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Eco-morphology of sagittal otoliths in five Macrouridae species from Central Mediterranean Sea

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Abstract

The increase in deep-sea exploitation and depletion related to fisheries activities has enhanced the importance of improving the knowledge about deep-sea species. Macrourids are an ecologically essential component of the bathyal community and are among the most abundant species in deep-sea environments worldwide. The present paper aims to investigate the *sagittae* morphology, morphometry, and shape of five Mediterranean Macrouridae species, investigating their intra and inter-specific variability. Shape and morphometric analyses highlighted the absence of directional bilateral asymmetry, with a morphometry and a mean contour shape changing among the investigated species. Despite this, statistically significant similarities were detected between *Coelorinchus caelorhincus* and *Coryphaenoides guentheri*, and between *Nezumia aequalis* and *Nezumia sclerorhynchus*. Otherwise, *Hymenocephalus italicus* showed the most marked differences in *sagittae*' features compared to the other species. The inter specific variability highlighted by results have confirmed the similarity in *sagittae*' shape and morphometry among both phylogenetically close species, and among those sharing similar depth distribution and feeding habits. Further analysis of the genetics, growth dynamics, feeding habits and environmental conditions experienced by species are required to confirm the environmental influence on *sagittae*, also comparing data from different Macrouridae populations.

Keywords Bathyal community, Deep environments, Otolith analysis, Sagittae, Shape analysis, Tyrrhenian Sea

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Introduction

Species belonging to Macrouridae family, commonly known as grenadiers or rattails, are among the most globally abundant for teleost in terms of biomass [1] and number of species (405 of valid species) [2]. These benthopelagic global distributed species inhabit a wide range of environments, from the continental shelves and slopes between 200 and 2000 m [3–5], to the abys-sal plains between 2000 and 6000 m [6, 7]. In the Mediterranean Sea, this family (composed by eight species, belonging to five genera *Coelorinchus*, Giorna, 1809, *Coryphaenoides*, Gunnerus, 1765, *Hymenocephalus*, Giglioli, 1884, *Nezumia*, Jordan, 1904, *Trachyrincus*, Giorna, 1809 [8, 9]) represents an essential component of the bathyal



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community in continental slope environments [10, 11]. Macrouridae species are a dominant component in terms of abundance below the 1000 m of depth [12], with temporal and spatial abundance trends in relation to each species [11]. Macrouridae, despite their low commercial value in Mediterranean basin, are caught by trawling fisheries, representing one of the major components of by-catch in deep-seas shrimps' fisheries [13–15]. The increases of deep environments exploitation and depletion due to fisheries activities [16-18], together with the essential role of species belonging to Macrouridae family for meso- and bathypelagic ecological dynamics [19, 20], have led to an improving attention of scientific community on these species; this could help in monitoring the effects of over-exploitation of demersal species assemblages and inhabited deep-sea habitats.

Otoliths are calcareous structures contained in teleost's inner ears. Both organs (one for side), fundamental in balance and hearing, are composed of three semicircular canals, three end organs (ampullae) and three otoliths' organs (sacculus, utriculus and lagena). These last contain otoliths, sagitta, lapillus and asteriscus, respectively. Sagittae, or sagittal otoliths, are the largest among them in non-ostariophysian fishes [21, 22], and they are widely used in many research fields: in paleontology and palaeoecology, to asses past marine teleost biodiversity and populations [23-26]; in fisheries science, to identify stocks, species and populations through otoliths shape analysis [27-33]; in ecology, being used for prey identification in stomach content analysis, and in ecomorphological studies for their intra-specific variability [34–51]; in taxonomy, for their species-specific morphology [52– 62] and in migratory and life cycle studies through microchemical analysis [63–66].

Several studies have been performed worldwide on otoliths of Macrouridae species [67-71], while in the Mediterranean Sea, studies were mainly focused on their populations structure and growth dynamics [72-74]. The present paper aims to investigate the intra and inter specific sagittal otoliths variability in five Macrouridae species (Hymenocephalus italicus, Giglioli, 1884, Nezumia sclerorhynchus, Valenciennes, 1838, Nezumia aequalis, Günther, 1878, Coryphaenoides guentheri, Vaillant, 1888, Coelorinchus caelorhincus, Risso, 1810) to evaluate the ecomorphological adaptation related to the bathymetric distribution and life habits of the studied species. By comparing otoliths weight and data obtained from shape and morphometrical analysis, it will be possible to evaluate: (i) the efficiency of shape analysis for stock discrimination in Mediterranean Sea Macrouridae species, (ii) the presence of directional bilateral asymmetry, (iii) the relationships between sagittal otoliths morphometries, shape and life habits of the species, and (iv) the reliability of *sagittae* features for species discrimination. Moreover, comparing data from results with those reported in literature from other geographical areas, it is also possible to improve the knowledge base on the intra-specific variability of the studied species at geographical scale. All these information are essential to better understand the taxonomy and ecology of Macrouridae species, and, for a better management and conservation of marine environment and resources of the deep Mediterranean Sea ecosystems.

Matherials and methods

Samples collection

A total of 144 individuals (35 C. guentheri, 20 C. caelorhincus, 24 H. italicus, 24 N. aequalis, 40 N. sclerorhynchus) collected from the Tyrrhenian Sea (Fig. 1) during October 2021 were obtained, already dead, by professional fisherman. The specimens belonging to the different studied species were identified according to the FAO taxonomic key for the identification of demersal teleosts' species [20]. More specifically, the taxonomic features useful for the identification of the different studied species were: (i) the length of the second dorsal's rays (more or less long than the anal ones), (ii) the distance between the anus, the anal fin and the pelvic fin insertion, (iii) the presence/absence of a serration in the second spiny ray of the first dorsal fin, (iv) the presence/absence of a ridge of spiny scales between the mouth and the operculum corner, (v) the rays' number of the pelvic fins, and (vi) the presence of scales in the inferior face of the head (near the mouth).

After landings, specimens were transferred to the laboratory, where each specimen was measured (total length, TL) and weighted (fish body weight, BW) (Table 1), and both sagittal otoliths were extracted. Then, sagittal otoliths were polished from tissues remains using 3% H₂O₂ for 15 min, and Milli-Q water [21, 28, 45]. Once dried, they were weighted and stored in plastic Eppendorf microtubes.

Each left and right sagittal otolith was photographed twice (one photo for each otolith face) under an Axiocam 208 colour camera (Carl Zeiss, Jena, Germany), installed on a stereomicroscope Zeiss Discovery V8 equipped. According to literature [21], the photos of the macular surface were acquired with the otoliths orientated vertically with respect to the longest axis to obtain *sulcus acusticus* images as clear as possible.

