adaptations of teleosts to various environmental conditions (Bostanci & Polat 2009; Bostanci et al. 2015; Tuset et al. 2018; Yedier & Bostanci 2021; De Souza Correa et al. 2022; Saygın et al. 2022). In addition, otolith morphology can play an important role in the identification of normal and abnormal (or aberrant) otoliths, providing researchers with information about life cycles of fish and their habitats (Yedier & Bostanci 2019; De Carvalho et al. 2019; Yedier & Bostanci 2020; Yedier 2022). Most of the above-mentioned studies emphasize that the determination of interspecific and intraspecific otolith shape, morphology, and morphometry is an important issue in terms of fish biodiversity and fisheries sustainability. Researchers have used various techniques such as shape factors and indices (Tuset et al. 2003; Ponton 2006), geometric morphometrics (Vergara-Solana et al. 2013), elliptic Fourier analysis (EFA) (Bird et al. 1986) and wavelet functions (WT) (Parisi-Baradad et al. 2010; Tuset et al. 2015) to describe the contour, shape, and morphology of otoliths. In recent years, the number of studies using wavelet functions (WT) has increased substantially, and many studies have been conducted on different fish species, such as *Trachurus picturatus* (Tuset et al. 2019), Lessepsian fishes (Tuset et al. 2020), *Sphyraena sphyraena* (Yedier 2021), *Psenopsis* spp. (Kumar et al. 2022), and *Lepidorhombus* spp. (Yedier et al. 2023a).

The Sparidae family is a ubiquitous fish taxon that includes more than 148 species in 37 genera worldwide (Nelson et al. 2016; Froese & Pauly 2023). *Pagellus*, a genus of the Sparidae family, has a wide geographical distribution in waters around the world, particularly in coastal ecosystems. The genus *Pagellus*, which has a high commercial value in the Turkish coastal waters of the Aegean Sea, is represented by three congeneric species: axillary seabream, *Pagellus acarne* (Risso, 1827), blackspot seabream, *Pagellus bogaraveo* (Brünnich, 1768), and common pandora, *Pagellus erythrinus* (Linneus, 1758). These three congeneric sparid fishes share a misleading morphological similarity, and their characters show some differences depending on the habitat, which may cause some problems in correctly distinguishing these species morphologically (Antonucci et al. 2009). Furthermore, although otoliths are widely used to study many commercial fish species, stocks, and populations, interspecific variations in otolith morphology of several congeneric fish species of the family Sparidae have not yet been fully elucidated. Therefore, the purpose of this study is to reveal morphological differences and similarities in sagittal otoliths according to both *Pagellus* species (*P. acarne, P. bogaraveo,* and *P. erythrinus*) and the longitudinal axis (left and right sides) of fish individuals. In this study, morphological differences and similarities in sagittal otoliths were determined by SEM analysis and wavelet analysis, contributing to the knowledge of the morphology of sagittal otoliths in congeneric *Pagellus* species distributed along the Turkish coasts of the Aegean Sea. This is the first study in which both the right and left otoliths of these three congeneric *Pagellus* species distributed along the Turkish coasts of the Aegean Sea are compared with 21 morphological characters and wavelet analyses.

2. Materials and methods

Samples of *Pagellus acarne*, *P. bogaraveo,* and *P. erythrinus* were collected from the Aegean Sea. The weight (to the nearest 0.01 g) and total length (to the nearest 0.01 cm) of fish samples from each species were recorded. Left- and right-side sagittal otoliths were extracted through an incision in the cranium to expose them, then cleaned and kept dry in storage containers. Otoliths on both sides were photographed using a stereomicroscope (S8APO; Leica) with a digital camera attachment (DFC 290; Leica) to determine their overall structure and morphology.

The otolith samples were attached to a stud using double-sided carbon tape. The immobilized otoliths were then coated with 13.5 nm thick gold, and imaging analysis was performed using scanning electron microscopy (SEM; Hitachi, SU 1510) at Ordu University Central Research Laboratory. The morphological features of the distal and proximal surfaces of sagittal otoliths were examined in detail using SEM. In this study, 21 morphological characters, such as otolith shape and width, anterior and posterior regions, distal and proximal surface, dorsal and ventral margins, sulcus acusticus type, opening and position, ostium shape and size, cauda shape and type, antirostrum type and size, rostrum type and size, notch, and excisura, were determined to describe the morphology of otoliths in *Pagellus* species (Fig. 1). The morphological features of the right and left otoliths in the fish specimens were defined using the terminology found in otolith atlases (Volpedo et al. 2017; Tuset et al. 2008).

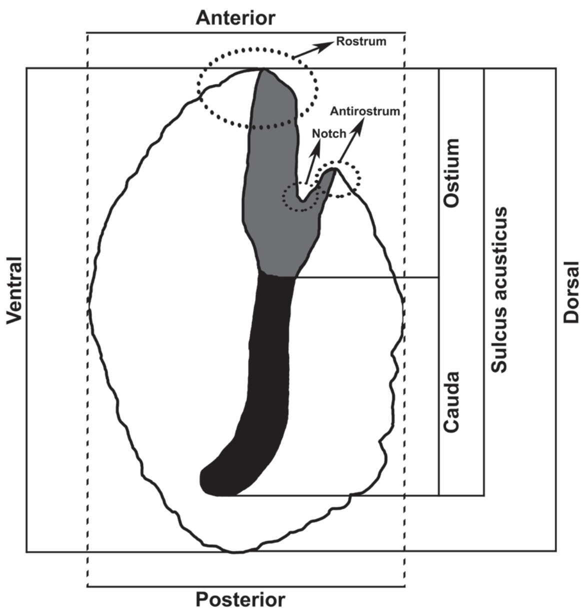

Figure 1

Mesial surface of the right otolith in *Pagellus* illustrating various morphological features and regions of the otolith.

