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Morphological comparison of otoliths in three species of the Sea Catfish family (Ariidae) from the Persian Gulf and the Oman Sea

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Abstract

Species of the family Ariidae exhibit high morphological similarity. In this study, the morphological features of lapillus otoliths were analyzed using shape indices and geometric morphometric techniques to compare three species of Sea catfish: *Arius maculatus*, *Netuma thalassina*, and *Plicofollis layardi* from the Persian Gulf and the Oman Sea. The comparison of shape indices revealed significant differences in the general shape and roundness indices for *A. maculatus*, as well as in the roundness and rectangularity indices for *P. layardi*, compared to the other species. The results indicated that the outline data were able to identify and differentiate these three species with an accuracy of 96.8%. Additionally, the results of the cluster analysis confirmed greater morphological differences in the outline of the otolith for *P. layardi* compared to the other two species. These results indicate that applying otolith morphological characteristics—particularly outline-based analyses—is an effective approach for discriminating among the examined species and contributes to improved taxonomic resolution within the family Ariidae.

Keywords: Lapillus, Outline, Shape indices, Taxonomy

Introduction

The family Ariidae, commonly known as Sea catfishes, is widely distributed across the globe, particularly in tropical and subtropical regions (Simier et al., 2021). Despite ongoing phylogenetic and taxonomic studies, the relationships among species within this family remain incompletely understood (Acero, 2004). Currently, 157 species belonging to 42 genera have been reported within this family (Fricke et al., 2025). In Iranian waters, Sea catfish are predominantly captured using bottom trawl nets and represent a significant portion of the total catch. Members of the Ariidae family are characterized by distinctive morphological features, including three pairs of barbels (maxillary, mandibular, and mental), closely spaced nostrils, short dorsal and anal fins, a forked caudal fin, an adipose fin, and heads covered with bony plates (Simier et al., 2021). These features not only help distinguish Ariidae from other families within the order Siluriformes but also highlight the complexity of their taxonomy. Five species from this family have been reported in the Persian Gulf and the Oman Sea: *Arius maculatus* (Thunberg, 1792), *Netuma bilineata* (Valenciennes, 1840), *Netuma thalassina* (Rüppell, 1837), *Plicofollis dusumieri* (Valenciennes, 1840) and *Plicofollis layardi* (Günther, 1866), with *N. thalassina* being the most abundant species in the region (Carpenter et al., 1998). Otoliths in bony fish are primarily composed of calcium carbonate in the

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aragonite form, along with trace and ultra-trace elements embedded in an organic matrix (Avigliano & Volpedo, 2016). These structures effectively serving as natural chronometers that record the growth and environmental history of fish, thus providing valuable insights for ichthyologists (Thomas & Swearer, 2019). The size and shape of otoliths are strongly specific to the sex and species of the fish (Khanali et al., 2021; Ferri, 2023; Khandan Barani & Alavi-Yeganeh, 2024; Shariati et al., 2024) and are commonly utilized in taxonomic studies (Khandan-Barani et al., 2024; Shariati et al., 2025). Environmental factors, along with ecological and behavioral characteristics, can also influence otolith shape (Ghanbarifardi & Zarei, 2021; Quigley et al., 2023). The inner ear of fish contains three types of otoliths: Sagitta, Lapillus, and Asteriscus. Among these, Sagitta otoliths are most frequently used in studies due to their larger size, interspecific morphological variation, and relative ease of access (Tuset et al., 2003). While Sagittae otoliths are generally larger in most bony fish families, in the orders Siluriformes, Characiformes, and Cypriniformes, Lapillus otoliths can be larger than Sagittae otoliths (González Dubox et al., 2023). Otolith shape indices, which define relationships based on the dimensions, perimeter, and area of the otolith, vary by species and facilitate species differentiation through statistical analysis (Tuset et al., 2003; Salehi Nejad Ranjbar et al., 2016). Geometric morphometrics is a precise and highly sensitive technique for distinguishing morphological traits across various organs, typically applied in two approaches: landmark-based and outline-based morphometrics (Damadi et al., 2024; Luo, 2024). Given the round shape of otoliths and the challenges associated with selecting specific points, outline-based techniques are often more effective for examining morphological variation. This method employs selected harmonic points around the otolith and applies Fourier ellipse analysis for shape comparison (Ponton, 2006; Ghanbarifardi & Zarei, 2021; Khandan Barani et al., 2024). The shape of otoliths has been widely analyzed using both traditional morphometric techniques and geometric methods (Páez-Collao et al., 2024; Shariati et al., 2025). Traditional morphometric methods, while useful, provide only indirect assessments of shape differences. In contrast, geometric methods, particularly Elliptic Fourier Analysis provides better capabilities for detecting all variations in form and small-scale individual differences in morphology of otolith (Martin et al., 2023). However simple description of otoliths by traditional morphometrics is inevitable for using in atlas and identification keys (Tuset et al., 2008). Considering the high morphological similarity in body form and external traits among species, this study for the first time, investigates the potential of lapillus otolith morphological traits—utilizing shape indices and outline methods as tools for identifying three species of Sea catfish (*A. maculatus*, *N. thalassina*, and *P. layardi*) in the southern waters of Iran.