Images elaboration and morphometric analysis

ImageJ 1.48p software [75] was used to perform otoliths measurements and to convert images into binary format for contour extraction.



Fig. 1 Map of the studied area, reporting the Tyrrhenian and the Ionian Sea, and highlighted by a red square, the fishing area where the studied specimens have been caught by local fishermen

Table 1 The calculated shape indexes, OL is otolith length (mm), OH is otolith height (mm), OP is otolith perimeter (mm), OS is otolith surface (mm²), SP is sulcus acusticus perimeter (mm), SS is sulcus acusticus surface (mm²), SL is sulcus acusticus length (mm), CL is cauda length (mm), CW is cauda width (mm), OSL is ostium length (mm) and OSW is ostium width (mm)

Shape Indexes	Formulas
Circularity	OP ² /OS
Rectangularity	$OS/(OL \times OH)$
Ellipticity	(OL-OH)/(OL + OH)
Aspect Ratio	OH/OL
Form Factor	4πOS/OP ²
Roundness	4OS/πOL ²
Otolith Length to Total Fish Length Ratio	OL/TL
Sulcus Acusticus Surface to Otolith Surface Ratio	SS/OS
Cauda Length to Sulcus Acusticus Length Ratio	CL/SL
Ostium Length to Sulcus Acusticus Length Ratio	OSL/SL

The performed otoliths measurements have been otolith length (OL, mm), otolith height (OH, mm), otolith perimeter (OP, mm), otolith surface (OS, mm²), otolith weight (OW, g), sulcus perimeter (SP, mm), sulcus surface (SS, mm²), sulcus length (SL, mm), cauda length (CL, mm), cauda width (CW, mm), ostium length (OSL, mm), ostium width (OSW, mm). Several shape indices, reported in Table 1, were also calculated according to literature [28, 55, 56, 76–78]: circularity, rectangularity, ellipticity, aspect ratio, form factor, roundness, otolith length to the total fish length ratio, *sulcus acusticus* surface to otolith surface ratio (expressed in percentage), *cauda* length to *sulcus acusticus* length ratio (expressed in percentage), *ostium* length to *sulcus acusticus* length ratio (expressed in percentage).

Otolith shape analysis

Shaper R (open-source software package running on R version 4.0.5, RStudio 2022.07.1 Build 554; R Gui 4.1.3 2022.03.10) was used to perform the otolith shape analysis, being a specific package for the investigation of the

intra and inter specific otoliths shape variability [79]. ImageJ software (version 1.53 k freely available at https:// imagej.nih.gov/ij/) was used to calibrate each image of the distal sagittae faces by determining, through the measure and setscale functions, how many pixels 1 mm corresponds based on the magnification used. All the images were then binarized by increasing the contrast, using the same software used for the calibration. Contour extraction was obtained using the appropriate detect.outline function of the shapeR package, setting the threshold argument to 0.05, and classifying the extracted outlines according to the individuals and otoliths information (e.g., species, otolith side). The get Measurements function was applied to calculate the otoliths measurements, based on the previously detected outlines. The extraction of Wavelet and Fourier coefficients were performed for the statistical analysis, adjusting them for the analysis of the allometric relationships between otolith shape and fish length. Using the generateShapeCoefficients function, all pictures of otoliths are aligned horizontally along their longest axis prior to transformation, ensuring that their areas are standardized to a value of 1. Polar coordinates are then determined by establishing a horizontal radial axis extending from the otolith centroid (calculated as the mean of the x and y coordinates of the outline) to the right, defining the 0° angle. From this reference point, radials are recorded in a counterclockwise direction up to 360°, maintaining equal angular distances between them. The Wavelet coefficients are derived using the wd and wr functions from the wavethresh R package (version 4.7.3 freely available at https://CRAN.R-project.org/package= wavethresh). To perform Fourier analysis, the Normalized Elliptic Fourier method is utilized via the iefourier and efourier function [80], which normalize the otoliths in terms of size and rotation before extracting the coefficients. The 10 Wavelet levels set by default in the package-specific function give a total of 64 Wavelet coefficients using the Daubechies least-asymmetric Wavelet [81], as well as 12 harmonics give 45 Elliptic Fourier coefficients that are normalized in relation to size and rotation. The coefficients that showed interaction between species and lengths (p < 0.05) were automatically omitted (4 Wavelet coefficients were removed) using the stdCoefs function to adjust the otolith shape based on allometric relationships with fish lengths [82].

The comparison between the mean *sagittae* shape of the analyzed species were obtained using Wavelet coefficients that allow the detection of shape differences in specific regions which might be located at a certain angle on the perimeter of the otolith. Such localized differences often cannot be detected based on analysis of Fourier coefficients, which provide overall information on otolith shape differences [79]. The deviation of the coefficients reconstruction from the otolith outline was analyzed to estimate the quality of the reconstruction (Supplementary materials 1_Figure S1). Finally, a g-plots R package's specific function was used to investigate how the position along the outline can influence the Wavelet coefficients variation (Supplementary materials 2_Figure S2).

Data analysis

Univariate and multivariate statistical methods were applied to conduct investigations on *sagittae* using Prism V.8.2.1 (Graphpad Software Ltd., La Jolla, CA 92037, USA), R vegan package V.2.5, and PAST V.4.

An unpaired t-test was used as a tool to investigate the occurrence of differences in morphometric parameters between right and left otoliths. Any otolith morphometric variations between the different species investigated were detected, after checking the normality and homogeneity of the variance, using a one-way analysis of variance (one-way ANOVA) followed by the Tukey's HSD test, and Linear Discriminant Analysis (LDA). Additionally, the correlation between the measured parameters and fish body weight (BW) and total length (TL) was tested using the Pearson correlation coefficient.

To explore the variation of otolith contours between the specimens, the shape indices were extrapolated and standardized Wavelet coefficients analyzed through an ANOVA-like permutation test and a Linear Discriminant Analysis (LDA) to obtain an overview of the differences in otolith shape between the species examined. The ANOVA results were confirmed and deeply investigated using a post-hoc PERMANOVA test to detect the significant differences between species.

Results

Morphometric and shape analysis

All the analyzed *sagittae*, belonging to the collected specimens (Table 2), have been described according to the terminology of Assis, Nolf, and Tuset et al. [23, 57, 83].

Hymenocephalus italicus specimens showed an overall elliptical and lobed shape of *sagittae*, with irregular margins slightly lobed anterodorsally, and generally equals length and heigh (Fig. 2A—E).