For wavelet analysis, otolith images were converted from JPG to TIFF format using Adobe Photoshop CS6 (ver. 13.1). Otolith images in TIFF format were uploaded to the system according to the application instructions on the AFORO website (Lombarte et al. 2006; Parisi-Baradad et al. 2010). The shape of otoliths was analyzed and digitized based on wavelet (WT) functions, a mathematical descriptor of contour shape analysis. The digitalization of otolith shapes using contour analysis yielded nine different wavelets for each otolith and a total of 512 equidistant Cartesian coordinates in each wavelet (Parisi-Baradad et al. 2005). In addition, a graphical feature, wavelet variance, was used to find regions with higher variability that could show different patterns in otolith shapes for both the left and right sides and the *Pagellus* species. A cluster analysis based on Euclidean distance was conducted using Ward's method to determine whether this variability could group the *Pagellus* species. Of the nine WT signals, analyses were performed based on data from the 4th WT signal, which was observed to provide the best representation of the otolith characters among the WT signals (Fig. 2). Principal component analysis (PCA) based on the variance–covariance matrix was performed to reduce the dimensionality of the 512 data obtained in the 4th WT for each individual without loss of information. In addition, six groups were formed using average otolith contour shapes to visualize shape differences between the *Pagellus* species and their left and right otoliths (group 1: left-side otolith shape of *P. acarne*; group 2: right-side otolith shape of *P. acarne*; group 3: left-side otolith shape of *P. bogaraveo*, group 4: right-side otolith shape of *P. bogaraveo*; group 5: left-side otolith shape of *P. erythrinus*; and group 6: right-side otolith shape of *P. erythrinus*). The effect of otolith size was removed using the residuals of the common within-group slopes of the linear regressions of each component on the otolith length, and a new PCA matrix was generated. Minitab (Ver. 19) and Past (Ver. 4.04) were used in all statistical analyses in the study.

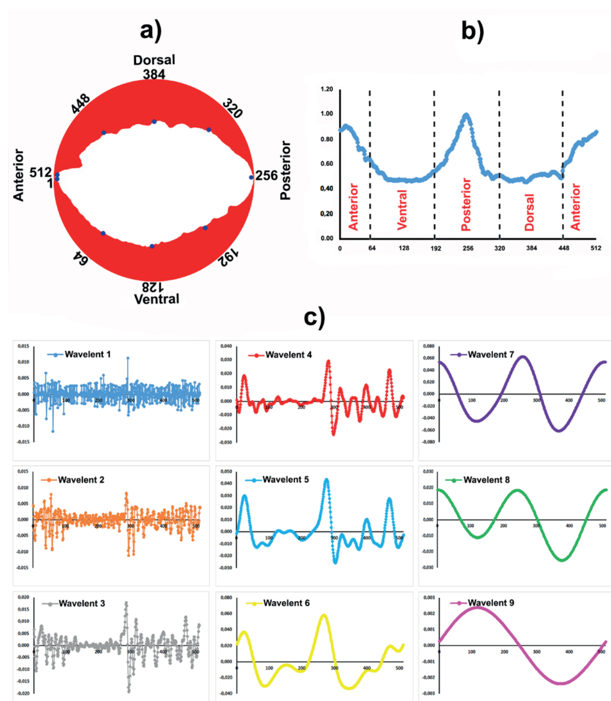

Figure 2

Wavelet signals and zones from the left and right otoliths in *Pagellus* species from the Aegean Sea. (a) otolith image with zones; (b) otolith contour wavelet functions with zones; (c) wavelets obtained in contour analysis.

3. Results

Mean body weight and total length values of individuals belonging to the *Pagellus* species are 13.91 ± 0.51 cm and 35.84 ± 5.35 g for *P. acarne*; 14.38 ± 0.26 cm and 39.79 ± 2.72 g for *P. bogaraveo*; and 13.44 ± 0.76 cm and 34.25 ± 5.18 g for *P. erythrinus*, respectively. Detailed evaluation of proximal and distal surfaces of the right and left otoliths in *Pagellus* species by SEM revealed that there was no significant morphological difference between the left and right otoliths in the *Pagellus* species in terms of the 21 morphological characters analyzed in the study (Table 1; Fig. 3). However, the study determined that morphological characters of otoliths showed some differences and similarities between the *Pagellus* species (Table 1; Fig. 3). Otolith characters such as otolith shape and width, anterior and posterior regions, dorsal and ventral margins, sulcus acusticus, cauda, antirostrum types, rostrum type and size, and notch differ among the *Pagellus* species and can be used for interspecific discrimination of *Pagellus* species (Table 1).

The left- and right-side otoliths of *P. acarne* are pentagonal to elliptic in shape and moderate in width (Table 1; Fig. 3). Dorsal margins of the left and right otoliths are sinuate to crenate, while ventral margins are crenate to irregular. Sulcus acusticus and cauda types are heterosulcoid and slightly curved in left and right otoliths, respectively. The excisura is wide with an acute notch. The antirostrum is absent or poorly defined with round or pointed shapes in otoliths on the left and right sides. The rostrum is short or long and narrow and well defined with a pointed shape in left- and right-side otoliths. The anterior region is angled to peaked and the posterior region is angled to oblique in left- and right-side otoliths in *P. acarne* (Table 1; Fig. 3).

The right- and left-side otoliths in *P. bogaraveo* are pentagonal to elliptic in shape and moderate in width

Table 1

Morphological comparisons between otoliths in *Pagellus* species (*P. acarne*, *P. bogaraveo*, and *P. erythrinus*) from the Aegean Sea and between left- and right-side otoliths in the same *Pagellus* species.

