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Species identification using otolith morphometry of four gurnards (Family: Triglidae) from Edremit Bay (Aegean Sea)

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Abstract: Shape and size analyses of otoliths are widely considered appropriate tools for distinguishing between fish species and for assessing populations and stocks. The present study aims to distinguish four gurnard species collected from Edremit Bay (Aegean Sea) based on variations in their otolith shape morphology. A total of 60 specimens of *Eutrigla gurnardus*, 64 of *Chelidonichthys lastoviza*, 60 of *Chelidonichthys lucerna,* and 60 of *Lepidotrigla cavillone* were analyzed. No significant intraspecific differences were noted in the morphology of the left and right otoliths, and so only the left sagittal otoliths were subjected to further analysis. Otolith shape was quantified using wavelet coefficients generated via the ShapeR software package. In addition, otolith size indices, including length (OL), height (OH), perimeter (OP), and area (OA), were extracted from digital images, while shape descriptors such as form factor (FF), aspect ratio (AR), ellipticity (E), circularity (C), roundness (RD), and rectangularity (R), were measured for each species. Significant interspecific differences were observed in the otolith shape and size indices ($p < 0.05$), and mean otolith shapes generated using wavelet-derived contours revealed obvious shape differences among the species. These findings were strongly supported by a canonical analysis of principal coordinates (CAP), with the first two axes together explaining 96.5% of the total variance (CAP1 = 84.7%, CAP2 = 11.8%). Overall, the results indicate that otolith shape analysis is a reliable tool for identifying gurnard species and provides a methodological baseline for future comparative studies in the Aegean Sea.

Key words: Otolith, morphology, Triglidae, ShapeR, Edremit Bay, Aegean Sea

1. Introduction

The Triglidae family, known as sea robins or gurnards, is composed of demersal fish species found in temperate and tropical seas and has been classified into 131 species across eight genera[1]. In the Mediterranean Sea, it is represented by eight species (Mutlu et al., 2024). Due to its rich diversity and the economic significance of certain species, it plays a crucial role among demersal fish stocks. It can be found in many different marine zones, ranging from continental shelf areas to deeper shelf slope regions (Colloca et al., 2019) but is typically found on sandy and muddy sea beds, where they primarily feed on epibenthic organisms (Platell and Potter, 1999).

The first studies of the family date back to the early 1900s, with the first documented research conducted in the Mediterranean (Priol, 1932, Ben-Tuvia, 1953; Dieuzeide, 1955; Tortonese, 1975). Several later studies have documented the age and growth characteristics of these species (Papaconstantinou, 1981, 1982a, 1982b, 1983, 1984, 1986; Baron, 1985; Papaconstantinou et al., 1992; Colloca et al., 1997; Faltas and Abdallah, 1997; Serena et al., 1998; Marano et al., 1999; Voliani et al., 2000; İşmen et al., 2004; Uçkun, 2005; İlhan and Toğulga, 2007; İlkyaz et al., 2010; Akalın and İlhan, 2013; Başusta et al., 2017; McCarthy et al., 2018; McCarthy and Marriott, 2018; Rodrigues, 2020), while studies of otolith morphometry in gurnards remain limited, both in the Mediterranean region and worldwide.

Teleosts have three pairs of otoliths: lapilli, asteriscus, and sagittae, with latter being the largest in most families (Harvey et al., 2000). The size and distinctiveness of sagittae make them one of the most informative calcified structures for the identification of species-specific traits (Platt and Popper, 1981; Reichenbacher et al., 2007). Owing to their structure, the three otolith pairs have long been regarded as closed records of the fish's life (Ozpicak and Akkaya, 2025). Their utility, however, is most evident at the family level, where they are commonly used to assess

[1] Froese R, Pauly D (2025). FishBase. Website https://fishbase.mnhn.fr/summary/FamilySummary.php?ID=266 [accessed 20 June 2025].

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the age and growth of teleosts for stock assessment and management purposes.

Otolith contour analyses of many different species have been conducted in the Mediterranean basin (Saygin, 2024; Yedier et al., 2025); however, studies focusing specifically on gurnards are scarce. Notable exceptions include studies of *Chelidonichthys lucerna* along the northwest coast of Portugal (Ferreira et al., 2019) and an analysis of the otolith contour shape of *Lepidotrigla cavillone* in the Mediterranean Sea (Bellodi et al., 2025). Other studies have investigated the changeability of the sulcus acusticus in the sagittae of *Chelidonichthys cuculus* (formerly *Aspitrigla cuculus*) and *Eutrigla gurnardus* in the north-central Adriatic Sea (Montanini et al., 2015a), while Şen and Daban (2024) examined the correlation between otolith morphology and body size in *C. lucerna*. In another study, Atak Sanduvaç and İşmen (2024) analyzed the otolith morphology and chemistry of *C. cuculus* and *C. lucerna* along the Turkish coast and in the Adriatic Sea (Montanini et al., 2015b).

Otoliths have been identified as an effective natural phenotypic marker aiding in the identification of fish species and populations (Montanini et al., 2015a). Otolith morphology is greatly influenced by various internal and external factors, including environmental conditions such as depth, temperature, substrate type, salinity, and feeding behavior (Gauldie, 1993; Lombarte and Lleonart, 1993; Cardinale et al., 2004; Capoccioni et al., 2011). Several techniques have been employed to date to investigate interspecific and intraspecific variations in otolith shape. These include shape indices based on traditional linear morphometrics (Tuset et al., 2003), elliptic Fourier analysis (EFA) (Lestrel, 1997), wavelet transform (WT)—a mathematical approach that captures one-dimensional contour features (Mallat, 2002; Ozpicak and Akkaya, 2025)—and geometric approaches (Pattanayak et al., 2024; Yildiz et al., 2024).

The present study examines and compares the shapes and sizes of sagittal otoliths among four gurnard species in the Aegean Sea. By identifying the unique characteristics of these species, we aim to enhance our understanding of their roles in the ecosystem and the distinctive traits of their otoliths in the Aegean Sea in the northeastern Mediterranean.

2. Materials and methods

2.1. Sampling

Gurnard specimens were collected over 11 months through bottom trawl surveys conducted in Edremit Bay in the northeastern Aegean Sea, from September 1999 to August 2000 (excluding February 2000) (Figure 1). Randomly selected sagittal otoliths of 60 specimens of *Eutrigla gurnardus* (Linnaeus, 1758), 64 specimens of *Chelidonichthys lastoviza* (Bonnaterre, 1788), 60 specimens of *Chelidonichthys lucerna* (Linnaeus, 1758), and 60 specimens of *Lepidotrigla cavillone* (Lacepède, 1801) were then analyzed. Gurnard species were identified following the method outlined by Whitehead et al. (1986). Fork length (FL) measurements were taken in centimeters (cm) using a measuring board accurate to 1 mm, while body weights were recorded in grams (g) using a digital balance with a precision of 0.01 g. For otolith examination, a pair of sagittal otoliths was extracted from the otic capsules of each specimen. The otoliths were stored in envelopes for future analysis after being cleaned and labeled.