Table 2Number of analyzed specimens (N), with the meantotal length (TL) and mean body weight (BW), reported for eachstudied species

	N	TL, mm	BW, g
H. italicus	24	91.87	2.46
N. sclerorhynchus	40	148.37	8.54
N. aequalis	24	139.79	7.98
C. guentheri	35	183.57	20.16
C. caelorinchus	20	164	17.25

The maximum length was infra median, while the maximum heigh was pre median. Both the dorsal and ventral margins were deeply asymmetric and convex. The dorsal one was crenate and slightly lobed, while the ventral was flat and smooth (Fig. 2B, C, E). The posterior region was bifid and slightly sharp, while the anterior was irregular to double peaked. The external face was concave, while the internal was convex. Rostrum and antirostrum were both triangular, small, and almost of the same size. Rostrum was generally longer than the antirostrum, antero-dorsally oriented. The excisura ostii was asymmetric, pointed and generally small. Excisura caudalis was deeper or deep as the excisura ostii. Pseudorostrum and pseudoantirostrum were triangular and almost of the same size, more dorsally oriented and pointed than rostrum and antirostrum. Sulcus acusticus was archaeosulcoid, median, with indistinct cauda and ostium, and a horizontal orientation (Fig. 2 A, D). The ostium and cauda measurements were not performed in sagittal otoliths of H. italicus specimens due to the peculiar sulcus acusticus structure showed by this species. Indeed, this is characterized by the absence of separation between ostium and cauda which make it impossible to measure separately their length and width [71]. The morphometrical parameters of sagittae calculated for H. italicus specimens are summarized in Table 3. The unpaired t-test did not detect the presence of bilateral asymmetry.

Nezumia sclerorhynchus specimens showed longer than higher *sagittae*, with an approximately oval overall shape. The general morphology of irregular polygon was characterized by a visible different angulation of the five sides (Fig. 3A—E).

The maximum *sagittae* length was not perfectly median in all the specimens, and the maximum heigh was always pre medial. The posterior region was more pointed than the anterior, that was characterized by a bilobed anterodorsal part (Fig. 3B, C). The dorsal and the ventral regions of the *sagittae* were both with convex margins, and an evident crenulation in the dorsal one. The *rostrum* was rounded and not clearly defined, and the anterior region of *sagittae* characterized by the absence of *antirostrum* and *excisura ostii* (Fig. 3B, C, E). *Sulcus acusticus* was generally homosulcoid and median, with *cauda* and *ostium* both oval, straight, almost equal in length and heigh (Fig. 3A). The *cauda* was characterized by a visible distance of its ending part from the posterior otolith's margin (Fig. 3D).

The morphometrical parameters of *sagittae* calculated for *N. sclerorhynchus* specimens are summarized in Table 3. The unpaired t-test did not detect the presence of a marked bilateral asymmetry, despite SS/OS% parameter varied significantly between the right and left side (p < 0.001).



Fig. 2 Medial view (**a**, **b**) and distal view (**c**) of right *sagitta* of *H. italicus*, with the reconstruction of the *sulcus acusticus*' outline (**d**) and the mean otoliths'shape (**e**); the outline in figure a represents the *sulcus acusticus*; *a* = antirostrum, *r* = rostrum, *ec* = excisura caudalis, *eo* = excisura ostii, *pa* = pseudoantirostrum, *pr* = pseudorostrum, *sa* = sulcus acusticus; scale bar: 1 mm

Table 3 Morphometric mean values of right sagittae of the investigated species with standard deviation (SD) and minimum (Min.) and maximum (Max.) range: otolith length (OL), otolith height (OH), otolith perimeter (OP), otolith surface (OS), otolith weight (OW), sulcus perimeter (SP), sulcus surface (SS), sulcus length (SL), sulcus height (SH), cauda length (CL), cauda width (CW), cauda perimeter (CP), cauda surface (CS), ostium length (OSL), ostium width (OSW), ostium perimeter (OSP), ostium surface (OSS), circularity (C), rectangularity (Re), ellepticity (E), aspect ratio (AR), form factor (FF), roundness (Ro), otolith length to total fish length ratio (OL/TL), sulcus acusticus surface to otolith surface ratio (SS/OS, expressed as percentage), cauda length to sulcus acusticus length ratio (CL/SL, expressed as percentage) and ostium length to sulcus acusticus length ratio (OSL/SL, expressed as percentage)