Morphological Characters	*Pagellus acarne*		*Pagellus bogaraveo*		*Pagellus erythrinus*	
	left side	right side	left side	right side	left side	right side
Otolith shape	pentagonal to elliptic	pentagonal to elliptic	pentagonal to elliptic	pentagonal to elliptic	pentagonal to oval	pentagonal to oval
Otolith width	moderate	moderate	moderate	moderate	broad	broad
Anterior region	angled to peaked	angled to peaked	angled to peaked	angled to peaked	angled to oblique	angled to oblique
Posterior region	angled to oblique	angled to oblique	oblique	oblique	oblique	oblique
Distal surface	concave	concave	concave	concave	concave	concave
Proximal surface	convex	convex	convex	convex	convex	convex
Dorsal margin	sinuate to crenate	sinuate to crenate	crenate to irregular	crenate to irregular	crenate	crenate
Ventral margin	crenate to irregular	crenate to irregular	crenate to irregular	crenate to irregular	sinuate	sinuate
Sulcus acusticus type	heterosulcoid, deep	heterosulcoid, deep	heterosulcoid, deep	heterosulcoid, deep	heterosulcoid, moderately deep	heterosulcoid, moderately deep
Sulcus acusticus opening	ostial	ostial	ostial	ostial	ostial	ostial
Sulcus acusticus position	median	median	median	median	median	median
Ostium shape	funnel-like	funnel-like	funnel-like	funnel-like	funnel-like	funnel-like
Ostium size	shorter than cauda	shorter than cauda	shorter than cauda	shorter than cauda	shorter than cauda	shorter than cauda
Cauda shape	tubular	tubular	tubular	tubular	tubular	tubular
Cauda type	slightly curved	slightly curved	slightly/strongly curved	slightly/strongly curved	strongly curved	strongly curved
Antirostrum type	round/pointed	round/pointed	round/pointed	round/pointed	round	round
Antirostrum size	small/absent	small/absent	small/absent	small/absent	small/absent	small/absent
Rostrum type	pointed	pointed	round/pointed	round/pointed	pointed	pointed
Rostrum size	long-narrow	long-narrow	short/long-broad	short/long-broad	short-broad	short-broad
Notch	acute	acute	acute	acute	shallow/absent	shallow/absent
Excisura	wide	wide	wide	wide	wide	wide

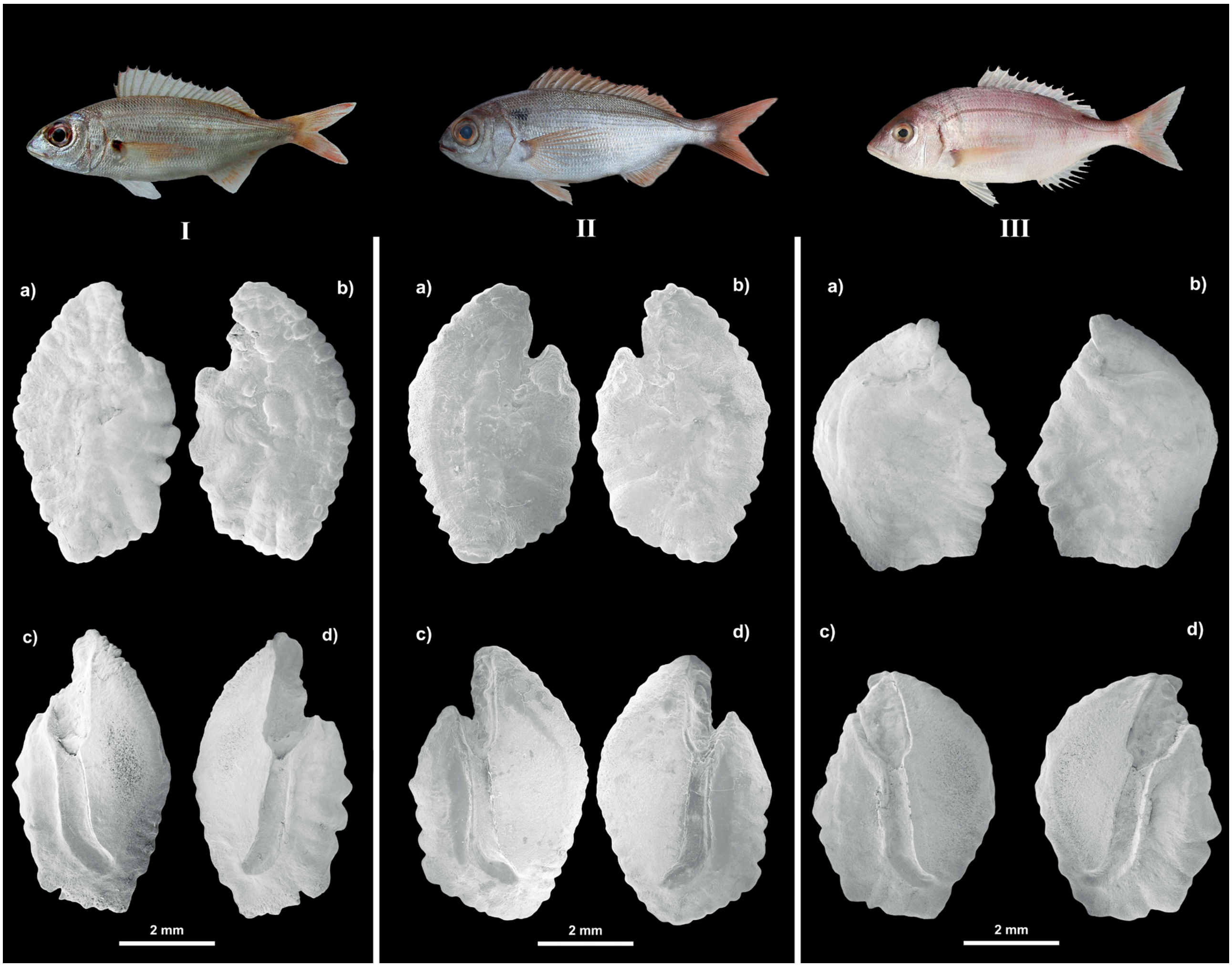

Figure 3

SEM images of the distal and proximal surfaces of left- and right-side otoliths in the three congeneric species. I – *Pagellus acarne*: distal surfaces of left- (a) and right-side (b) otoliths; proximal surfaces of left- (c) and right-side (d) otoliths; II – *Pagellus bogaraveo*: distal surfaces of left- (a) and right-side (b) otoliths; proximal surfaces of left- (c) and right-side (d) otoliths; III – *Pagellus erythrinus*: distal surfaces of left- (a) and right-side (b) otoliths; proximal surfaces of left- (c) and right-side (d) otoliths.

(Table 1; Fig. 3). Ventral and dorsal margins of the left and right otoliths are crenate to irregular. Sulcus acusticus and cauda types are heterosulcoid and slightly or strongly curved in the left and right otoliths, respectively. The excisura is wide with an acute notch. The antirostrum is absent or poorly defined with round or pointed shapes on the left and right sides. The rostrum is long and broad and well defined with round or pointed shapes in left- and right-side otoliths. The anterior region is angled to peaked and the posterior region is oblique in the left- and right-side otoliths in *P. bogaraveo* (Table 1; Fig. 3).