2.2. Otolith imaging

High-resolution otolith images were obtained using a Dino-Lite AM4113T digital microscope and DinoCapture 2.0 image processing software[2]. The sagittal otoliths of each specimen were placed on a black-bottomed petri dish with the proximal face of the otolith facing upward and the rostrum pointing to the left. Prior to the shape analysis, each otolith image was examined and edited using GIMP image manipulation software (Version 2.10.38)[3], with all images saved in the JPEG format (Figure 2).

2.3. Contour extraction

The properties of the otoliths from the four gurnard species and their contour extraction were obtained using the ShapeR 1.0-1 package (Libungan and Pálsson, 2015) in R software, version 4.4.3 (R Core Team, 2025)[4]. The ShapeR package extracts the otolith contour from its image by utilizing color contrasts, providing a visual representation of its shape. The contour of each otolith was extracted using the "detect outline" function with a threshold parameter of 0.2. All outlines were saved in the PNG format and visually inspected to ensure that the detected contours accurately matched the actual edges of the otoliths.

Variations in otolith shapes among gurnards were assessed using wavelet transformation of the otolith outlines. The outlines were extracted, smoothed and decomposed into 63 wavelet coefficients using the ShapeR package in R. These coefficients were then standardized to account for allometric growth by regressing them against fish fork length (FL), and the residuals were used in the subsequent analysis. As part of the standardization procedure, seven wavelet coefficients (1, 6, 7, 12, 13,

[2] AnMo Electronics Corporation (2023). DinoCapture 2.0 imaging software for Dino-Lite digital microscopes [online]. Website: https://www.dino-lite.com [accessed 13 June 2025].

[3] GIMP Development Team (2024). GIMP (GNU Image Manipulation Program), version 2.10.38 [online]. Website https://www.gimp.org [accessed 11 July 2025].

[4] R Core Team (2025). R: A language and environment for statistical computing [Software]. R Foundation for Statistical Computing [online]. Website https://www.R-project.org [accessed 10 June 2025].

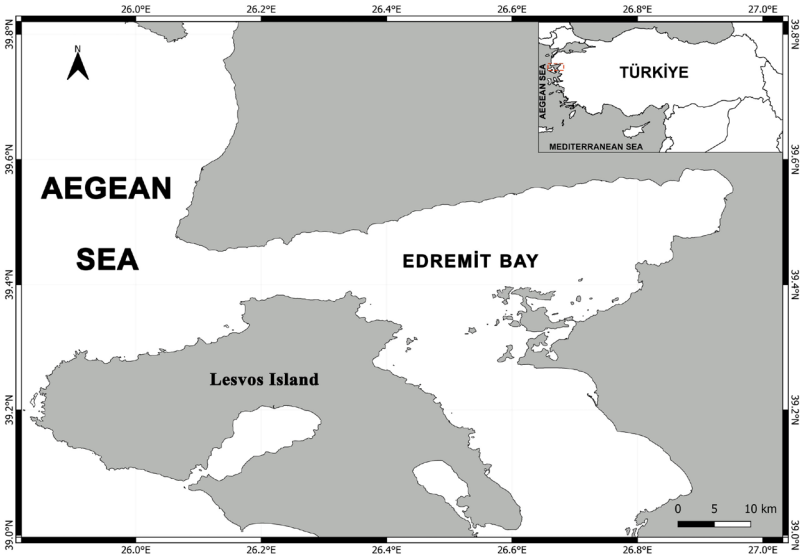


Figure 1. Sampling area (Edremit Bay).

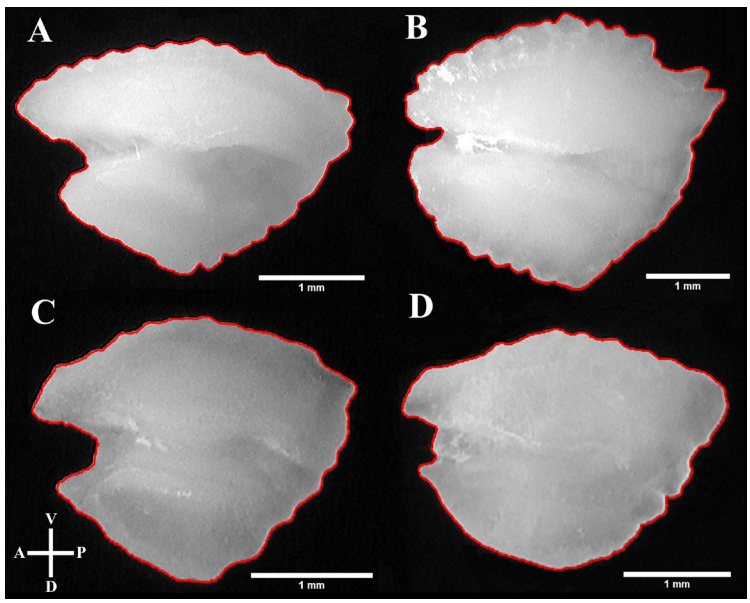


Figure 2. Sagittal otoliths of gurnards (A- *C. lastoviza* (14.5 cm), B- *C. lucerna* (18.9 cm), C- *E. gurnardus* (12.1 cm) and D- *L. cavillone* (12.0 cm)) are shown in the imaging and shape extraction orientation (D: dorsal, V: ventral, A: anterior, and P: posterior), with the red line representing the ShapeR-derived outline.

15, 30) were excluded based on their significant length related variation in the package, and the remaining 56 standardized wavelet coefficients were subjected to further multivariate analyses. These coefficients were analyzed with a canonical analysis of principal coordinates (CAP) (Lleonart et al., 2000; Park et al., 2023).

2.4. Otolith shape and size analysis

The ShapeR package was utilized for the automatic measurement of various otolith size parameters, including otolith length (OL, mm), otolith height (OH, mm), otolith perimeter (OP, mm), and otolith area (OA, mm²). A paired t-test was conducted to identify any differences between the left and right sagittae, and when no significant differences were found ($p > 0.05$), the null hypothesis (right = left) was supported, and the left sagittae were selected for further evaluation (Table S1). Otolith shape indices, such as form factor (FF), roundness (RD), aspect ratio (AR), circularity (C), rectangularity (R), and ellipticity (E), were calculated

using mathematical expressions provided in Table 1 (Tuset et al., 2003, 2006; Ponton, 2006).

2.5. Data analysis

Since otolith morphometric indices are largely influenced by fish length, all variables were standardized using the formula of Elliott et al. (1995) and Lleonart et al. (2000) to eliminate the allometric influence of fish length on otolith shape and size:

$$M_s = M_o \left(\frac{\bar{x}}{x}\right)^b,$$

where M_s = standardized (size-adjusted) measurement; M_o = size and shape measurement (mm); $\bar{x}$ = average size parameter (FL) for all datasets; x = observed size parameter (FL) of each fish species; and b = slope of the regression between log M_o and log x.