	H. italicus	N. sclerorhynchus	N. aequalis	C. guentheri	C. caelorinchus
	Min.—Max	Min.—Max	Min.—Max	Min.—Max	Min.—Max
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
OL	2.58—4.77	3.65—7.78	5.37 ± 1.22	5.26—10.21	6.38—9.4
	3.66 ± 0.6	5.42 ± 1.18	3.62—7.28	8.29 ± 1.10	7.76 ± 0.83
ОН	2.76—4.81	2.25—5.06	2.25—4.8	5.27 ±0.55	4.5—5.9
	3.88 ±0.58	3.45 ±0.69	3.46 ± 0.8	3.79—6.18	5.1 ±0.37
OP	9.97—18.88	10.98—28.81	10.61—21.33	16.52—28.69	23.85 ± 1.95
	14.48 ± 2.45	17.12 ±4.16	15.93 ±3.34	24.87 ± 3.05	19.41—27.25
OS	5.15—14.8	5.92—28.52	5.66—24.42	13.24—40.81	18.85—35.88
	9.81 ±2.80	13.75 ±5.73	13.6 ± 5.93	30.05 ±6.71	27.25 ±4.74
ow	0.01—0.04	0.01—0.06	0.01 - 0.06	0.02 - 0.14	0.04—0.11
	0.02 ±0.01	0.02 ±0.01	0.02 ± 0.01	0.08 ± 0.03	0.07 ± 0.02
SP	3.61—6.92	2.82—13.12	3.97—12.20	8.1—17.8	11.78—20.23
	5.15 ±0.97	7.63 ±2.39	6.91 ±2.36	13.87 ±2.62	15.1 ± 2.79
SS	0.76—2.2	0.28—7.09	0.44—4.97	1.18—10.63	3.84—13.51
	1.33 ±0.45	2.22 ±1.65	1.71 ± 1.22	5.97 ±2.36	7.38±3.25
SL	1.49—3.13	1.29—5.61	1.71—5.17	3.85—8.34	4.5—8.99
	2.25 ±0.46	3.40 ±1.04	3.02 ± 1	6.41 ± 1.2	6.7 ± 1.23
SH	0.53—1.01 0.76 ±0.14	_	_	_	-
CL	-	0.63—2.4 1.48 ±0.44	0.68—2.09 1.3 ±0.43	0.87—3.53 2.57 ±0.7	2.32—3.99 3.07±0.57
СН	-	0.27—1.49 0.67 ±0.27	0.25 - 1.31 0.68 ± 0.26	0.3—1.44 0.96 ±0.34	0.6—1.81 1.02±0.33
СР	-	1.44—6.08 3.6 ± 1.1	1.83—5.04 3.16 ± 1.04	2.09—8.31 5.86 ± 1.62	5.05—9.98 7.14 ± 1.51
CS	-	0.1—2.6 0.77 ±0.55	0.14—1.53 0.6±0.4	0.18—3.66 1.78 ±0.9	1.07—5.17 2.57 ± 1.2
OSL	-	0.65—2.80 1.71 ±0.54	0.93—2.66 1.59±0.54	1.33—4.5 3.11 ±0.77	2.3—4.77 3.47 ±0.82
OSH	-	0.22—1.55 0.75 ±0.33	0.3—1.44 0.72 ±0.32	0.26—1.94 1 ±0.33	0.7—1.68 1.05 ±0.3
OSP	-	1.48—6.89 4.09 ± 1.34	2.09—6.65 3.77 ± 1.27	2.93—10.55 6.96 ± 1.74	5.4—11.97 8.04 ± 2.09
OSS	-	0.08-3 1.01 ±0.73	0.19—2.69 0.82±0.64	0.31—5.87 2.35 ± 1.2	1.44—6.87 3.19 ± 1.66
с	18.36—25.24	17.93—52.51	17.44—27.12	19.02—23.3	19.2—26.89
	21.75 ± 1.94	22.36 ±5.35	19.82 ± 1.79	20.86 ± 1.05	21.11 ± 1.75
Re	0.65—0.71	0.68—0.75	0.66—0.72	0.63 - 0.72	0.65—0.72
	0.68 ±0.02	0.71 ±0.02	0.7 ±0.02	0.68 ± 0.02	0.68 ±0.02
E	-0.07 - 0	0.18—0.31	0.15—0.28	0.16—0.27	0.17—0.27
	-0.03 ± 0.02	0.22 ±0.03	0.22 ±0.03	0.22 ±0.03	0.21 ±0.03
AR	0.99—1.16	0.53—0.7	0.56 - 0.74	0.57 - 0.72	0.57—0.7
	1.07 ±0.05	0.64 ±0.04	0.65 ± 0.04	0.64 ± 0.04	0.66 ±0.04
FF	0.5—0.69	0.24 - 0.7	0.46—0.72	0.54—0.66	0.47—0.66
	0.58 ±0.05	0.58 ± 0.08	0.64 ±0.05	0.6 ±0.03	0.6±0.04
Ro	0.83—1.04	0.47—0.62	0.49—0.66	0.48—0.63	0.52—0.62
	0.92±0.06	0.57 ± 0.04	0.57 ±0.04	0.55 ±0.04	0.57 ±0.03
OL/TL	0.03—0.05	0.03 - 0.05	0.03—0.05	0.04—0.06	0.03—0.06
	0.04 ±0.01	0.04 ± 0.004	0.04 ±0.004	0.05 ±0.004	0.05 ±0.01

	H. italicus	N. sclerorhynchus	N. aequalis	C. guentheri	C. caelorinchus
SS/OS%	0.1—0.17 0.14 ±0.02	4.71—28.64 15.05 ± 5.86	0.06—0.2 0.11 ±0.04	0.07—0.3 0.19±0.05	16.51—38.5 26.11 ±7.63
CL/SL%	-	35.35—52.88 43.9 ±4.23	0.36—0.51 0.43 ±0.05	0.19—0.53 0.4 ± 0.06	39.92—81.21 46.39 ± 9.01
OSL/SL%	-	41.59—59.54 50.19 ± 3.95	0.4—0.82 0.53 ±0.09	0.3—0.56 0.48±0.06	43.2—101.22 52.22 ±12.27

Table 3 (continued)



Fig. 3 Medial view (**a**, **b**) and distal view (**c**) of right *sagitta* of right *sagitta* of *N. sclerorhynchus*, with the reconstruction of the *sulcus acusticus'* outline (**d**) and the mean otoliths'shape (**e**); the outline in figure a represents the *sulcus acusticus* with the *colliculi* (coloured in blue). *cc* = culliculum cauda, *co* = colliculum ostii, *r* = rostrum, *pr* = pseudorostrum, *sa* = sulcus acusticus; scale bar: 1 mm

Nezumia aequalis specimens showed an approximately oval *sagitta*, with a slightly polygonal morphology, characterized by lobed margins, more irregulars in the dorsal region than in the ventral one. The polygonal morphology was highlighted by the presence of five differently angled sides (Fig. 4A—E).

The maximum *sagittae* length was not perfectly median in all the specimens, and the maximum heigh was always pre medial. The ventral region was pointed, while the anterior one was rounded and asymmetric (Fig. 4B, C, E). The dorsolateral part of the dorsal region was bilobed, while the ventral part was curved and deeply irregular. *Rostrum* was scarcely visible and rounded, while the *antirostrum* was totally absent, such as, consequently, the *excisura ostii* (Fig. 4B, C, E). The mesial *sulcus acusticus* was slightly heterosulcoid, with larger *cauda* than the *ostium*, located in a median position (Fig. 4A, D).

The morphometrical parameters of *sagittae* calculated for *N. aequalis* specimens are summarized in Table 3. The unpaired t-test did not detect the presence of bilateral asymmetry.

Coryphaenoides guentheri specimens showed a pentagonal shape, characterized by a triangular dorsal region and a trapezoidal ventral one (Fig. 5A—E).