The left- and right-side otoliths in *P. erythrinus* are pentagonal to oval in shape and wide (Table 1; Fig. 3). Dorsal margins of the left and right otoliths are crenate, while ventral margins are sinuate. Sulcus acusticus and cauda types are heterosulcoid and strongly curved in the left and right otoliths, respectively. The excisura is wide and the notch is shallow or absent. The antirostrum is absent or poorly defined with a round shape on the left and right sides. The rostrum is short and broad and well defined with a pointed shape in the left- and right-side otoliths. The anterior region is angled to oblique and the posterior region is oblique in the left- and right-side otoliths of *P. erythrinus* (Table 1; Fig. 3).

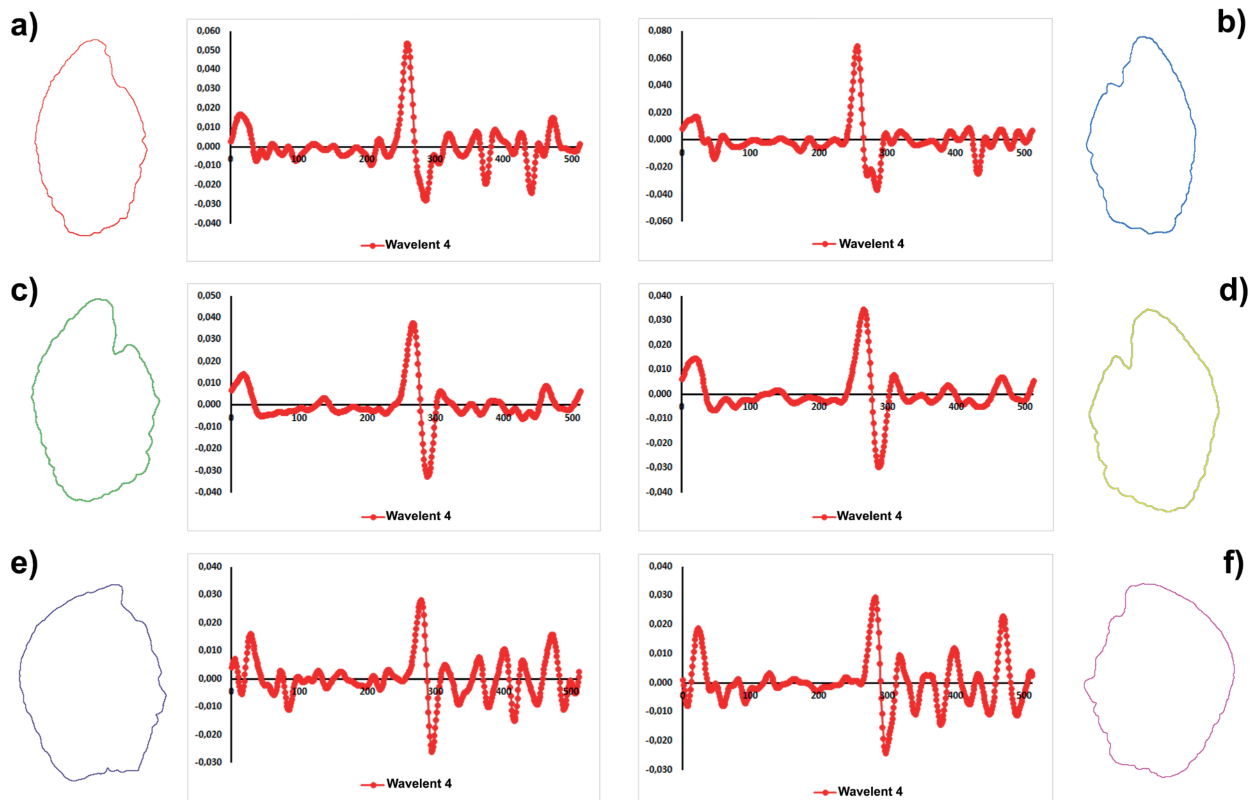

Figure 4

Signals of wavelet 4 from the left- and right-side otoliths in *Pagellus* from the Aegean Sea: (a) left- and right-side (b) otolith signals in *P. acarne*; (c) left- and right-side (d) otolith signals in *P. bogaraveo*; (e) left- and right-side (f) otolith signals in *P. erythrinus*.

The graphical illustration of wavelet 4 showed specific variations associated with prominent features of the otolith contour in the *Pagellus* species (Fig. 4). ANOVA showed three zones of high variability both between the left- and right-side otoliths of the same *Pagellus* species and between the three *Pagellus* species (Fig. 5): postero-dorsal, antero-dorsal, and antero-ventral. The results of principal component analysis (PCA) based on PC scores of the 4th WT signal assigned samples of each group to a separate group, confirming the existence of morphological differences between otoliths in *Pagellus* species (Fig. 6). The first three principal components accounted for 78.4%, 19.3%, and 1.2% of the total variance, respectively. PC1 and PC2 provided clear discrimination between the *Pagallus* species. Cluster analysis grouped the *Pagellus* species based on PCs (Fig. 7). There were two main clusters, one containing only *P. erythrinus* and the other cluster containing *P. acarne* and *P. bogaraveo* (Fig. 7). Cluster analysis recognized *P. acarne* and *P. bogaraveo* as more similar taxa based on otolith shape analysis (Fig. 7).