The standardized otolith shape and size indices were initially tested for normality and homogeneity of variances using the Kolmogorov–Smirnov (K-S) test and Levene's test, respectively. If the data did not meet the assumption of normality ($p < 0.05$), a nonparametric Kruskal–Wallis test was employed to compare otolith shape and size parameters across the four gurnard species. When significant differences were found in the otolith measurements, Dunn's post hoc test was conducted following the Kruskal–Wallis test (Park et al., 2023). Additionally, an ANOVA-like permutation test and CAP were applied to the wavelet coefficients using the ShapeR package to further evaluate species-level shape differences.

The Kaiser–Meyer–Olkin (KMO) measure and Bartlett's test of sphericity (BTS) were employed to assess the data's suitability for factor analysis. The KMO index, ranging from 0 to 1, indicates sampling adequacy, in which values above 0.5 are considered acceptable (Kaiser, 1974), values between 0.5 and 0.7 reflecting moderate adequacy, values of 0.7–0.8 signifying good adequacy, and values exceeding 0.8 denoting excellent adequacy. A principal component analysis (PCA) was also conducted to examine morphometric variations and to visualize the differentiation patterns among otoliths across species, based on the full set of standardized otolith morphometric variables.

All statistical analyses and graphical representations were performed in the R software environment (R Core Team, 2025)[4]. The following R packages were utilized: ggpubr (0.6.0), rstatix (0.7.2), cowplot (1.1.3), glue (1.8.0), RColorBrewer (1.1-3), scales (1.3.0), ggplot2 (3.5.1), FSA (0.10.0), psych (2.5.3), knitr (1.50), car (3.1-3), dplyr (1.1.4), FactoMiner (2.11), and factoextra (1.0.7).

3. Results

The otoliths of *E. gurnardus* were smaller in all size parameters than other gurnards (mean OL ± SD = 2.50 ± 0.47 mm, mean OH ± SD = 2.04 ± 0.37, OP ± SD = 7.54 ± 1.51 mm and OA ± SD = 3.70 ± 1.18 mm^2), while their mean RD ± SD (0.74 ± 0.05) were higher. On the other hand, C. *lucerna* were larger in all otolith size parameters (mean OL ± SD = 4.13 ± 0.41 mm, mean OH ± SD = 3.21 ± 0.29 mm, mean OP ± SD = 11.80 ± 1.15 mm and mean OA ± SD = 9.30 ± 1.65 mm^2) than other species (Table S2).

The standardized otolith shape and size indices exhibited significant differences among the four gurnard species, as determined by the Kruskal–Wallis test ($p < 0.0001$; Table S3). Notably, all measured variables, including otolith length (OL), height (OH), area (OA), perimeter (OP), form factor (FF), roundness (RD), aspect ratio (AR), circularity (C), rectangularity (R), and ellipticity (E), differed significantly among the four species ($p < 0.001$). Post hoc Dunn's tests with Bonferroni correction provided detailed pairwise comparisons between the four species (Figure 3), revealing that the large otoliths of *C. lucerna* were significantly different in size (OL, OH, OA, OP) than all the other species ($p < 0.0001$; Table S4). While the shape indices of most species pairs differed significantly, there were some notable exceptions. For instance, roundness (RD) did not differ between *C. lastoviza* and *C. lucerna*, and form factor (FF) was similar between *C. lastoviza*, *C. lucerna*, *and L. cavillone*.

The KMO value measure of sampling adequacy was 0.609 for otolith measurements in the study. When combined with a statistically significant Bartlett's test (p

Table 1. Size and shape indices of otoliths with corresponding equations.

Size parameters	Shape indices	Equation
Otolith length, *OL* (mm)	Form factor, *FF*	$(FF) = (4\pi \times OA)/OP^2$
Otolith height, *OH* (mm)	Roundness, *RD*	$(RD) = (4 \times OA)/(\pi \times OL^2)$
Otolith perimeter, *OP* (mm)	Aspect ratio, *AR*	$(\mathrm{AR}) = OL/OH$
Otolith area, *OA* (mm^2)	Circularity, *C*	$(C) = OP^2/OA$
	Rectangularity, *R*	$(R) = OA/(OL \times OH)$
	Ellipticity, *E*	$(E) = (OL - OH)/(OL + OH)$