The maximum otolith length was median, while the maximum heigh was pre median. The dorsal margin was irregular and slightly crenulated, while the ventral one was smooth. Both the posterior and the anterior regions were pointed (Fig. 5B, C, E). The *rostrum* was small, rounded and anteriorly directed, not always clearly



Fig. 4 Medial view (**a**, **b**) and distal view (**c**) of right *sagitta* of *N. aequalis*, with the reconstruction of the *sulcus acusticus*' outline (**d**) and the mean otoliths'shape (**e**); The outline in figure a represents the *sulcus acusticus* with the *colliculi* (coloured in blue). cc = culliculum cauda, co = colliculum ostii, r = rostrum, pr = pseudorostrum, sa = sulcus acusticus; scale bar = 1 mm



Fig. 5 Medial view (**a**, **b**) and distal view (**c**) of right *sagitta* of *C. guentheri*, with the reconstruction of the *sulcus acusticus*' outline (**d**) and the mean otoliths'shape (**e**); The outline in figure a represents the *sulcus acusticus* with the *colliculi* (coloured in blue). cc = culliculum cauda, co = colliculum ostii, cp = canalis postcaudalis, pc = pseudocolliculum, r = rostrum, pr = pseudorostrum, sa = sulcus acusticus; scale bar = 1 mm

differentiated. *Extremo posterior* was slightly pointed, median and horizontal. *Sulcus acusticus* was very large, pseudo-ostiocaudal, medial and horizontal. *Cauda* and *ostium* were approximatively of the same shape, with *ostium* slightly longer than *cauda* (Fig. 5A). It was detected the presence of a *canalis postcaudalis*, a *colliculum* heteromorphico and a medial *pseudocolliculum* (Fig. 5B).

The morphometrical parameters of *sagittae* calculated for *C. guentheri* specimens are summarized in Table 3. The unpaired t-test did not detect the presence of bilateral asymmetry.

Caelorhincus caelorhincus specimens showed *sagittae* longer than higher, with a pentagonal shape, characterized by sides differently angled, a triangular dorsal region and a trapezoidal ventral one. The posterior and anterior region were pointed, with the posterior longer and more pointed than the anterior one (Fig. 6A - E).

The maximum otolith length was median, while the maximum heigh was pre median. Dorsal and ventral margins were not symmetric and both convex. The margins were crenulated, with crenulations more evident in smaller otoliths (Fig. 6B, C, E). While larger otoliths were smoother than the smaller ones. The *rostrum* was short, rounded in median position, while *antirostrum* and excisura *ostii* were not differentiated. *Extremo posterior*

was rounded, median and horizontal. The *sulcus acusticus* was superficial, heterosulcoid, horizontal and median. *Cauda* was longer than *ostium*, and both were rectangular and with the same height (Fig. 6A, D).

The morphometrical parameters of *sagittae* calculated for *C. caelorhincus* specimens are summarized in Table 3. The unpaired t-test did not detect the presence of bilateral asymmetry, despite CL/SL% varied significantly between the right and left side (p < 0.001).

Inter-specific differences in morphology and shape

Since no striking differences in the morphometry of the *sagittae* were detected between the right and left sides, only the morphometric parameters of the right otoliths were used and subjected to ANOVA to evaluate the interspecific variations.

Generalizing, some species have shown similar characteristics, for example *C. caelorhincus* and *C. guentheri*, or even the congeneric species *N. aequalis* and *N. sclerorhynchus. H. italicus*, on the other hand, appeared more dissimilar from the other species investigated. This was already evident from the comparison of specimens body parameters, such as total length and body weight. The detailed results obtained through the ANOVA are reported in Table 4.



Fig. 6 Medial view (**a**, **b**) and distal view (**b**) of right *sagitta* of *C. caelorhincus*, with the reconstruction of the *sulcus acusticus*' outline (**d**) and the mean otoliths'shape (**e**); The outline in figure a represents the *sulcus acusticus* with the *colliculi* (coloured in blue). cc = culliculum cauda, co = colliculum ostii, r = rostrum, pr = pseudorostrum, sa = sulcus acusticus; scale bar = 1 mm

The investigated species show clear differences in the parameters of the otoliths (OW, OS, OP, OL, OH), *sulcus acusticus* (SS, SP, SL), *ostium* (OS, OP, OL) and *cauda* (CS, SP). The most recurrent pattern is the similarity between *C. caelorhincus* and *C. guentheri*, between *N. aequalis* and *N. sclerorhynchus* and finally *H. italicus* which distances itself from the other two groups, as highlighted by the LDA (Fig. 7).

Results from Pearson correlation between sagittae and body parameters (TL and BW) of the analyzed specimens are reported in Supplementary materials 3_Table S1. In C. caelorhincus specimens, the presence of significant correlations was observed between TL and OW, OH/OL, OL/TL, SS/OS and OSL/SL, and between BW and OW, OH/OL, OL/TL and SS/OS. The analyzed C. guentheri specimens showed significant correlations between TL, OW and OH/OL, and between BW and OW, OH/OL, and SS/OS. In N. aequalis, TL showed significant correlations with OW, OL/TL and SS/OS, while BW with OW and SS/OS. In the analyzed N. sclerorhynchus specimens, both TL and BW was significantly correlated to OW, OL/ TL, SS/OS and CL/SL, while the TL values of *H. italicus* were significantly correlated to OW and SS/OS, differently to BW, which was not correlated to SS/OS.

A comparison among the mean contours of the studied species, obtained from the shape analysis performed at inter specific level, is provided in Fig. 8. The shape *H. italicus* specimens showed the most different shape of the *sagittae*, with a peculiar oval contour and prominent dorsal al posterior region, visibly lobed. *C. caelorinchus* and *C. guentheri* showed a similar contour, characterized by a pentagonal shape, with differences in the prominence of the *rostrum*. Conversely, both *N. aequalis* and *N. sclerorhynchus* showed an approximately overall oval shape, with differences in the irregularity of the margins.

The results of the ANOVA performed on the shape indices highlighted the differences between the investigated species, showing the same pattern observed in the measurements of the *sagittae* previously mentioned. The results were also confirmed by LDA (Fig. 9), which showed a greater distance for the *H. italicus* species, and by the post-hoc PERMANOVA test, which showed a significant similarity (p > 0.05) between *C. caelorinchus* and *C. guentheri*, and between *N. aequalis* and *N. sclerorhynchus* (Supplementary materials 4_Table S2).

Discussion

Findings of the present paper reported an overall morphology of the investigated *sagittae* not in line with data from literature for the same species, with differences on both morphometry and morphology. Despite this, results from the shape analysis confirmed its reliability for the discrimination of the main otolith contour in the studied species.

Hymenocephalus italicus specimens from the western and central Mediterranean Sea show smaller values of aspect ratio, circularity and rectangularity [57] than those reported in the present study. The differences were longer sagittae, with higher surface and perimeter values, and a more oval shape, in *H. italicus* specimens analyzed in the present study. Also, smaller rostrum, less pointed, and most prominent antirostrum, pseudorostrum and pseudoantirostrum, differentiated the sagittae of the H. italicus from the Tyrrhenian Sea from those reported in literature from western and central Mediterranean Sea, and Atlantic Portuguese waters [83]. The descriptions provided by Schwarzans [71] highlighted a marked variability in sagittae compression and margins regularity between eastern, western Atlantic Ocean and Indian Ocean. As suggested by results, the analyzed specimens showed dorsal and ventral margins generally smooth, in line with those from western Atlantic Ocean, described by Schwarzans [71].