Materials and Methods

Sampling

Specimens of *Netuma thalassina* were collected from the coasts of the Persian Gulf (Hormuz Island; 27°03'N, 56°32'E), while specimens of *Arius maculatus* and *Plicofollis layardi* were captured from the Oman Sea (Chabahar Port; 25°32'N, 60°58'E), occasionally between 2024 and 2025. Initial identification of the specimens was conducted using established identification keys (Carpenter et al., 1998; Alexandre & Menez, 2007; Marceniuk & Menezes, 2007). Ten specimens of each identified species were selected for otolith extraction which all were from adult specimens.

Otolith Extraction

To extract the lapillus otoliths, a transverse section was made at the back of the head, near the fish's gill arch. The otoliths of the right side were then washed for 2 minutes in a 1% sodium hydroxide solution to remove contaminants (Kinacigil et al., 2000). Photographs of the intact otoliths were taken using an SZM-2 stereomicroscope equipped with a digital camera in the research laboratory at the University of Sistan and Baluchestan.

Shape indices

Following photography, the length, width, perimeter, and area of each otolith were measured using Digimizer software (Version 5.7.2). Subsequently, shape indices were calculated, including Aspect ratio, Circularity, Ellipticity, Form factor, Rectangularity and Roundness (Table 1) (Tuset et al., 2003). The Form factor (F) assesses the degree of irregularity of the outer margin, with a value of 0.1 representing a perfect circle, while values less than 0.1 indicate greater irregularity. The Aspect Ratio (AR) reflects that higher values correspond to more elongated otoliths. The Roundness (RD) and Circularity (C) indices measure the similarity of otolith shapes to a perfect circle. The Ellipticity index (E) reflects the proportionality of variations along the axes, while the Rectangularity index (R), which ranges from 0 to 1, indicates that values closer to one represent a perfect quadrilateral shape (Tuset et al., 2003). The normality and homogeneity of the dataset were evaluated using Shapiro–Wilk and Levene's tests, respectively ($p < 0.05$) (Ghotbi Jokandan et al., 2015). Indices were analyzed through one-way analysis of variance (ANOVA). Duncan's post-hoc multiple comparison test was utilized to determine significant differences among the species ($p < 0.05$).

Table 1. Shape Indices Examined in Otoliths

Aspect Ratio (AR) = OL/OW
Circularity = P^2/A
Ellipticity (E) = $(OL-OW)/(OL+OW)$
Form-factor (F) = $(4\pi A)/P^2$
Rectangularity (R) = $A/(OL*OW)$
Roundness (RD) = $(4A)/(\pi OL^2)$

Otolith length, OW: Otolith width:OL Perimeter, P A: Area,

Outline Analyses

Elliptical Fourier Analysis (EFA) was used to assess otolith shape variability as described by Kuhl and Giardina (1982) and Ferson et



al. (1985). The outlines of the otoliths were traced using the software tools tpsUtil (version 1.83), tpsDig (version 2.12) (Rohlf, 2000; Rohlf, 2013), and PAST (version 4.16c) (Hammer & Harper, 2001). Using tpsDig, the x- and y-coordinates of 300 evenly spaced landmarks along the otolith boundary were extracted. The resulting TPS file was analyzed with PAST. To account for variations in size, rotation, and initial tracing position, the Fourier harmonic coordinates (x and y) were standardized following the recommendations of Ferson et al. (1985) and Rohlf and Slice (1990), and EFA principal components were then extracted. Elliptic Fourier Descriptor (EFD) coefficients were used to conduct Principal Component Analysis, Discriminant Analysis, and hierarchical clustering (UPGMA method) using PAST v5.2.1 and SPSS 23. Otolith shape was estimated based on the superimposed coordinates projected in a PCA using thin-plate splines. Additionally, various sections and the external morphology of the otoliths were compared descriptively, following the terms provided by Tuset et al. (2008) (Figure 1).