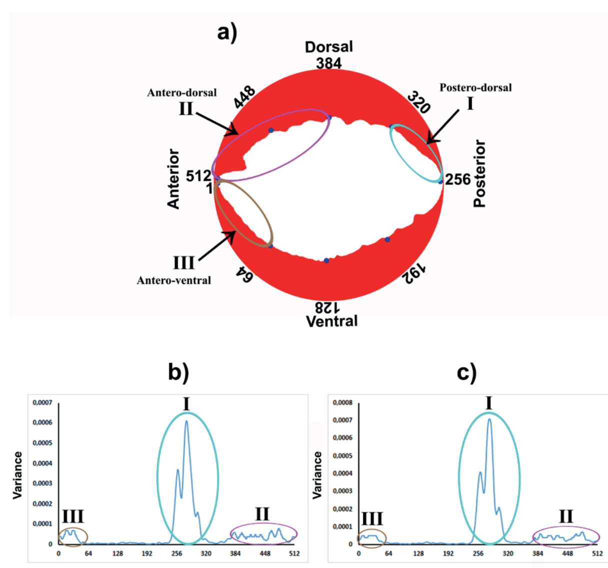

Figure 5

High variation zones in otoliths of *Pagellus* species (a) and variance distribution of the 4th wavelet signal of the left- (b) and right-side (c) otoliths in *Pagellus* species.

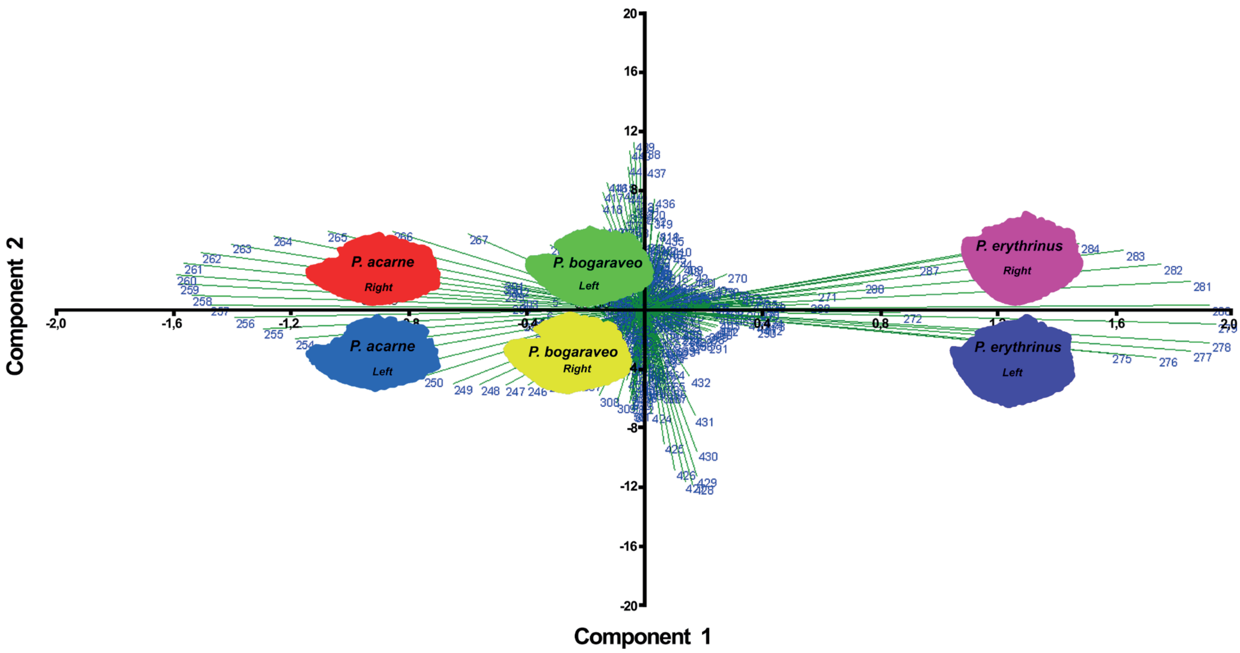

Figure 6

Principal component analysis (PC1 versus PC2) of the left- and right-side otolith shapes computed for the *Pagellus* species.

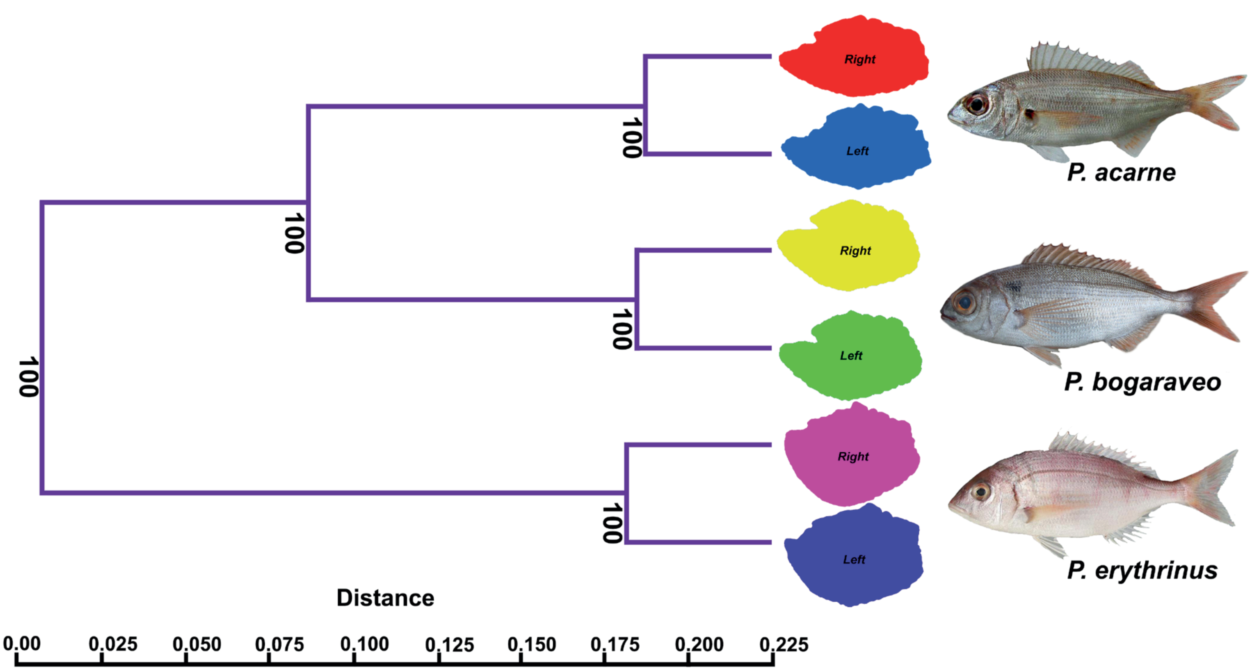

Figure 7

Cluster analysis for the left- and right-side otoliths in the three *Pagellus* species – *P. acarne*, *P. bogaraveo*, and *P. erythrinus* from the Aegean Sea.

