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Does habitat dominate phylogeny in fish otolith ecomorphology? Insights from Sepetiba Bay, Brazil

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ABSTRACT

This study investigated morphological variation in fish otoliths from a tropical bay in southeastern Brazil to assess whether shape is primarily determined by phylogenetic relatedness or by habitat-specific environmental pressures. We tested the hypothesis that habitat-driven functional convergence can override phylogenetic constraints, leading to similar otolith morphologies in ecologically similar but unrelated species. *Sagitta* otoliths from 15 species collected in Sepetiba Bay (RJ) were analyzed using elliptical Fourier analysis and traditional morphometric descriptors. Cluster analysis revealed nine morphogroups. Several groups reflected phylogenetic structure, including Sciaenidae, Gerreidae, Paralichthyidae, and *Stellifer rastrifer*, which retained family-typical traits despite representing a single species. In contrast, other groups comprised species from different families (e.g., Trichiuridae-Serranidae, Triglidae-Haemulidae, Sciaenidae-Achiridae), indicating habitat-driven convergence. *Cetengraulis edentulus* and *Symphurus tessellatus* formed isolated groups, likely reflecting lineage-specific or niche-related adaptations. Overall, otolith morphology reflects a balance between evolutionary history and environmental filtering, with phylogeny as the dominant influence and habitat acting as a consistent secondary driver of shape variation.

1. Introduction

Otoliths are calcified structures located within the sacculus, lagena, and utriculus compartments of the membranous labyrinth in the inner ear of teleost fishes. They form during embryonic development and grow incrementally through the deposition of calcium carbonate ($CaCO_3$) within an organic matrix (Campana, 1999). Each otolith pair, *sagittae*, *asterisci*, and *lapilli*, differs in location, size, shape, microstructure, and function (Popper and Coombs, 1982; Secor et al., 1992; Schulz-Mirbach et al., 2014). Among these pairs, *sagittae* are the most extensively studied, mainly because their typically larger size facilitates collection and analysis (Schulz-Mirbach et al., 2011; Moraes et al., 2023). Due to their species-specific morphology, otoliths are widely used in various scientific applications.

Their mass and overall shape are closely linked to auditory function, as different morphologies can influence endolymph flow dynamics and the functioning of the sensory epithelium within the semicircular canals (Schulz-Mirbach et al., 2019). These structures are essential for detecting sound, motion, and spatial orientation (Popper and Lu, 2000; Ramcharitar et al., 2006). Otoliths vibrate in response to external stimuli while suspended in the endolymph and attached to the sensory epithelium by a thin membrane. Because of their higher density relative to surrounding tissues, otoliths move out of phase with the rest of the inner ear structures, thereby stimulating mechanoreceptive hair cells. These cells convert mechanical vibrations into neural signals, a process fundamental to hearing and balance in fishes, highlighting the functional complexity of the vestibular system (Popper, 2003; Ladich and Popper, 2004; Soares and Niemiller, 2013).

Beyond overall contour, the morphology of the otolith acoustic *sulcus*, including its position, aperture, and shape, varies considerably among species, reflecting functional adaptations (Schulz-Mirbach et al., 2019). These features are closely associated with the presence, absence, or size of the *rostrum* (a protrusion on the anterior margin below the ostium opening) and the *antirostrum* (a smaller adjacent structure formed by an excision). Together, these elements define the ostium–excisura region, a key area involved in vibration detection (Bardhan et al., 2021). Recent studies on Neotropical fishes have shown that dorso-rostral variation, particularly in ostium development,

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correlates with environmental factors such as turbidity, hydrological flow, and habitat fragmentation (Santos et al., 2024).

Otoliths are central to studies of fish stock differentiation (Cadrin and Friedland, 1999; Duarte-Neto et al., 2008), age and growth estimation (Santos et al., 2017), and several other applications. Moreover, otolith morphology has become an important taxonomic tool for species identification (Tuset et al., 2006; Afanasyev et al., 2017; Vaz-dos-Santos et al., 2023; Santos et al., 2024; Wilhelm et al., 2025). In recent decades, ecomorphological research has increasingly examined how otolith shape and size relate to environmental factors and fish behavior.

Otolith shape can be influenced by environmental conditions such as food availability, water temperature, depth, and substrate type (Cardinale et al., 2004; Gauldie and Crampton, 2002; Mérigot et al., 2007), as well as by genetic, ontogenetic, and ecological factors (Lombarte et al., 2003; Campana, 2005; Sadighzadeh et al., 2014; Tuset et al., 2015). Contour analysis is a key approach for distinguishing inter- and intraspecific morphotypes (Duarte-Neto et al., 2008; Santos et al., 2017). Basic descriptors such as circularity, rectangularity, and ellipticity are commonly used to characterize otolith contours, alongside more advanced geometric techniques, such as wavelet functions, which decompose shapes across multiple spatial scales (Parisi-Baradad et al., 2005; Vasconcelos et al., 2025).

Otolith size and shape can reflect ecological adaptations related to habitat use and swimming capacity. Larger otoliths are often observed in benthic and deep-water species, where enhanced sensitivity to substrate-transmitted acoustic cues is advantageous (Lombarte and Cruz, 2007; Lombarte et al., 2010). By contrast, pelagic species, particularly migratory ones, tend to possess smaller *sagittae* and well-developed *rostra*, traits linked to higher locomotor efficiency in open-water environments (Tuset et al., 2008). *Rostrum* development is also habitat-dependent: it is frequently absent or poorly developed in species inhabiting soft substrates and remains small in those associated with hard bottoms (Volpedo and Echeverría, 2003). Additionally, otolith morphology may vary with swimming behavior; fishes with simple or restricted movements typically exhibit more spherical or ellipsoidal otoliths, whereas more complex contours are generally associated with greater mobility (Schellart and Popper, 1992; Reichenbacher et al., 2009).