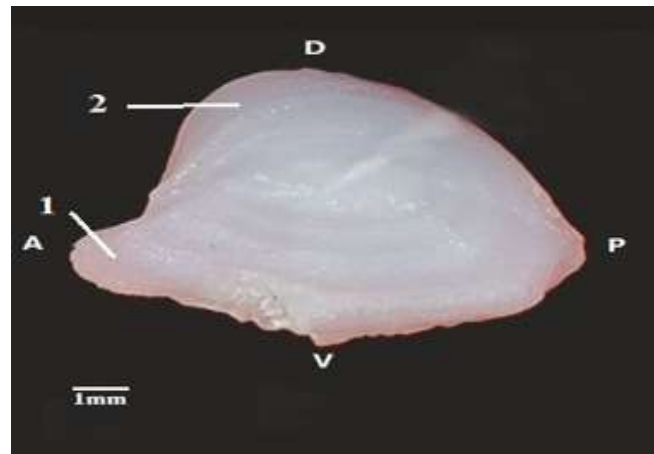


Figure 1. Terms used to describe the proximal face of the lapillus otoliths: Rostrum (1); Antirostrum (2); Anterior margin (A); Posterior margin (P); Ventral margin (V); Dorsal margin (D), (Tuset et al., 2008).

Results

There were depressions and protrusions in the ventral margin of the lapillus otoliths, across all three species (Figure 2), while the dorsal margin was smooth and flattened. In *A. maculatus*, the number of depressions and protrusions was greater than in the other two species. The rostrum of the otolith in *A. maculatus* was larger, wider, and characterized by a rough surface. In contrast, the rostrum of *N. thalassina* was pointed and exhibited a completely smooth surface. Additionally, in *P. layardi*, the rostrum was elongated and curved upward, with the antirostrum demonstrating greater elongation toward the left in comparison with the other two species. Results related to the morphological indices are presented in Table 2. The average Form factor for the three species ranged from 0.83 to 0.86, with *A. maculatus* exhibiting a significantly lower value compared to *N. thalassina* and *P. layardi*. The mean Aspect ratio index for the three studied species ranged between 0.79 and 0.80, showing no significant differences among the species ($p > 0.05$). The average Roundness index ranged from 1.11 to 1.23, with *P. layardi* exhibiting the highest value, which was significantly different from the other species ($p < 0.05$). The Circularity index ranged from 14.5 to 15.06, with *A. maculatus* demonstrating a higher value of 15.06, which was statistically significant among studied species ($p < 0.05$). The Ellipticity index, reflecting oval and egg-shaped characteristics among the species, ranged from 0.10 to 0.11, with no significant differences observed among the three species ($p > 0.05$). Regarding the Rectangularity index, the average values for the examined otoliths varied between 0.70 and 0.76, with *P. layardi* showing higher values that were significantly different ($p < 0.05$). The first two principal components accounted for a total of 78.38% of the variance ($PC_1 = 59.83\%$ and $PC_2 = 18.55\%$). The scatter plot of the outline data for the lapillus otoliths, analyzed using principal component analysis (PCA), is presented in Figure 3. This plot illustrates samples from the three species of the Sea catfishes, indicating the otoliths of *P. layardi* distinctly separated from those of *A. maculatus* and *N. thalassina*. Additionally, the otoliths of *A. maculatus* and *N. thalassina* were separated from each other, although with slight overlap. Figure 4 presents the results of the cluster analysis of otolith outline data, performed using UPGMA method and Euclidean distance. This analysis clearly distinguished the samples belonging to the three species of the Ariidae. The first separation was observed at a distance of nine between *P. layardi* and the other two species; *N. thalassina* and *A. maculatus*. A greater similarity in outline was noted between *A. maculatus* and *N. thalassina*, which separated at a distance of two from each other. Thin-plate spline of the otolith of every species is shown on the right side of the cluster. The results of the discriminant function analysis using the outline data (Table 3) indicated a satisfactory level of agreement between the predicted groupings and the actual groups, achieving an accuracy of 96.8%.