4. Discussion

In the present study, three congeneric *Pagellus* species (*P. acarne, P. bogaraveo,* and *P. erythrinus*) from the Aegean Sea are compared using the otolith morphological characters and otolith wavelet analysis. The otolith morphology of three congeneric *Pagellus* species has been described (Tuset et al. 2008), but has not been described in detail for both the left- and right-side otoliths in the *Pagellus* species. This is the first study to provide detailed otolith morphological characters and wavelet analysis for the left- and right-side otoliths in the three congeneric *Pagellu*s species from the Aegean Sea. In addition, morphological differences were observed between the *Pagellus* species in the postero-dorsal, antero-dorsal, and antero-ventral parts of the otoliths, as well as between the left- and right-side otoliths of these regions (Fig. 5).

Some similar and different patterns in otolith morphology were observed in the three *Pagellus* species studied. Among the *Pagellus* species, nine of the 21 otolith characters examined in this study show similar morphological features, and these characters are not suitable for use in interspecific differentiation of the *Pagellus* species. For instance, otolith morphological characters such as distal and proximal surfaces, sulcus acusticus opening, sulcus acusticus location, ostium shape and size, cauda shape, antirostrum size, and excisura did not differ between the *Pagellus* species (Table 1). Similar results were obtained by Tuset et al. (2008) and Çiçek et al. (2021). Such morphological differences in fish species may be related to the organic matrix and the way calcium carbonate is accumulated during the sagittal development of fish species (Volpedo & Echevarria 2003). The morphological difference between the *Pagellus* species is mostly manifested in *P. erythrinus*, which has a more rounded shape compared with *P. acarne* and *P. bogaraveo*. The shape of the otolith varies between pentagonal to elliptic in *P. acarne* and *P. bogaraveo*, but is pentagonal to oval in *P. erythrinus*. In terms of otolith shape, it is possible to distinguish *P. erythrinus* from *P. acarne* and *P. bogaraveo*. This situation, which was observed in the study of the otolith shape in *Pagelus* species, was also reported in previous studies (Tuset et al. 2008; Çiçek et al. 2021). The dorsal and ventral margins, as well as the anterior and posterior regions of the otolith show considerable morphological variation among the three *Pagellus* species. In addition, there are differences and irregularities in the grades of lobation of these parts of the otolith in individuals of the same species. Such differences can be encountered in fish species of different sizes. Similar results have been reported in the literature for *Saurida tumbil* (Jawad 2008) and *Atherina boyeri* (Bostanci & Yedier 2018). In the present study, the overall morphology of the rostrum is narrow, short or long and pointed in *P. acarne*, broad and long, round or pointed in *P. bogaraveo,* and broad, short and pointed in *P. erythrinus*. The information on the rostrum shape and characteristics of the *Pagellus* species is consistent with the information found in the literature (Tuset et al. 2008; Çiçek et al. 2021).

The analysis of otoliths in the three congeneric *Pagellus* species revealed that the otolith morphology was generally similar to that described in previous studies conducted in the Western Mediterranean Sea and the Atlantic Ocean (Tuset et al. 2008; Çiçek et al. 2021), but the current study found that some otolith characters differed from those reported in the above studies. It is assumed that this may be due to habitat differences and genetic variations of the species. SEM images of the otoliths of these three *Pagellus* species obtained in studies conducted in other geographical regions are given in otolith atlases (Tuset et al. 2008; Nolf 2013; Çiçek et al. 2021) and are generally similar to the images obtained in our study. However, detailed SEM images of both the proximal and distal surfaces of the right and left otoliths in these three congeneric *Pagellus* species are not available in the literature. This is the first study to conduct a comprehensive morphological examination of the left and right otoliths in the three congeneric *Pagellus* species. Therefore, our study contributes to eliminating this paucity of information on the three species in this field. In this study, the general otolith morphology of each species was obtained from the distal and proximal surfaces of the otoliths of *P. bogaraveo, P. acarne,* and *P. erythrinus*, and the information obtained from these otoliths generally overlaps with the information in the literature (Tuset et al. 2008; Çiçek et al. 2021). For instance, the shape of the sulcus is heterosulcoid and the ostium is shorter than the cauda. Another morphological feature that can support the ecology of fish species with sagittae is the condition of the ostium and cauda in the sulcus acusticus. For instance, in the current study, we found that the ostium and cauda structure penetrated deep into the sagittae carbonate structure in *P. acarne* (Fig. 8) and *P. bogaraveo* (Table 1; Fig. 9). However, in *P. erythrinus* samples, the ostium and cauda did not penetrate as deep as in the other two *Pagellus* species (Table 1; Fig. 10). As for the characteristics of the ostium and cauda, some of the observed differences, such as minor differences between the right and left sagitta and the absence of a notch and antirostrum in the sagitta, may correspond to the ecology and feeding behavior of the *Pagellus*

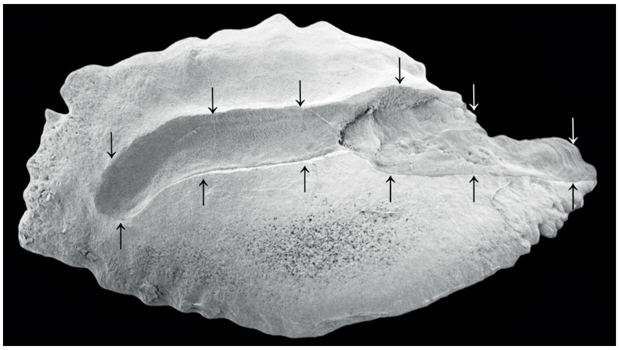

Figure 8

Proximal surface of the left sagittal otolith in *Pagellus acarne*. Arrows indicate the sulcus area on the sagittal otolith.