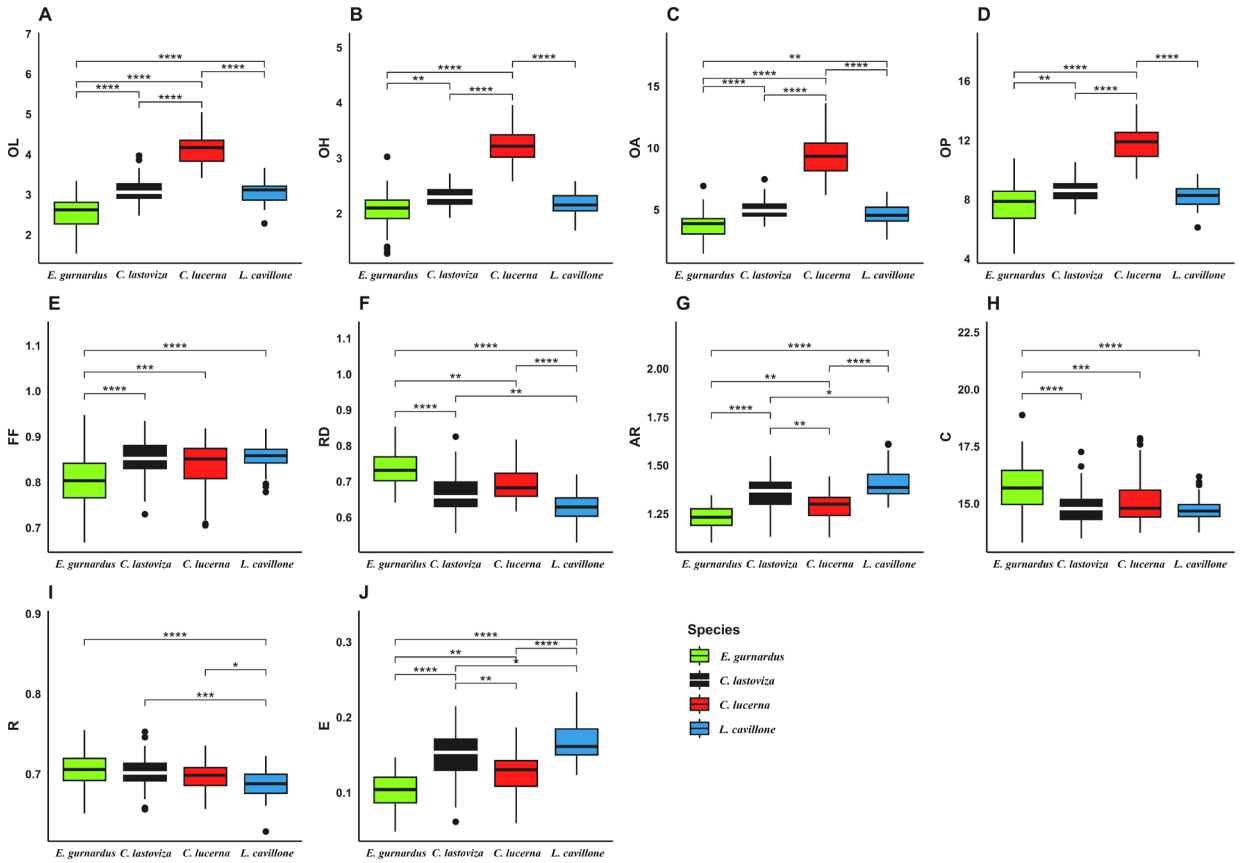


Figure 3. Box plots illustrating otolith size indices analysed using the nonparametric Kruskal–Wallis test followed by Dunn's post hoc comparisons. Significance is represented as follows: **** ($p < 0.0001$), *** ($p < 0.001$), **($p < 0.01$), * ($p < 0.05$), no asterisks ($p > 0.05$).

< 0.05), the results can be considered suitablfor factor extraction. The first principal component (Dim1), which loaded heavily on all otolith size parameters (OL, OH, OA, OP), explaining 41.2% of the variation, is interpreted as a size axis, while the second axis (Dim2), which reflects the RD, R, E and AR, accounted for an additional 31.6% of the variation (Figure S). Among the species, *C. lucerna* formed a distinct cluster along the positive side of Dim1, separate from the other species. In contrast, *E. gurnardus* and *C. lastoviza* showed partial overlap, indicating similarities in otolith morphology. *L. cavillone* was more dispersed and distributed along the negative side of both dimensions, reflecting greater variability in its otolith shape characteristics (Figure 4; Table S5).

The full rotation (0°–360°) of mean otolith contours reconstructed from wavelet coefficients (Figure 5) revealed species-specific shape patterns among the four gurnards, while a permutation-based ANOVA uncovered statistically significant differences in otolith shapes (df = 3, sum of squares = 59.896, F = 36.398, p = 0.001). *E. gurnardus* displayed a more pronounced expansion in the ventral region (angles 90–180°), while *C. lastoviza* showed a smoother and more symmetrical outline across the entire angular range. In contrast, *L. cavillone* exhibited a sharper inward curvature of around 180°, indicating a narrower mid-region than the other species. The dorsal area, especially between 270° and 300°, appeared to be relatively consistent across species, with minimal deviations in contours. Notably, *C. lucerna* presented a broader outline at the anterior end (near 0°), differentiating it from the other gurnards.

A CAP was conducted to assess the differences in otolith shapes between species, utilizing wavelet descriptors (see Figure 6). The first canonical axis (CAP1) explained 84.7% of the total variation, while the second axis (CAP2) accounted for an additional 11.8%. *E. gurnardus* was located primarily on the positive side of CAP1, indicating significant shape differences along this axis. In contrast, *C. lucerna* formed a separate cluster that was predominantly in the negative region of CAP2, highlighting its unique morphological profile. *C. lastoviza* and *L. cavillone,* on the other hand, exhibited partial overlap within the ordination space.

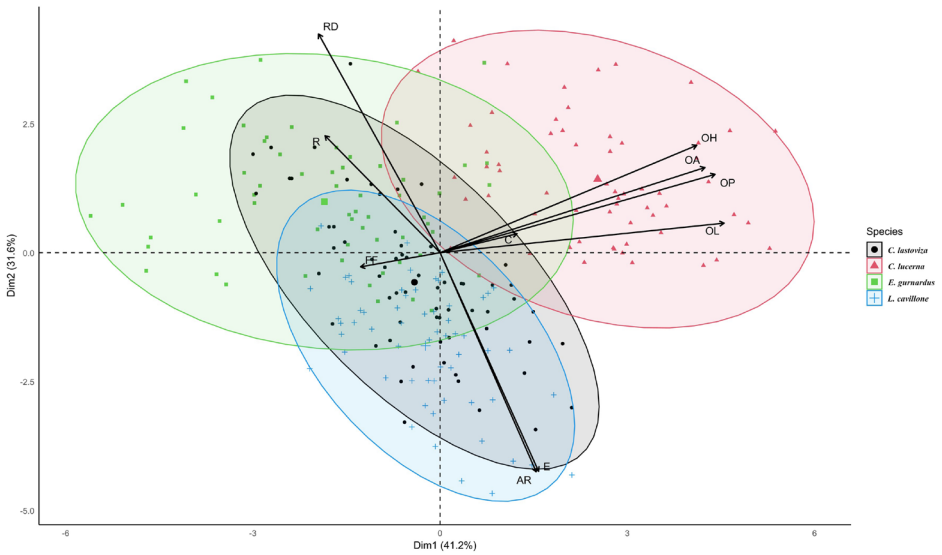


Figure 4. Biplot of principal component analysis (PCA) showing otolith size and shape indices in gurnard species.

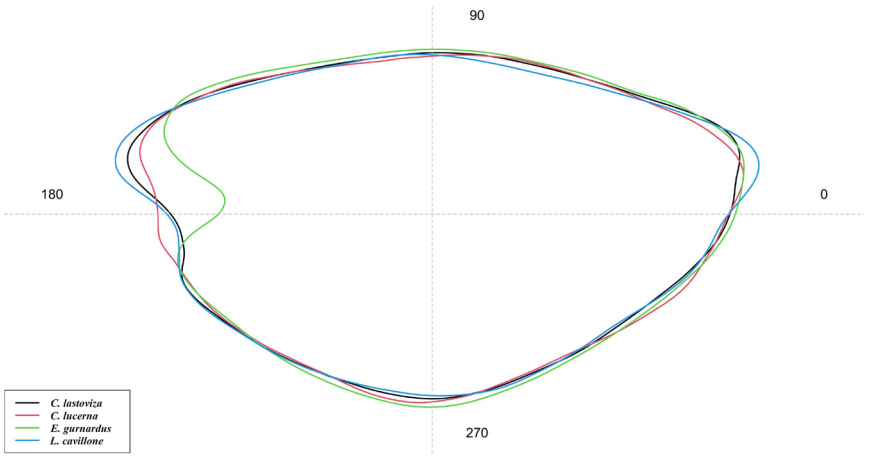


Figure 5. Reconstruction of the mean otolith shape of gurnards based on wavelet coefficients. The numbers correspond to angles expressed in degrees.

4. Discussion

The present study has examined variations in otolith shape and size among four gurnard species from Edremit Bay in the Aegean Sea to identify their unique otolith characteristics. The angular distribution of wavelet coefficients (Figure 7) revealed the most significant interspecific differences (mean ± SD) to be near 180°, highlighting this region as critical for differentiation. Angular sectors between 60° and 120°, and 300° and 320° showed relatively low variability, suggesting greater shape stability among the species in these regions. The intraclass correlation (ICC) curve indicated increased discriminatory power near 180° and again near 360°, identifying these angles as key contributors to the differences in otolith shape among the species.