Figure 2. Dorsal View of the Lapillus Otolith in the Family Ariidae (Sea Catfish)) A (*A. maculatus*) 'B (*N. thalassina*) 'C (*P. layardi*)

Table 2. Comparison of otolith shape indices among the three studied species (Mean \pm Standard Deviation)

Indices	<i>P. layardi</i>	<i>N. thalassina</i>	<i>A. maculatus</i>
Form factor	0.85 \pm 0.01 ^b	0.86 \pm 0.01 ^b	0.83 \pm 0.02 ^a
Aspect ratio	0.04 ^a 0.79 \pm	0.80 \pm 0.04 ^a	0.04 ^a 0.79 \pm
Roundness	1.23 \pm 0.09 ^b	1.11 \pm 0.05 ^a	\pm 0.09 ^a 1.15
Circularity	14.71 \pm 0.28 ^a	14.58 \pm 0.30 ^a	0.43 ^b 15.06 \pm
Ellipticity	-0.11 \pm 0.02 ^a	-0.10 \pm 0.02 ^a	0.02 ^a -0.11 \pm
Rectangularity	0.76 \pm 0.04 ^b	0.70 \pm 0.03 ^a	\pm 0.04 ^a 0.71

* Different letters indicating to the significant difference

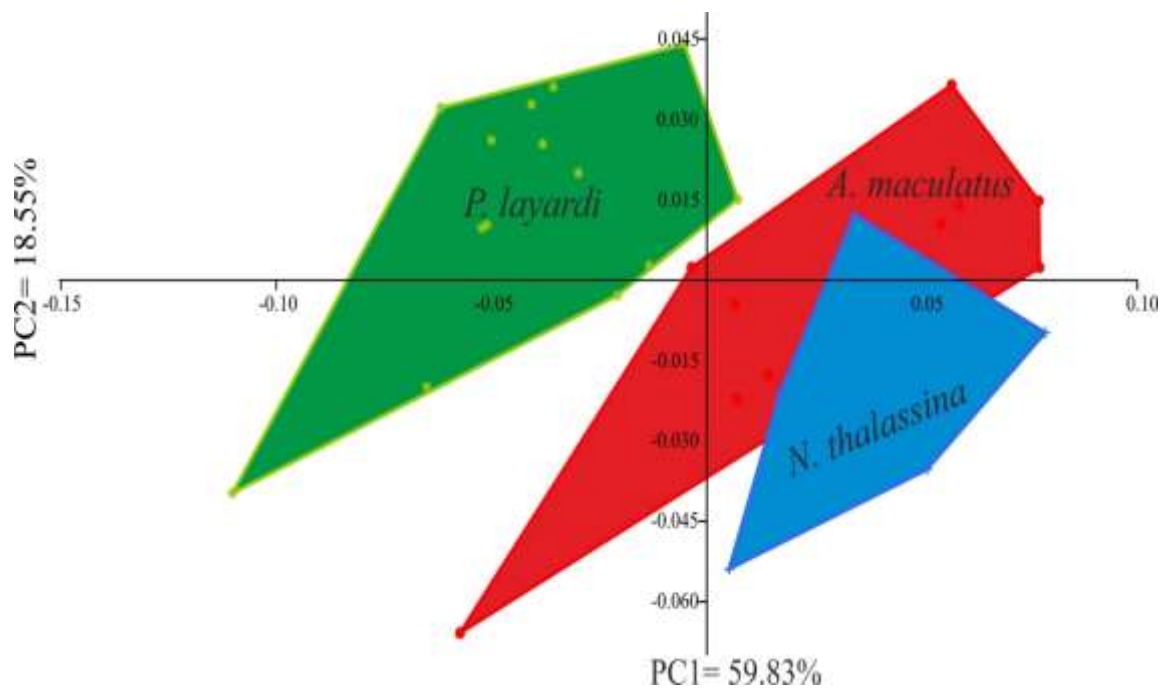


Figure 3. Scatter plot using outline data of lapillus otoliths in three sea catfish species based on Principal Component Analysis.