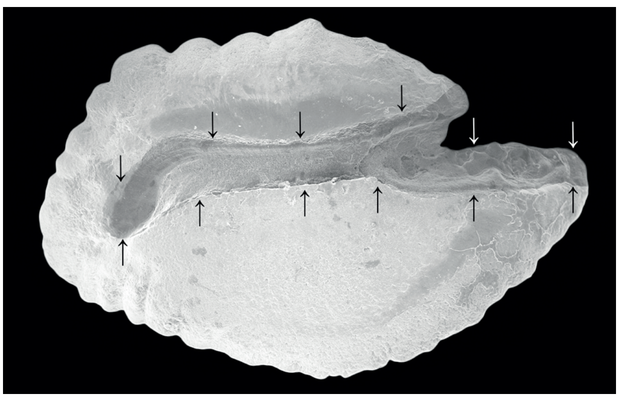

Figure 9

Proximal surface of the left sagittal otolith in *Pagellus bogaraveo*. Arrows indicate the sulcus area on the sagittal otolith.

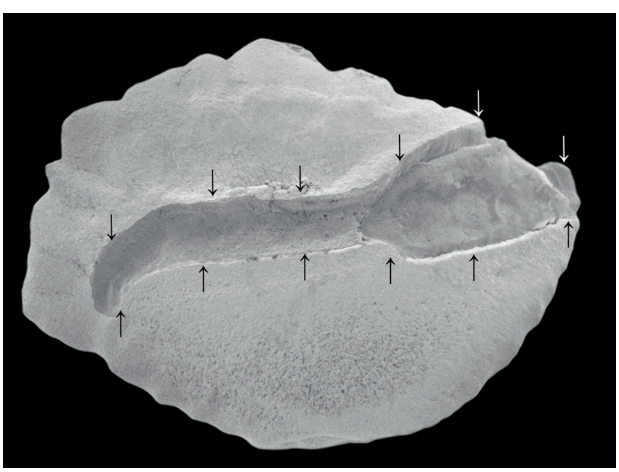

Figure 10

Proximal surface of the left sagittal otolith in *Pagellus erythrinus*. Arrows indicate the sulcus area on the sagittal otolith.

species specialized in different strategies. The deeper sulcus acusticus in *P. acarne* and *P. bogaraveo* observed in our study may be related to the depth distribution of the species, as in the case of *P. bogaraveo*, but it also corresponds to high mobility associated with feeding behavior, as in both *P. acarne* and *P. bogaraveo*. Different depths of the sulcus acusticus in fish species may affect the relative movement of otoliths with the macula sacculi and cause the thickness of the otolithic membrane to change (Schulz-Mirbach et al. 2019). As a result, different thicknesses of the otolithic membrane in fish species may cause differences in the mechanical resistance between the sensory epithelium and the otolith, resulting in differences in fish sensory functions (Popper et al. 2005). In summary, the results show that the sulcus acusticus and otolith morphological differences between *P. bogaraveo* and the other two *Pagellus* species may also be related to differences in habitats, soundscape, and feeding habits.

Fish occurring in demersal areas may increase the surface of their sagittae in the anterior regions and margins compared to fish found in pelagic areas. The increase in surface area in these parts of the otoliths also results in an increase in the size of otoliths (D'Iglio et al. 2021). However, when looking at this increase from an ecological perspective, it may be related to the life cycle and habitat selection of the species. Differences in the life cycles and habitat preferences of the *Pagellus* species may be responsible for morphological variations in sagittae. In addition, this situation highlights the relationship between sagitta characteristics and environmental and biological factors. It has been reported in the literature that the percentage of fish species with large otoliths tends to increase with depth, excluding the abyssal depth (Lombarte & Cruz 2007; D'Iglio et al. 2021), and the current study of the *Pagellus* species also confirms this information. A comparison of the size of otoliths from individuals of similar total length belonging to the three *Pagellus* species conducted in our study revealed that *P. bogaraveo* had the largest otolith, followed by *P. acarne* and *P. erythrinus*, respectively. It has been reported that the range of depth in the distribution areas of *Pagellus* species varies. For instance, *P. bogaraveo* occurs to a depth of up to 700 m (usually 150–300 m), *P. acarne* up to 500 m (usually 40–100 m), and *P. erythrinus* up to 300 m (20–100) (Frose & Pauly 2023). *P. bogaraveo* specimens analyzed in the current study also had otoliths larger than the other two *Pagellus* species due to their demersal habits. It is essential for fish species living in demersal environments to have larger sagittal otoliths to support improved sound perception, acoustic communication, and sense of balance to compensate for light reduction (Buran et al. 2005; Lombarte & Cruz 2007; Kéver et al. 2014).

The results of PCA on the wavelength data showed that a reasonably high level of discrimination can be obtained for the six analyzed groups (left-side otolith of *P. acarne*, right-side otolith of *P. acarne*, left-side otolith of *P. bogaraveo*, right-side otolith of *P. bogaraveo*, left-side otolith of *P. erythrinus*, and right-side otolith of *P. erythrinus*). The results of the current study demonstrate that wavelet analysis is a useful mathematical procedure for use in species identification and discrimination. This is emphasized in many studies in which this method was used (Parisi-Baradad et al. 2005; Sadighzadeh et al. 2012; Yedier et al. 2023a). The present study found that otolith regions with high morphological variability can be identified reliably and easily with the wavelet method. This also means that information on the shape of the entire otolith may not be required for the identification of fish species or stocks, or for ecomorphological or ontogenetic studies. In otolith studies, researchers studying relevant genera and species of fish can save time and obtain more detailed information if they examine parts of otoliths with morphologically variable regions instead of all parts. The use of innovative approaches such as wavelength analysis in the identification and discrimination of fish species may allow new and more detailed information to be added to the literature on related species and genera. It is very important to better understand the functioning of aquatic ecosystems and the ecological roles played by fish species, especially in distinguishing congeneric fish species and determining their activities in the ecosystem (Aguzzi et al. 2013; Meakin & Qin 2011; Colmenero et al. 2010).