Regarding N. sclerorhynchus, specimens showed morphometrical values of aspect ratio, circularity, and rectangularity much higher than those reported from Western and central Mediterranean Sea, resulting in a more oval and less polygonal sagittae shape described in literature than those obtained by results of the present study [57]. Otherwise, specimens from the off Portuguese West coast [84] present longer and heavier otoliths, resulting in more lanceolate to oval sagittae, with a very pointed rostrum, as confirmed also by description of Assis from Atlantic Portuguese waters [83]. Differently, specimens form Northwest Atlantic Ocean, described by Campana [85], are characterized by *sagittae* with a more regular oval shape and smoother margins than those from the studied area. Also N. aequalis specimens showed much higher values for aspect ratio, circularity and rectangularity than those reported in literature from the western and central Mediterranean Sea [57], resulting in oval and high sagittae. Differently, specimens from Portuguese waters [83, 84] described in literature have longer sagittae, characterized by a more pointed posterior region and a more lanceolate shape.

Coryphaenoides guentheri individuals showed aspect ratio values similar to those reported from western and central Mediterranean Sea [57], with marked differences in circularity and rectangularity. These differences in shape indexes result in a different shape, with specimens from the studied area that exhibited a pentagonal shape, very distant from the oval one reported by Tuset et al. [57] and by Campana from the Northwest Atlantic Ocean [85]. Also *C. coelorhincus* specimens showed

Table 4 Results of ANOVA carried out on the investigated species between morphometric parameters of right sagittae, with significant results setted at <i>p</i> < 0.05 (otolith
length (OL), otolith height (OH), otolith perimeter (OP), otolith surface (OS), otolith weight (OW), sulcus perimeter (SP), sulcus surface (SS), sulcus length (SL), sulcus height (SH),
cauda length (CL), cauda width (CW), cauda perimeter, CP, cauda surface, CS, ostium length, OSL, ostium width, OSW, ostium perimeter, OSP, ostium surface, OSS, circularity, C,
rectangularity, Re, ellipticity, E, aspect ratio, AR, form factor, FF, roundness, Ro, otolith length to total fish length ratio, OL/TL, sulcus acusticus surface to otolith surface ratio, SS/OS,
expressed as percentage, cauda length to sulcus acusticus length ratio, CL/SL, expressed as percentage, and ostium length to sulcus acusticus length ratio, OSL/SL, expressed as
percentage)

	201									
	C. <i>caelorhincus</i> vs C. guentheri	C. <i>caelorhincus</i> vs N. aequalis	C. caelorhincus vs N. sclerorhyncus	C. caelorhincus vs H. italicus	C. guentheri vs N. aequalis	C. guentheri vs N.sclerorhyncus	C. guentheri vs H. italicus	<i>N. aequalis vs</i> N. sclerorhyncus	N. <i>aequalis vs</i> H. italicus	N. sclerorhyncus vs H. italicus
	p value	p value	p value	p value	p value	p value	p value	p value	p value	p value
MO	0.1323	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	> 0.9999	0.9649	0.9437
os	0.3744	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	> 0.9999	0.1285	0.051
ОР	0.7999	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	0.6132	0.5354	0.0168*
OL	0.3828	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	0.9998	< 0.0001*	< 0.0001*
НО	0.863	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	> 0.9999	0.1412	0.0658
Ro	0.3395	> 0.9999	> 0.9999	< 0.0001*	0.3519	0.1383	< 0.0001*	0.9996	< 0.0001*	< 0.0001*
Ë	0.3562	0.9997	> 0.9999	< 0.0001*	0.4177	0.1892	< 0.0001*	0.9997	< 0.0001*	< 0.0001*
ш	0.5478	0.8241	0.4618	< 0.0001*	0.9947	> 0.9999	< 0.0001*	0.986	< 0.0001*	< 0.0001*
U	0.9986	0.6618	0.6025	0.9624	0.7284	0.2486	0.8231	0.0191*	0.2187	0.9463
Re	0.8184	0.1241	0.0001*	0.8325	0.0016*	< 0.0001*	> 0.9999	0.2825	0.0039*	< 0.0001*
AR	0.4613	0.8082	0.4975	< 0.0001*	0.9868	> 0.9999	< 0.0001*	0.9937	< 0.0001*	< 0.0001*
OL/TL	0.3775	0.0006*	< 0.0001*	0.0061*	0.0472*	< 0.0001*	0.2426	0.7119	0.9636	0.2667
SS	0.0832	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	0.8527	0.9629	0.4061
SP	0.3386	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	0.7553	0.0746	0.0006*
SL	0.8392	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	0.6038	0.0811	0.0003*
os	0.0295	< 0.0001*	< 0.0001*		< 0.0001*	< 0.0001*		0.9057		
ОР	0.0813	< 0.0001*	< 0.0001*		< 0.0001*	< 0.0001*		0.8592		
oL	0.2232	< 0.0001*	< 0.0001*		< 0.0001*	< 0.0001*		0.9		
Ю	0.9402	0.0059*	0.0053*		0.0088*	0.0066*		0.9864		
S	0.0023*	< 0.0001*	< 0.0001*		< 0.0001*	< 0.0001*		0.8409		
P	0.005*	< 0.0001*	< 0.0001*		< 0.0001*	< 0.0001*		0.5673		
Ъ	0.0079*	< 0.0001*	< 0.0001*		< 0.0001*	< 0.0001*		0.5469		
Н	0.8948	0.0021*	0.0003*		0.0043*	0.0004*		0.9983		
SS/OS %	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	0.0083*	0.0008*	0.0518	0.603	0.7848
% CL/SL %	0.0006*	0.2639	0.4308		0.1369	0.0132*		0.9493		
% OSL/SL	0.2256	0.9453	0.7521		0.0433*	0.6615		0.3255		
* <i>p</i> <0.05										



Fig. 7 Linear Discriminant Analysis (LDA) between morphometric parameters (OW, OS, OP, OL, OH, SS, SP, SL, OS, OP, OL) and cauda (CS, SP).) of the investigated species



Fig. 8 Mean shape of sagittae contours of the investigated species, based on standardized Wavelet coefficients. CC is C. coelorhiincus, CG is C. guentheri, HI is H. italicus, NA is N. aequalis, NS is N. sclerorhynchus

similar values of aspect ratio and highly different values of circularity and rectangularity than those reported in literature from the western and central Mediterranean Sea [57]. The overall shape showed also an evident variability, with specimens from the studied area that exhibited a pentagonal shape, clearly different from the elliptic to trapezoidal one reported in literature by Tuset et al. [57]. Otherwise, comparing results to data from the Portuguese Atlantic waters [83], it appears evident a similarity between the *sagittae* of the two populations. Both are characterized by a polygonal shape, with differences in the organization of *rostrum* and *antirostrum*.