Previous studies analyzing otolith shape and size among gurnards report both consistencies and notable differences with the present study (Table 2). For *C. lucerna*, the mean OL (4.13 mm) and OH (3.21 mm) recorded in the present study were higher than those reported by Şen and Daban (2024) in their study in the Sea of Marmara (1.80 mm and 1.53 mm, respectively), suggesting potential ontogenetic or regional variation (N= 7, mean TL= 77.23 mm). Furthermore, the otoliths of the species from Edremit Bay were generally larger than those found in Black Sea and other Aegean Sea populations (Atak Sanduvaç and İşmen,

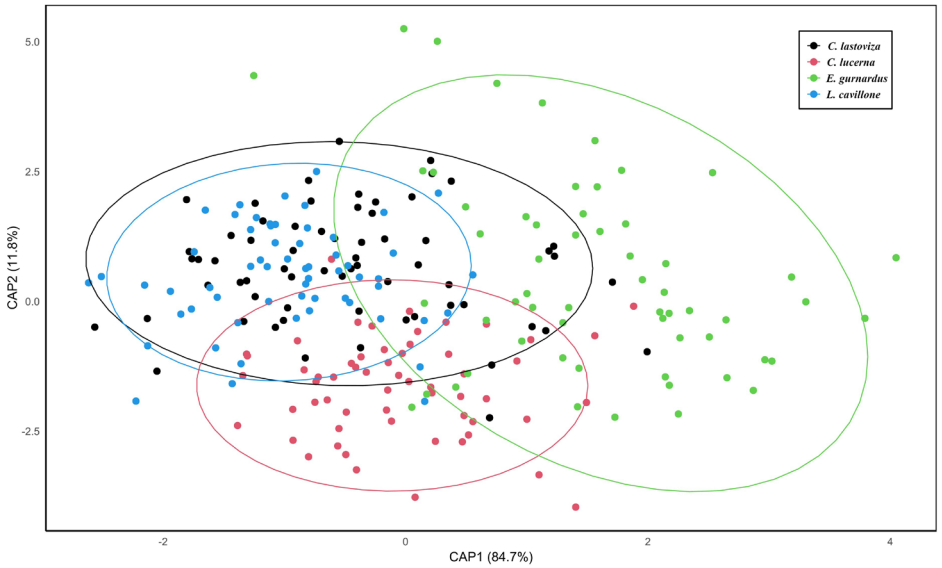


Figure 6. Canonical analysis of principal coordinates (CAP) of gurnards based on wavelet coefficients.

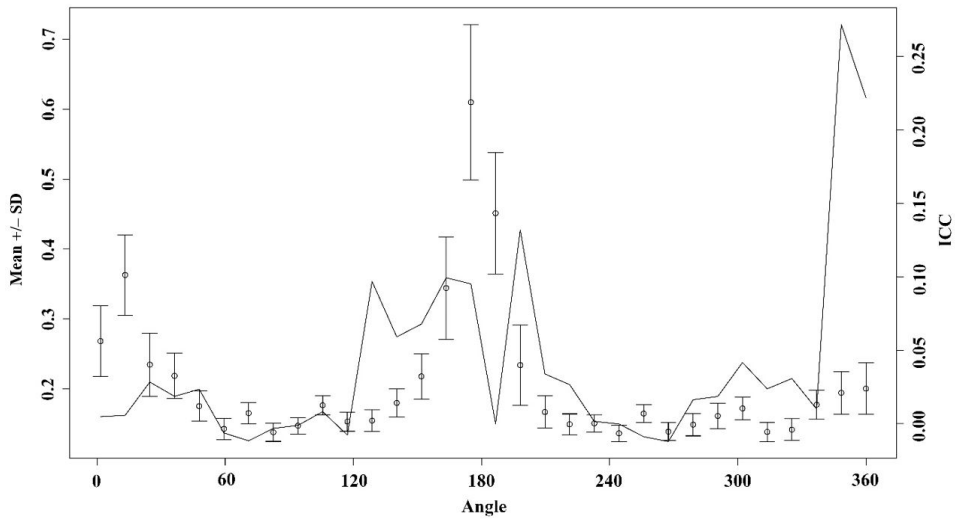


Figure 7. Wavelet coefficient means and standard deviations (SD) for otoliths pooled from four gurnard species. The solid black line represents the intraclass correlation coefficient (ICC) that quantifies variance between species.

2024), particularly in OA (9.30 mm²). In terms of shape indices, the *C. lucerna* in Edremit Bay showed relatively higher FF (0.84), RD (0.69), and AR (1.29), indicating a moderately elongated otolith form, consistent with the Mediterranean specimens reported by Atak Sanduvaç and İşmen (2024). For *L. cavillone*, the otolith size values in the present study were comparable to those reported by Bellodi et al. (2025) from Sicily and Sardinia. While the mean OL (3.06 mm) in Edremit Bay closely matched that of the Sicilian population (2.98 mm), the shape indices such as AR (1.41) and E (0.17) were slightly higher in Edremit Bay, suggesting more elongated and elliptical otoliths in the northeastern Mediterranean population. This study provides some of the first quantitative otolith morphometric data for *E. gurnardus* and *C. lastoviza* in the northeastern Mediterranean. The otoliths of *E. gurnardus* were the smallest among the examined species, with a mean OL of 2.50 mm and OA of 3.70 mm², reflecting its relatively smaller body size. However, the otoliths displayed a higher FF (0.80) and RD (0.74), indicating a compact and circular outline. In contrast, the otoliths of *C. lastoviza* were of more moderate size than the other

Table 2. Previous data on otolith shape and size indices of selected Mediterranean gurnard species.