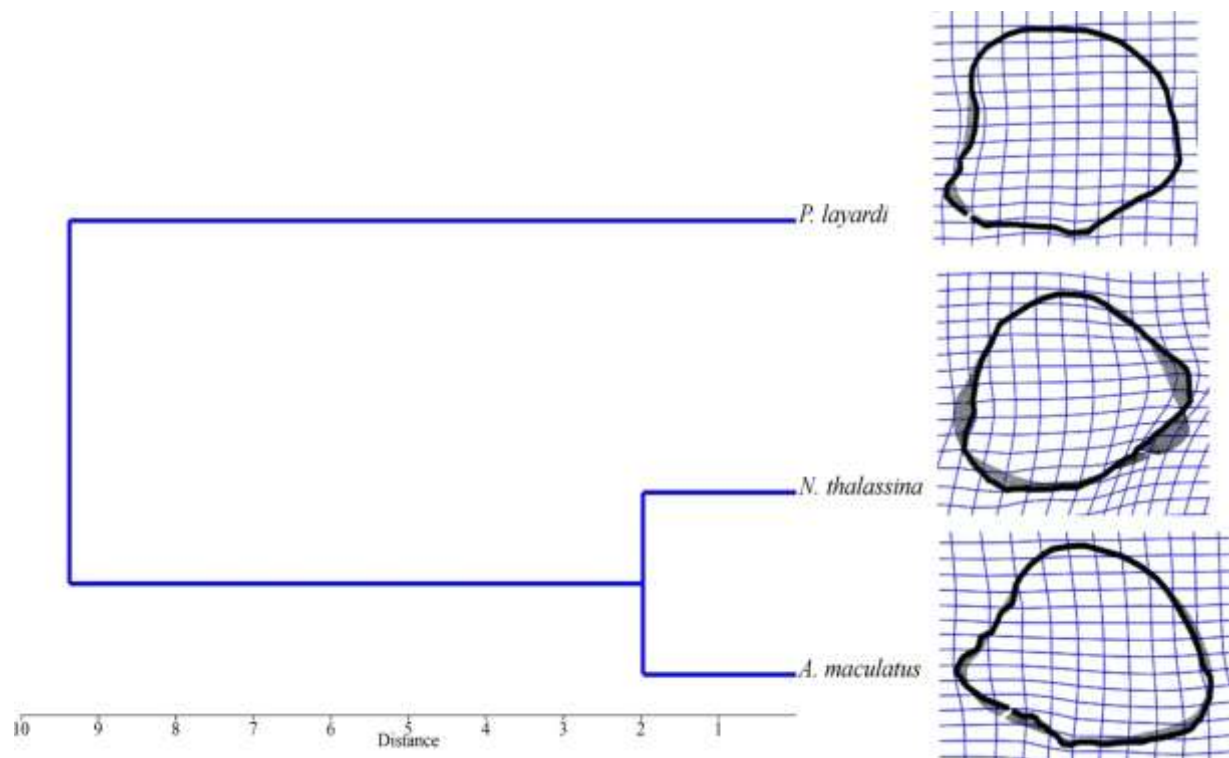


Figure 4. Cluster analysis comparing otolith shape in three Sea catfish species: *Arius maculatus*, *Netuma thalassina*, and *Plicofollis layardi*. Thin-plate spline of the otolith of every species is shown on the right side of the cluster.

Table 3. Predicted group membership using outline data of otoliths in the three Sea catfish species using Discriminant Analysis. 96.8% of cross-validated grouped cases correctly classified.

Species	Predicted Group Membership		
	1	2	3
1. <i>Arius maculatus</i>	100.0	0.0	0.0
2. <i>Netuma thalassina</i>	14.3	85.7	0.0
3. <i>Plicofollis layardi</i>	0.0	0.0	100.0