These discrepancies between literature data [23, 57, 71] in *sagittae* morphology and morphometry, and those showed by our results could be related to several aspects (e.g., differences in analyzed samples number and size



Fig. 9 Linear Discriminant Analysis (LDA) between Wavelet descriptors calculated for the investigated species. Ellipses include 95% confidence interval

class composition, differences in environmental features and species' life habits between the different geographical areas). However, to completely assess, quantify and describe these differences, they are necessary direct analyses on *sagittae* belonging to a statistically significant sample of specimens inhabiting different geographical areas. The comparison with literature data, despite it highlights the presence of morphological heterogeneity in sagittae, it is not enough to quantify and describe this variability at population level. Moreover, genetic, trophic and population studies, at the largest as possible temporal scale, are required to find significant correlations between otolith variability and population features. According to literature, the inter population sagittae differences can be related to the genetic variability among the populations of the studied species, and to the differences in environmental conditions experienced by individuals inhabiting different geographical areas (such as the water temperature, that is lower in the Atlantic and Western Mediterranean, than in the Tyrrhenian Sea) [86, 87]. It is impossible to find direct correlations explaining the differences detected with literature data useful to explain how much these are related to geographical changes in environmental features, rather than inter population changes in life habits or genetic. Exploring valuable literature dealing with this topic, it is widely reported how otoliths are influenced by both genetic and environmental habitats' conditions [88]. According to Vignon and Morat [88], the overall otoliths' form is regulated by genetics, while the quantity of deposited calcium carbonate during otolith formation is under environmental control. Indeed, somatic and otolith growth (which influence otoliths' shape, morphology and morphometry) are deeply related to metabolic expression and physical constrain, which in turn are sensitive to environmental conditions, such as water temperature, food availability and composition, depth and soundscape organization and complexity [89-93]. The interactions between genetic and environment could act as a driver, resulting in an inter population variability of sagittae shape and morphometry, as highlighted by the phenotypic variations that allow to the better adaptability of individuals to different habitats [94]. As stated by Vignon and Morat [88], several otoliths morphometrical features (such as the presence of antirostrum and the morphology of the ros*trum*) are under the genetic control, while overall otolith contour can be shaped by contrasting environmental features. The absence of literature data regarding shape analyses performed on different Macrourids' populations make difficult to quantify and identify the contour differences allowing to the inter population variability in grenadiers' species. Data from literature deal with the general otolith's morphology and morphometry [57, 71, 83-85], but, according to several authors [28, 95, 96], shape indexes can be considered as good indicators for stock and populations separation in several species. Moreover,

Macrourids species show a high plasticity in feeding habits and depth distribution between different geographical area [11, 73, 97, 98]. They are considered as generalist feeders which can adapt their diet to the preys' availability experienced in the different areas, an essential adaptation to the low productive deep environment inhabited by these species [19, 99, 100]. Also exploring their genetic structure, species belonging to Macrouridae family can exhibit significant degrees of genetic divergence between different geographic areas [101, 102]. The detected variability in sagittae features, added to the potential heterogeneity in environmental conditions between geographical areas, could suggest the presence of different populations and stocks of the studied species. Otherwise, the absence of genetic and life habits data supporting this assumption make impossible to confirm it. According to literature, high inter population variability it was reported for other Macrouridae species between the Atlantic and Pacific Oceans [103], with species from the Atlantic Ocean which showed larger *sagittae* than the Pacific one. They were also detected differences related to the bathymetric distribution and the geographic distribution of the analyzed Macrourids species, between the ratio of otolith length and head length. Indeed, authors reported a significant decrease in species inhabiting grater depths and significant differences between specimens of Coryphaenoides yaquinae Iwamoto & Stein, 1974 inhabiting Eastern and Western Pacific Ocean. These differences in size were related to environmental factors, and specifically with depth, being the Pacific populations distributed at grater depths than the Atlantic ones and being Atlantic waters warmer than the Pacific ones. This was related to differences in growth rates between shallow and deep populations. Indeed, it is well known as fast growth is reported for teleost species and populations inhabiting warmer, more eutrophic, superficial waters [104-106]. Somatic growth rate strongly influences the otoliths growth, resulting in larger sagittae reported for species characterized by a fast growth [93]. Improve the knowledge base on the populations structure of these species within the Mediterranean basin, elucidating their growth dynamics and sagittae features, is essential for conservation porpoises, being Macrourids fundamental for the well-being of the deep marine ecosystems worldwide.

At inter specific level, present paper confirmed the reliability of *sagittae* to discriminate between the different Macrouridae species, assessing the high variability in morphology, morphometry, and shape between the investigated species. These findings were in line with those reported by Moore et al. [61], which demonstrated, in the geographical area of Ross and Amundsen Sea, the

accuracy of otolith shape as a rapid, simple, and reliable tool for species differentiation between two grenadiers' species, Macrourus caml, McMillan, Iwamoto, Stewart & Smith, 2012, and Macrourus whitsoni, Regan, 1913. Other authors successfully applied sagittae shape analysis and otolith morphometry to identify cryptic species, such as Tuset et al. [107] for rockfishes (Sebastidae family), Sadighzadeh et al. [108] for snapper species (Lutjanidae family) and Lombarte et al. [54] for Mediterranean Sea gobies (Gobidae family). A correct identification at species level is fundamental, especially for the main harvested teleost with a high ecological value and diversity, such as Macrouridae family. For instance, in the fisheries sampling programs, the accuracy of species identification can affect the reported catch and landings by vessels, the biological data collection and, consequently, the efficiency of the entire management design (see Moore et al. [61], and references therein). The morphological identification of the studied species (especially discern among species belonging to Nezumia genus, and between Coelorhincus and Coryphaenoides species), and of many of those belonging to the Macrouridae family, can be challenging and time consuming especially for an untrained eye. Otoliths can strongly improve this process, giving a reliable, and relatively rapid, tool for a correct species discrimination. This is essential for the validity of fisheries programs dealing with grenadiers' species conservation, being their fisheries widely diffused, either as target species or as by-catch, world-wide [11, 18]. Moreover, these species play a vital role in several deep marine communities, being close to the top predator in the food chain, controlling preys' populations, being among the main preys of several demersal predators [35, 36, 109], and influencing the dynamics of the entire communities [19]. For all these reasons the conservation and discrimination of Macrourids' populations is fundamental for the wellbeing of the deep communities and, consequently, for the entire marine ecosystem.