			Otolith shape and size indices									
			OL (mm)	OH (mm)	OP (mm)	OA (mm²)	FF	RD	AR	C	R	E
Author	Area	Species	Min-max (mean ± SD)	Min-max (mean ± SD)	Min-max (mean ± SD)	Min-max (mean ± SD)	Min-max (mean ± SD)	Min-max (mean ± SD)	Min-max (mean ± SD)	Min-max (mean ± SD)	Min-max (mean ± SD)	Min-max (mean ± SD)
Şen and Daban (2024)	Sea of Marmara	*C. lucerna*	1.64–1.96 (1.80)	1.40–1.65 (1.53)								
Atak Sanduvaç and İşmen (2024)	Black Sea	*C. lucerna*	2.64–3.85 (3.32 ± 0.26)	2.27–3.01 (2.63 ± 0.18)	9.33–17.36 (11.83 ± 1.38)	4.44–8.44 (6.25 ± 0.79)	0.30–0.71 (0.57 ± 0.08)	0.62–0.88 (0.72 ± 0.06)	1.02–1.51 (1.27 ± 0.09)	17.66– 41.79 (22.57 ± 3.96)	0.60– 0.76 (0.72 ± 0.03)	0.01– 0.20 (0.12 ± 0.04)
	Sea of Marmara		2.97–4.15 (3.58 ± 0.25)	2,48– 3.31 (2.77 ± 0,19)	10.67–14.46 (12.10 ± 0.85)	5.29–9.16 (7.05 ± 0.79)	0.45–0.69 (0.61 ± 0.05)	0.59– 0.80 (0.70 ± 0.05)	1.12–1.46 (1.29 ± 0,08)	18.28–28.05 (20.90 ± 2.09)	0.66–0.78 (0.71 ± 0.03)	0.06–0.19 (0.13 ± 0.03)
	Aegean Sea		2.67–4.12 (3.51 ± 0.31)	2.40–3.17 (2.74 ± 0.17)	9.89– 16.16 (11.93 ± 1.18)	4,61– 8.95 (6.72 ± 0.85)	0.43– 0.73 (0.60 ± 0.06)	0.57– 0.82 (0.70 ± 0.06)	1.07– 1.48 (1.28 ± 0.09)	17.18– 29.19 (21.30 ± 2.34)	0.63– 0.78 (0.70 ± 0.03)	0.03– 0.19 (0.12 ± 0.04)
	Mediterranean Sea		2.87– 4.03 (3.32 ± 0.27)	2.38– 3.06 (2.67 ± 0.16)	10.33– 16.01 (11.64 ± 1.27)	5.22–8.12 (6.28 ± 0.72)	0.34–0.67 (0.59 ± 0.06)	0.63–0.85 (0.73 ± 0.06)	1.08–1.38 (1.25 ± 0.08)	18.79– 37.43 (21.71 ± 3.54)	0.65– 0.77 (0.71 ± 0.03)	0.04– 0.16 (0.11 ± 0.03)
Bellodi et al. (2025)	Mediterranean Sea (Sardinia)	*L. cavillone*	1.73–4.42 (3.22 ± 0.49)	1.24–3.16 (2.31 ± 0.35)	4.57–12.62 (9.00 ± 1.43)	1.43–8.46 (5.16 ± 1.48)						
	Mediterranean Sea (Sicily)		2.36–3.81 (2.98 ± 0.30)	1.70–2.74 (2.16 ± 0.21)	6.49–10.33 (8.20 ± 0.85)	2.82–6.89 (4.38 ± 0.83)						
This study	Aegean Sea (Edremit Bay)	*E. gurnardus*	1.57–3.20 (2.50 ± 0.47)	1.31–2.56 (2.04 ± 0.37)	4.48–9.85 (7.54 ± 1.51)	1.49–5.56 (3.70 ± 1.18)	0.67–0.94 (0.80 ± 0.06)	0.64–0.85 (0.74 ± 0.05)	1.09–1.35 (1.23 ± 0.06)	13.42–18.78 (15.71 ± 1.23)	0.65–0.75 (0.70 ± 0.02)	0.05–0.15 (0.10 ± 0.02)
		C. lastoviza	2.47–3.95 (3.08 ± 0.31)	1.91–2.71 (2.28 ± 0.18)	6.96–10.49 (8.55 ± 0.80)	3.58–7.41 (4.96 ± 0.82)	0.73–0.93 (0.85 ± 0.04)	0.55–0.82 (0.67 ± 0.06)	1.13–1.54 (1.35 ± 0.09)	13.54–17.29 (14.82 ± 0.73)	0.66–0.75 (0.70 ± 0.02)	0.06–0.21 (0.15 ± 0.03)
		C. lucerna	3.45–5.19 (4.13 ± 0.41)	2.63–4.07 (3.21 ± 0.29)	9.60–14.93 (11.80 ± 1.15)	6.42–14.44 (9.30 ± 1.65)	0.71–0.92 (0.84 ± 0.05)	0.61–0.81 (0.69 ± 0.05)	1.13–1.44 (1.29 ± 0.07)	13.68–17.79 (15.09 ± 1.05)	0.66–0.73 (0.70 ± 0.02)	0.06–0.18 (0.13 ± 0.03)
		L. cavillone	2.25–3.66 (3.06 ± 0.29)	1.67–2.55 (2.17 ± 0.19)	6.03–9.68 (8.20 ± 0.75)	2.49–6.32 (4.59 ± 0.76)	0.78–0.92 (0.85 ± 0.03)	0.53–0.72 (0.62 ± 0.04)	1.28–1.61 (1.41 ± 0.08)	13.69–16.19 (14.72 ± 0.51)	0.63–0.72 (0.68 ± 0.02)	0.12–0.23 (0.17 ± 0.03)

SD: standard deviation.

species examined (OL = 3.08 mm, OA = 4.96 mm^2) and had relatively higher shape indices (FF = 0.85, AR = 1.35), suggesting a slender and defined shape.

A contour analysis performed by Bellodi et al. (2025) revealed notable morphological differences in the *L. cavillone* collected from the waters around Sardinia and Sicily (Mediterranean Sea) in terms of their OA, OL, OH, and OP values. Specifically, the Sicilian population displayed a more noticeable inward curvature toward the centroid, especially around the excisura and post-rostrum regions. This pattern aligns with the findings of the present study (Figure 5).

Variations in otolith morphology among different species may be linked to their ecological preferences, particularly in terms of depth range and prey availability. These variations also reflect ecomorphological adaptations both to biotic factors, such as food sources, and abiotic factors, such as temperature, within their ecological niches. Interestingly, these adaptations may sometimes take precedence over ontogenetic influences on shape (Tuset et al., 2003; Lombarte et al., 2010; Tuset et al., 2016).

In the present study, *C. lucerna* was found to have larger otoliths than the other gurnard species examined. *C. lucerna* is a demersal species that occupies a broad depth range and exhibits a well-defined pattern of seasonal bathymetric migration during both its juvenile and adult stages. It tends to aggregate in shallower coastal zones during the spring and summer months and then progressively move toward deeper offshore habitats in the winter (Montanini et al., 2017; Carbonara and Follesa, 2019; Ferreira et al., 2024). As a general trend, fish that inhabit deeper waters are expected to have larger otoliths (Tuset et al., 2003). The size and shape of fish otoliths can be influenced by the type of prey consumed, as the nutritional composition of the prey affects the energy and nutrient intake of the fish, which, in turn, impacts somatic growth and otolith development (Mille et al., 2016; Qiao et al., 2022; Park et al., 2023).

Overall, otolith shape exhibits species-specific patterns in fish, making it a valuable tool for taxonomic identification and classification (Avigliano et al., 2018). This approach has been applied in other areas of fisheries biology, where it is recognized as an effective method for distinguishing fish stocks (Campana and Casselman, 1993). Within this study into the differentiation of gurnard species collected from Edremit Bay (Aegean Sea) based on otolith morphology, the pronounced interspecific variability demonstrates the reliability of otolith shape analysis as an efficient method for species identification within this biogeographically significant area.