Although no significant differences were observed in our study between the left and right otoliths in the *Pagellu*s species in terms of distinct morphological characters, we determined that there were statistical differences between the contours of the left and right otoliths of the same species in the wavelength analysis. These results confirm the high sensitivity of wavelength analysis in the analysis of otolith shape. In studies of otoliths, when assessing left and right otoliths, researchers will benefit from assessing contours of otoliths using wavelet analysis in addition to morphological assessment. The results of published studies on otoliths in *Pagellus* species indicate that there are differences in the left and right otoliths in *P. acarne* and *P. erythrinus*, especially in their morphometric data (Merji et al. 2018; D'Iglio et al. 2021; Yedier et al. 2023b). However, the difference between the right and left sagittae in *P. bogaraveo* was determined for the first time in this study. The differences between the left and right otoliths are extensively reported in the literature on flatfish species. Such differences have also been reported for other round fish species, such as *Clupea harengus* (Bird et al. 1986), *Scomberomorus niphonius* (Zhang et al. 2016), *Diplodus puntazzo* (Bostanci et al. 2016), and *Liza ramada* (Rebaya et al. 2017). In the literature, such asymmetrical differences in otoliths are generally associated with genetic and environmental stressors (Trojette et al. 2015; Jawad et al. 2023; Yedier et al. 2023c). Since the functional morphology of otoliths is still not fully understood, it is difficult to find a direct link between the small differences between left and right otoliths and the ecology of the species. However, the relationship between otolith characteristics and the habitat of the species should be examined through various eco-functional factors, such as the feeding behavior of the species, for better conservation. For instance, most members of the Sparide family in benthic zones feed primarily on benthic organisms such as benthic crustaceans, brachyuran crabs, and polychaetes that often hide in sandy substrate (Fanelli et al. 2011; Froese & Pauly 2023). For this reason, members of this family can perform various behaviors such as swallowing sediment, digging in the sand and pushing sideways and forward with the head and body to find and catch prey (D'Iglio et al. 2021). This type of behavior in benthic and benthopelagic species may affect the morphology and growth of sagittae, causing some minor differences between right and left sagittal otoliths.

Since insufficient understanding of the functional morphology and physiology of otoliths does not allow us to find a direct relationship between eco-functional characteristics of fish and their otolith morphology, it is difficult to eco-morphologically assess the interspecific differences in sagittal morphology based on the results obtained in this study on sagittal otoliths of the three congeneric *Pagellus* species. However, the wavelet shape analysis and PCA (Figs 5 & 6) revealed clear differences in the morphology and shape of sagittal otoliths among the three congeneric *Pagellus* species. In addition, considering the various ecological, functional and biological characteristics of each species, it was revealed that the sagittae morphology in these three congeneric *Pagellus* species examined by both SEM analyzes and wavelet analyzes is suitable for their ecology and lifestyle. The similarities and differences between the morphological characters of the otoliths in the *Pagellus* species found in our study may be evidence of the ecomorphological adaptation of sagittae in the *Pagellus* species to demersal and pelagic environments. This hypothesis can be confirmed by comparing the ecology and modes of life of the three *Pagellus* species with the morphology of otoliths in the species. In the present study, the sagittal otoliths in *P. acarne* are similar in shape to those in other pelagic species reported in the literature, with a long and narrow rostrum compared to the other two *Pagellus* species. Considering the feeding activity and life cycle, *P. acarne* shows the most pelagic habits compared to the other two *Pagellus* species (D'Iglio et al. 2021). In addition, otoliths in *P. acarne* showed more similar characteristics to *P. bogaraveo* than *P. erythrinus*. This may be due to the similarity in feeding habits and ecology of *P. acarne* and *P. bogaraveo* compared to *P. erythrinus* (Morato et al. 2001; Savoca et al. 2019). The results of this study revealed that there are significant differences in the shape of otoliths in *P. erythrinus* when they are compared with those in the other two *Pagellus* species. Of these three *Pagellus* species, *P. erythrinus* has the most important morphological difference with the shortest rostrum otoliths. *P. erytrinus* is the species with the most benthic habits compared to other *Pagellus* species, preying mainly on epibenthic and infaunal species. In addition, *P. erythrinus* is the most diverse species in terms of ecology and life cycle among the three *Pagellus* species examined, and this species is also closely associated with the benthic environment. The difference in the lifestyle of *P. erythrinus* compared to the other two *Pagellus* species is also consistent with the results of the wavelet analysis in our study. The contour of the sagittal otoliths in *P. erythrinus* has a more circular appearance compared to the other two species. *P. bogaraveo* is a demersal species living in the deep biocenosis and feeding in both mesopelagic and benthic environments. The sagittal otolith in *P. bogaraveo* is similar in shape to the otolith in *P. acarne*. The feeding habits and ecology of these species are in line with the similarities and differences in the sagittal shape of the two *Pagellus* species. In addition, PCA analysis confirmed that the greatest difference in shape among the three *Pagellus* species pertained to *P. erythrinus*. The differences and similarities in otolith morphology in the three congeneric *Pagellus* species assessed in the present study are also consistent with the relationships between sagittal morphological parameters, depth and habitat characteristics of different fish species previously reported by Lombarte & Cruz (2007).

The PCA results showed that the shape of the sagitta in *P. erythrinus* was significantly different compared to the otoliths in *P. bogaraveo* and *P. acarne*. These features determined in our study may provide a key to understanding the ecomorphology of sagittae and sulcus acusticus in the life cycle and environmental adaptation of the *Pagellus* species. The study discusses otolith morphologies in the *Pagellus* species with a wide range of morphological features. In addition, *Pagellus* otoliths were assessed using wavelet shape analysis. It was found that these features show high differentiation between the species and can be used to identify the *Pagellus* species. Furthermore, it is crucial to consider the asymmetry between sagitta pairs in the *Pagellus* species, which was clearly revealed by wavelet analysis in our study. Ignoring this asymmetry between otolith pairs in future studies may lead to incorrect discrimination and identification of fish stocks and populations. Further, advancing our knowledge of the variation in the morphology and morphometry of sagittal otoliths among the three congeneric *Pagellus* species may provide a basis for further research into the functional and morphological aspects of fish otoliths. In terms of fisheries management, obtaining more detailed information on fish growth and changes in sagittal otoliths can help understand the population connectivity, stock structure, and dynamics of these fish species. This is essential for the management of commercially valuable fish stocks, such as *Pagellus* species, and for developing stock management strategies.

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