The main inter specific differences between the investigated species were related to the *sulcus acusticus*, the general otolith morphology and the mean contours. The similarity detected between congeneric species (*N. aequalis* and *N. sclerorhynchus*) or phylogenetically close genera (*Coelorhynchis* and *Coryphaenoides*), which constituted two statistically significant and clearly detectable patterns of similarity, was in line with literature [110– 112]. It is widely reported for several teleost groups (e.g., gobies, rockfishes, Scianidae species) [54, 107, 113, 114] how both phylogenetic divergences, together with environmental factors and life habits, have a strong effect on otoliths' shape and their morphological/morphometrical

features. C. coelorhynchis and C. guentheri, in addition to be phylogenetically close, share several aspects of their life habits, such as the feeding strategy, being both benthic predators [97, 99], and the distribution depth, being both mainly distributed, and with highest abundance values, between 400 and 500 m in the Mediterranean basin [11]. Abundance and biomass of *N. sclerorhynchus* and *N. aequalis* increase with depth, with a maximum depth of distribution until 1600 m reported in the Tyrrhenian and in the Ionian Sea [115, 116]. Also, they mainly feed on benthic preys, with N. sclerorhynchus which occasionally show some benthopelagic habits [100, 117, 118], according to their generalist opportunistic predator behavior. According to results, C. coelorhynchis, C. guentheri, N. sclerorhynchus and N. aequalis specimens showed general oval contours of the sagittae, which could be strictly related to their benthic feeding habits. Indeed, the foraging techniques, together with feeding habits and diet composition experienced by the species, can shape the feature of sagittae, such as biochemistry, growth, mean contour, morphology and morphometry [107, 114, 119]. The absence of data on the diet composition of the studied species from the investigated area make impossible to find direct correlations between feeding strategies and otoliths features to confirm this hypothesis. Conversely, the differences, resulting in a pentagonal shape, more lanceolate, in C. coelorhynchus and C. guentheri, and a more oval shape, with a marked irregularity of the margins in species belonging to Nezumia genera, could be influenced by the differences in their depth distribution [47, 48, 120, 121]. Moreover, morphometrical parameters of sulcus acusticus and sagittae showed significant differences between Nezumia species and, Coryphaenoides and Coelorhynchus species. Specimens belonging to the last two species showed markedly higher values of otoliths surface, length, weight, and sulcus acusticus surface and length, than those belonging to Nezumia genera. According to literature [43], species belonging to abyssal communities (between 1000 and 2000 m) show a decrease in otoliths size, if compared with the belonging to demersal communities, until the 750 m of depth. N. sclerorhynchus and N. aequalis inhabit deeper habitats than C. coelorhynchus and C. guentheri, with a close relation with the abyssal environment that could influenced the detected differences in morphometry and mean contours. The decrease in sagittae size related to the increase of habitats depths it was also reported for others Macrouridae species from the Atlantic and Pacific Ocean [103], with the similarity in otoliths length assessed for species with a similar depth distribution. Authors suggested that environment can control otoliths' size, being temperature and carbonate solubility strictly influenced by depth. Hymenocephalus italicus shows the highest abundance values between 400 and 500 m of depth, with a decrease in abundance below the 600 m [73], and a maximum distribution depth reported in some Mediterranean areas at 1200 m [115]. It shows pelagic habits, preying mainly on copepods, planktonic amphipods and pelagic crustaceans [11, 100, 117, 118]. It performs wide vertical movement, following the prey along the water column, and being capable to inhabit also deep environments [100, 122]. This differences in life habits could be reflected in *sagittae* features. Indeed, otoliths belonging to this species showed the higher distance to the other two groups (*Nezumia* sp group and *Coelorhynchus/Coryphaenoides* group) in LDA analyses performed on morphometric and shape indexes, as reported in Figs. 7 and 9.

In conclusion, present paper has provided the first accurate description of sagittae belonging to Macrouridae species from the studied areas. On our best knowledge, it was the first time in which shape analysis was applied on the studied species, providing evidence on the reliability of mean contours to discriminate among the species and, eventually, different populations. Moreover, findings reported the absence of directional bilateral asymmetry in all the investigated species, showing several differences in morphometry and morphology with literature data from other geographical areas. At inter specific level, it was stated the statistically significant distance between Nezumia sp group, Coelorhynch us/Coryphaenoides group and H. italicus group regarding data from both morphometrical and shape analysis. This confirmed the dual influence of phylogenetic and environment on otoliths development and features, being the three groups of species characterized by similarity in life habits and phylogenetically closeness. Further analyses are required to understand and detect the direct correlations between genetics, life habits, environment and sagittae features at intra and inter specific level. It will be essential to provide information on the genetics, feeding habits and depth distribution of the studied species from the investigated area to understand their influence on sagittae shape and morphometry. Moreover, it will be interesting to compare growth dynamics, environmental and genetical data of different populations to add valuable information and new insights on the otoliths' ecomorphology and inter-population differences. This is of the utmost importance to better understand the dynamics allowing for the stocks and populations separation in demersal and abyssal teleost fishes. Improve the knowledge base on Macrouridae inter specific and inter populations differences, and on the dynamics allowing to these variations, is fundamental to understand at all their life habits and improve their conservation for the well-being of the entire Mediterranean marine ecosystem.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

Claudio D'Iglio, Investigation, Data curation, Writing-Original draft; Sergio Famulari, Formal analysis, Software, Data curation; Dario Di Fresco, Mariachiara Costanzo and Alex Carnevale, Methodology and Investigation; Marco Albano, Data curation, Writing—review and editing; Nunziacarla Spanò, Supervision, Validation; Serena Savoca, Data curation, Formal analysis, Software, Writing—review and editing; Gioele Capillo Conceptualization, Supervision, Visualization, Writing-review and editing. All authors read and approved the final manuscript.

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Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate

Not applicable. Fish specimens were obtained already dead. No experiments were conducted, nor were surgical procedures performed. No procedures caused lasting harm to sentient fish, nor were sentient fish subjected to chemical agents. The care and use of collected animals complied with animal welfare guidelines, laws, and regulations set by the Italian Government.

Consent for publication

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