Discussion

The family Ariidae, comprising Sea catfish species, poses significant challenges in terms of classification and taxonomy due to the high morphological similarities among its members. Such similarities have led to instances of misidentification, as noted by Bavithra et al. (2024). Three species of the Ariidae family were examined in this study focusing on differences in otolith morphology. The observed differences in morphological indices can be attributed to various factors. For instance, the lower average Form factor index in *A. maculatus* compared to *N. thalassina* and *P. layardi* correlates with greater protrusion in the rostrum area and the relative irregularity of the otolith margin in this species. Conversely, the higher Roundness index values for *P. layardi*, which differed significantly from the other two species, can be attributed to the non-pronounced antirostrum, resulting in a reduced length relative to the others. Additionally, the circularity index for *A. maculatus* was significantly higher due to its more pronounced rostrum and posterior protrusion, which resulted in lower regularity compared to a perfect circle. The Rectangularity index for *P. layardi* also showed significant differences, as the curvature in the upper and posterior edges of the otolith was less pronounced than in the other two species, resulting in a shape closer to a rectangle. The variations in the shapes of the rostrum and antirostrum (Figure 2), further influenced the calculated morphological indices. Despite no significant differences in all morphological indices across the three species, the Form factor and circularity indices effectively distinguished *A. maculatus*, while the roundness and rectangularity indices were useful in differentiating *P. layardi* from the other species. This suggests that the morphological features of the otoliths, when combined with other morphological traits, can enhance the precision of species identification within the family Ariidae. The lapillus otolith shape has emerged as a particularly powerful taxonomic feature for identifying sea catfishes from the northeastern Pacific (Arroyo-Zúñiga et al., 2022). Similarly, Tuset et al. (2003) demonstrated the utility of morphological indices in distinguishing species of the genus *Serranus*, while Shariati et al. (2025) highlighted their effectiveness in differentiating endemic Caspian Sea sprat species. Based on research findings, otolith morphology often corresponds to habitat type: rectangular shapes are characteristic of pelagic fishes. Species living on soft substrates tend to possess circular or polygonal otoliths with an indistinct rostrum. Conversely, species from hard substrates commonly exhibit stretched otoliths featuring a clear rostrum. Nevertheless, this relationship between shape and habitat is not universally observed in all fish species (Tuset et al., 2016). Otolith morphometric characters have used to compare species of



Ariidae, Farooq and Panhwar (2023) measured lapillus otolith height, length, weight, and thickness in nine sea catfish from the coastal waters of Pakistan. Their results suggested that the combined use of fish biometry and otolith shape parameters is an effective tool for catfish identification. In another study, Johari et al., (2025) compared shape indices and morphometric measurements of ventral side of otoliths in 12 Ariid Catfish from Northern Brunei marine waters. They delineated otoliths into two major groups; the rounded group comprised species from the genera *Hexanematichthys*, *Netuma* (two species), and *Plicofollis* (two species), while the elongated group included species from *Arius* (three species), *Batrachocephalus*, *Cryptarius*, *Kyataphisa*, and *Osteogeneiosus*. Generally, Otoliths are considered as efficient tool for identifying fish species because of the high degree of species-specific morphological characteristics (Torres et al., 2000; Lombarte and Cruz, 2007; Tuset et al., 2012). The outline data in our study accurately identified and differentiated the three species from the family Ariidae with a precision of 96.8%. This high level of accuracy underscores the potential of Fourier analysis in capturing morphological variations and its efficiency in describing otolith shape. Thus, the effectiveness of the outline trait of otoliths in identifying Sea catfish in this study is considered satisfactory. Cluster analysis further revealed that *P. layardi* was distinctly separated from *A. maculatus* and *N. thalassina*, indicating a greater morphological distance, while *A. maculatus* and *N. thalassina* exhibited more similarity to each other. This phenomenon may be linked to the deviation of the antirostrum and the flattened nature of the posterior area. The landmark-based geometric survey method is used to compare the sexual dimorphism of *Aphaniops hormozensis* (Mouludi-Saleh et al., 2020) and populations of *Alburnus sellal* (Ghafouri et al., 2021) and Haemulidae (Damadi et al., 2024). The outline method is used to compare the otoliths of three species of mudskippers from the Persian Gulf and the Oman Sea (Ghanbarifardi & Zarei 2021), members of the Sillaginidae (Khandan Barani et al., 2024) and three species of the *Clupeonella* (Shariati et al., 2025). The use of hard anatomical structures such as otoliths has been very important in species identification and studies of different fish populations (Tuset et al., 2008). The effectiveness of the morphological indices and geometric morphometric techniques in comparing the morphology of lapillus otoliths among these three species of Sea catfish from the Persian Gulf and the Oman Sea suggests that utilizing these traits, in conjunction with other morphological features such as bony plates in the roof of the mouth and molecular traits, can help address some of the ambiguities in taxonomic studies of this family in the marine waters of Iran. By integrating multiple approaches, we can enhance our understanding of species diversity and improve the accuracy of classifications within the Ariidae family.

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