5. Conclusion

This study has demonstrated the utility of otolith shape and size analyses for distinguishing gurnard species, revealing clear interspecific differences in the otolith morphological features of the investigated species. While modern genetic techniques can provide clear species separation, otolith shape analyses can be considered a more cost-effective and complementary approach. Further studies of how ecological interactions lead to differences in shape and how these differences relate to factors such as habitat, depth distribution, and diet could improve our understanding of gurnard species in the Aegean Sea. The present study revealed some intraspecific variations in otolith shape indices and the results of a wavelet analysis, although future studies in which an elliptic Fourier analysis (EFA) approach is adopted may provide additional insights. Overall, the findings of this study can contribute to species identification, and reveal that otolith morphology can serve as a useful complementary tool in the region.

Data availability

Datasets are available from the author upon reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical statement

No ethical contravention occurred from the beginning to the end of this research (including laboratory work).

Author contributions

AMŞ: Writing – original draft, methodology, formal analysis, data curation, visualization, conceptualization; Dİ: Writing – review and editing, methodology, data curation, conceptualization, supervision; AÜ: Writing – review and editing, investigation, data curation, conceptualization; SA: Writing – review and editing, investigation, data curation, conceptualization.

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Supplementary file

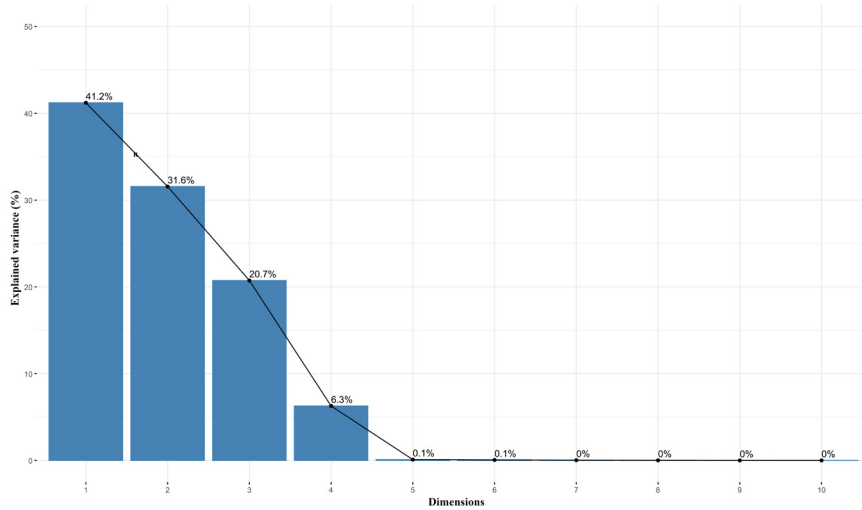


Figure S. Scree plot of explained variance and eigenvalues from PCA.

Table S1. Comparison of left and right otoliths.

Parameters	**n**	**$L_{Sagittae}$**	**$R_{Sagittae}$**	**p > 0.05**
		Mean ± SD	**Mean ± SD**	
OL (mm)	244	3.1926 ± 0.6955	3.1926 ± 0.5286	0.7190
OH (mm)	244	2.4242 ± 0.5274	2.4301 ± 0.5300	0.0705
OP (mm)	244	9.0131 ± 1.9626	8.9880 ± 1.9368	0.1071
OA (mm^2)	244	5.6259 ± 2.4377	5.6402 ± 2.4716	0.2465

Table S2. Descriptive statistics of gurnard otoliths.

	Original data (mm)				**Standardised data (mm)**			
Species	***E. gurnardus***	***C. lastoviza***	***C. lucerna***	***L. cavillone***	***E. gurnardus***	***C. lastoviza***	***C. lucerna***	***L. cavillone***
	Min-max (mean ± SD)	**Min-max (mean ± SD)**	**Min-max (mean ± SD)**	**Min-max (mean ± SD)**	**Min-max (mean ± SD)**	**Min-max (mean ± SD)**	**Min-max (mean ± SD)**	**Min-max (mean ± SD)**
OL (mm)	1.57–3.20 (2.50 ± 0.47)	2.47–3.95 (3.08 ± 0.31)	3.45–5.19 (4.13 ± 0.41)	2.25–3.66 (3.06 ± 0.29)	1.52–3.33 (2.52 ± 0.43)	2.47–3.96 (3.08 ± 0.31)	3.40–5.04 (4.14 ± 0.41)	2.27–3.65 (3.06 ± 0.29)
OH (mm)	1.31–2.56 (2.04 ± 0.37)	1.91–2.71 (2.28 ± 0.18)	2.63–4.07 (3.21 ± 0.29)	1.67–2.55 (2.17 ± 0.19)	1.26–3.01 (2.05 ± 0.34)	1.91–2.71 (2.28 ± 0.18)	2.57–3.95 (3.21 ± 0.28)	1.68–2.57 (2.17 ± 0.19)
OP (mm)	4.48–9.85 (7.54 ± 1.51)	6.96–10.49 (8.55 ± 0.80)	9.60–14.93 (11.80 ± 1.15)	6.03–9.68 (8.20 ± 0.75)	4.31–10.74 (7.59 ± 1.41)	6.95–10.49 (8.55 ± 0.80)	9.35–14.41 (11.82 ± 1.12)	6.07–9.70 (8.20 ± 0.75)
OA (mm^2)	1.49–5.56 (3.70 ± 1.18)	3.58–7.41 (4.96 ± 0.82)	6.42–14.44 (9.30 ± 1.65)	2.49–6.32 (4.59 ± 0.76)	1.40–6.88 (3.73 ± 1.15)	3.59–7.42 (4.96 ± 0.82)	6.14–13.57 (9.30 ± 1.59)	2.52–6.41 (4.60 ± 0.78)
FF	0.67–0.94 (0.80 ± 0.06)	0.73–0.93 (0.85 ± 0.04)	0.71–0.92 (0.84 ± 0.05)	0.78–0.92 (0.85 ± 0.03)	0.67–0.95 (0.80 ± 0.06)	0.73–0.93 (0.85 ± 0.94)	0.70–0.92 (0.84 ± 0.05)	0.73–0.93 (0.85 ± 0.04)
RD	0.64–0.85 (0.74 ± 0.05)	0.55–0.82 (0.67 ± 0.06)	0.61–0.81 (0.69 ± 0.05)	0.53–0.72 (0.62 ± 0.04)	0.64–0.85 (0.73 ± 0.05)	0.55–0.82 (0.67 ± 0.06)	0.62–0.82 (0.69 ± 0.05)	0.55–0.82 (0.67 ± 0.06)
AR	1.09–1.35 (1.23 ± 0.06)	1.13–1.54 (1.35 ± 0.09)	1.13–1.44 (1.29 ± 0.07)	1.28–1.61 (1.41 ± 0.08)	1.10–1.34 (1.23 ± 0.06)	1.13–1.54 (1.35 ± 0.09)	1.13–1.44 (1.29 ± 0.07)	1.13–1.54 (1.35 ± 0.09)
C	13.42–18.78 (15.71 ± 1.23)	13.54–17.29 (14.82 ± 0.73)	13.68–17.79 (15.09 ± 1.05)	13.69–16.19 (14.72 ± 0.51)	13.27–18.85 (15.75 ± 1.14)	13.45–17.24 (14.81 ± 0.72)	13.70–17.84 (15.10 ± 1.05)	13.45–17.24 (14.81 ± 0.72)
R	0.65–0.75 (0.70 ± 0.02)	0.66–0.75 (0.70 ± 0.02)	0.66–0.73 (0.70 ± 0.02)	0.63–0.72 (0.68 ± 0.02)	0.65–0.75 (0.70 ± 0.02)	0.66–0.75 (0.70 ± 0.02)	0.66–0.73 (0.70 ± 0.02)	0.66–0.75 (0.70 ± 0.02)
E	0.05–0.15 (0.10 ± 0.02)	0.06–0.21 (0.15 ± 0.03)	0.06–0.18 (0.13 ± 0.03)	0.12–0.23 (0.17 ± 0.03)	0.05–0.15 (0.10 ± 0.02)	0.06–0.21 (0.15 ± 0.03)	0.06–0.19 (0.13 ± 0.03)	0.06–0.21 (0.15 ± 0.03)

Table S3. Comparison among the species (Kruskal–Wallis test).

Variable	χ^2	df	p value	Significant difference
OL	166.94	3	5.82e-36	****
OH	146.84	3	1.27e-31	****
OA	158.09	3	4.74e-34	****
OP	144.32	3	4.42e-31	****
FF	35.91	3	7.83e-08	****
RD	102.76	3	3.96e-22	****
AR	113.02	3	2.46e-24	****
C	35.91	3	7.83e-08	****
R	24.26	3	2.20e-05	****
E	112.27	3	3.56e-24	****

**** indicates $p < 0.0001$.

Table S4. Dunn's post hoc test results with Bonferroni correction.

Variable	Comparison	Adjusted p-value	Significant difference
OL	*C.lastoviza-C.lucerna*	1.292945e-13	****
	C.lastoviza-E.gurnardus	4.737109e-07	****
	C.lucerna-E.gurnardus	9.148453e-37	****
	C.lastoviza-L.cavillone	1.000000e+00	ns
	C.lucerna-L.cavillone	8.802956e-14	****
	E.gurnardus-L.cavillone	1.879758e-06	****
OH	*C.lastoviza-C.lucerna*	7.252661e-14	****
	C.lastoviza-E.gurnardus	2.666039e-03	**
	C.lucerna-E.gurnardus	1.307482e-27	****
	C.lastoviza-L.cavillone	1.609934e-01	ns
	C.lucerna-L.cavillone	8.849247e-22	****
	E.gurnardus-L.cavillone	1.000000e+00	ns
OA	*C.lastoviza-C.lucerna*	3.238087e-13	****
	C.lastoviza-E.gurnardus	9.771719e-06	****
	C.lucerna-E.gurnardus	4.802478e-33	****
	C.lastoviza-L.cavillone	7.416028e-01	ns
	C.lucerna-L.cavillone	2.821464e-18	****
	E.gurnardus-L.cavillone	8.141217e-03	**
OP	*C.lastoviza-C.lucerna*	1.242554e-14	****
	C.lastoviza-E.gurnardus	4.170450e-03	**
	C.lucerna-E.gurnardus	4.290408e-28	****
	C.lastoviza-L.cavillone	7.946852e-01	ns
	C.lucerna-L.cavillone	9.012873e-20	****
	E.gurnardus-L.cavillone	3.796312e-01	ns
FF	*C.lastoviza-C.lucerna*	1.000000e+00	ns
	C.lastoviza-E.gurnardus	3.391621e-06	****
	C.lucerna-E.gurnardus	8.071564e-04	***
	C.lastoviza-L.cavillone	1.000000e+00	ns
	C.lucerna-L.cavillone	7.426681e-01	ns
	E.gurnardus-L.cavillone	5.073273e-07	****
RD	*C.lastoviza-C.lucerna*	5.166347e-02	ns
	C.lastoviza-E.gurnardus	2.380997e-09	****
	C.lucerna-E.gurnardus	2.133819e-03	**
	C.lastoviza-L.cavillone	1.333995e-03	**
	C.lucerna-L.cavillone	2.986624e-09	****
	E.gurnardus-L.cavillone	7.399398e-22	****
AR	*C.lastoviza-C.lucerna*	1.621566e-03	**
	C.lastoviza-E.gurnardus	1.735723e-12	****
	C.lucerna-E.gurnardus	1.913400e-03	**
	C.lastoviza-L.cavillone	2.831848e-02	*
	C.lucerna-L.cavillone	1.164460e-09	****
	E.gurnardus-L.cavillone	1.296030e-22	****
C	*C.lastoviza-C.lucerna*	1.000000e+00	ns
	C.lastoviza-E.gurnardus	3.391621e-06	****
	C.lucerna-E.gurnardus	8.071564e-04	***
	C.lastoviza-L.cavillone	1.000000e+00	ns
	C.lucerna-L.cavillone	7.426681e-01	ns
	E.gurnardus-L.cavillone	5.073273e-07	****
R	*C.lastoviza-C.lucerna*	1.000000e+00	ns
	C.lastoviza-E.gurnardus	1.000000e+00	ns
	C.lucerna-E.gurnardus	4.610358e-01	ns
	C.lastoviza-L.cavillone	5.841081e-04	***
	C.lucerna-L.cavillone	3.016048e-02	*
	E.gurnardus-L.cavillone	2.862211e-05	****
E	*C.lastoviza-C.lucerna*	1.737582e-03	**
	C.lastoviza-E.gurnardus	2.080076e-12	****
	C.lucerna-E.gurnardus	1.961538e-03	**
	C.lastoviza-L.cavillone	2.895649e-02	*
	C.lucerna-L.cavillone	1.366276e-09	****
	E.gurnardus-L.cavillone	1.770422e-22	****

**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns: $p > 0.05$.

Table S5. Factor loadings of PCA analysis using the Jolliffe criterion (eigenvalue ≥ 1.0).

Variable	**PC1**	**PC2**	**PC3**
OL	0.972	0.123	0.193
OH	0.879	0.445	0.153
OA	0.907	0.353	0.213
OP	0.941	0.325	0.057
FF	–0.272	–0.059	0.945
RD	–0.416	0.907	0.003
AR	0.329	0.908	0.130
C	0.264	0.079	–0.945
R	–0.394	0.485	0.378
E	0.337	–0.906	0.136
Eigenvalues	4.121	3.156	2.074
Variance (%)	41.211	31.561	20.737
Cumulative contribution ratio (%)	41.211	72.772	93.509