The "habitat over phylogeny" hypothesis proposes that ecological factors, particularly habitat characteristics, exert a stronger influence on morphological traits than shared evolutionary history. Although not entirely new to ichthyology, studies since the 1980s and 1990s have highlighted the role of the environment conditions in shaping fish morphology (e.g., Gauldie, 1988; Lombarte, 1992). The explicit formulation and broader recognition of this idea came with Cadotte et al. (2009), who, in plant ecology, integrated phylogenetic and functional information to explain community assembly. Since then, this framework has been widely cited and adapted across diverse taxa. More recently, fish studies such as Nakamura and Soares (2021) have adopted this perspective, demonstrating that ecological factors, particularly habitat features, can outweigh phylogenetic history in explaining morphological variation. Accordingly, similarities in otolith morphology among species occupying comparable habitats may represent adaptive responses to environmental pressures, potentially overriding phylogenetic signals (*e.g.*, Vignon and Morat, 2010, in tropical fishes). This interpretation is especially relevant in heterogeneous coastal systems, where habitat-driven morphological convergence may reflect functional adaptations to specific ecological niches. In Sepetiba Bay, for example, high turbidity and predominantly muddy substrates may act as selective forces shaping otolith form. Convergent patterns among species from distinct ecological guilds (e.g., demersal and benthopelagic) may thus arise from similar habitat constraints. In such environments, a more robust *rostrum*, rather than the elongated profiles typical of pelagic habitats, could enhance acoustic reception or stabilize the otolith within soft, dynamic substrates. Previous studies support this view, showing that environmental variables such as substrate type, depth, and turbidity can strongly influence otolith morphology, sometimes overriding phylogenetic signals (Volpedo and Echeverría, 2003; Reichenbacher et al., 2009).

In this study, we aim to determine whether ecological factors, particularly habitat characteristics, exert a stronger influence on otolith shape than phylogenetic relatedness. Specifically, we test the *habitat-over-phylogeny* hypothesis by evaluating whether otolith morphology, both overall shape and key structures (e.g., *rostrum, antirostrum, sulcus acusticus*), reflects functional adaptations to habitat demands such as swimming behaviour, substrate type, and environmental stability. Based on this premise, we expect species occupying similar ecological niches (e.g., demersal or benthopelagic) to display convergent otolith shapes regardless of phylogenetic proximity. Conversely, if the habitat-over-phylogeny hypothesis is not supported, we expect phylogeny to emerge as the primary driver of otolith morphology, with habitat playing a secondary, yet modulatory, role.

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2. Methodology

2.1. Study area

Sepetiba Bay (Fig. 1) is located on the southeastern coast of Brazil (22°54′–23°04′S, 43°34′–44°10′W), bordering the Southwest Atlantic Ocean. It is bounded to the north and east by the mainland, to the south by the Marambaia Sandbank, an environmentally protected area, and to the west by Ilha Grande Bay. With an elongated ellipsoidal shape and an area of approximately 450 km^2, the bay encompasses a variety of coastal habitats, including mangroves, sandbanks, mudflats, and small estuaries, making it a highly heterogeneous environment (Kjerfve et al., 2021).

Bay waters are generally shallow, with average depths below 5 m, particularly in the inner and marginal zones. Deeper areas occur near the Marambaia Sandbank, where natural channels or subsidence zones can reach up to 20 m. These deep sectors are frequently used by fishing fleets, especially for shrimp trawling. Average salinity is ~32, decreasing near river estuaries. Water temperature averages ~24 °C, ranging from ~19 °C in winter to ~27 °C in summer, and dissolved oxygen remains close to 8 mg/L (Cunha et al., 2006). Annual rainfall is ~2300 mm, with a rainy season from December to March and a drier period from July to October. According to the Köppen–Geiger classification, the region corresponds to the Tropical Af zone, characterized by hot, humid conditions and low thermal amplitude (Alvares et al., 2013). Water circulation is primarily driven by tides (Leal Neto et al., 2006; Kjerfve et al., 2021) and wind. Neap tides average ~0.3 m, while spring tides reach ~1.1 m and may exceed 1.4 m (Kjerfve et al., 2021), classifying Sepetiba Bay as a microtidal system (Davies, 1964).

The region exhibits a marked contrast between preserved natural areas (e.g., mangroves and the Marambaia Sandbank) and anthropized zones along the northern continental margin, including urban, industrial, and port areas that contribute to the influx of organic and inorganic pollutants. The bay's main freshwater input is the Guandu River, which carries water originally diverted from the Paraíba do Sul River. This river supplies drinking water to the Rio de Janeiro metropolitan region and has a mean discharge ranging from 70 to 150 m^3 /s (Araújo, 1998). Numerous small streams and drainage canals also transport pollutants from the northern urban–industrial zone, reducing water quality in the northern and central sectors of the bay, including Pedra de Guaratiba (Fonseca et al., 2013; Castelo et al., 2021). In contrast, conditions near Ponta da Pombeba, within the protected southern sector adjacent to Marambaia Island and closer to the oceanic inlet, are comparatively better, with higher water quality and weaker urban influence (Copeland et al., 2003; Pereira et al., 2024). Even so, polluted dredged material has been transported toward distal sectors, spreading contaminants from inner areas to regions near Ponta da Pombeba (Saibro et al., 2023). Local geomorphology and circulation patterns favor the retention of metals

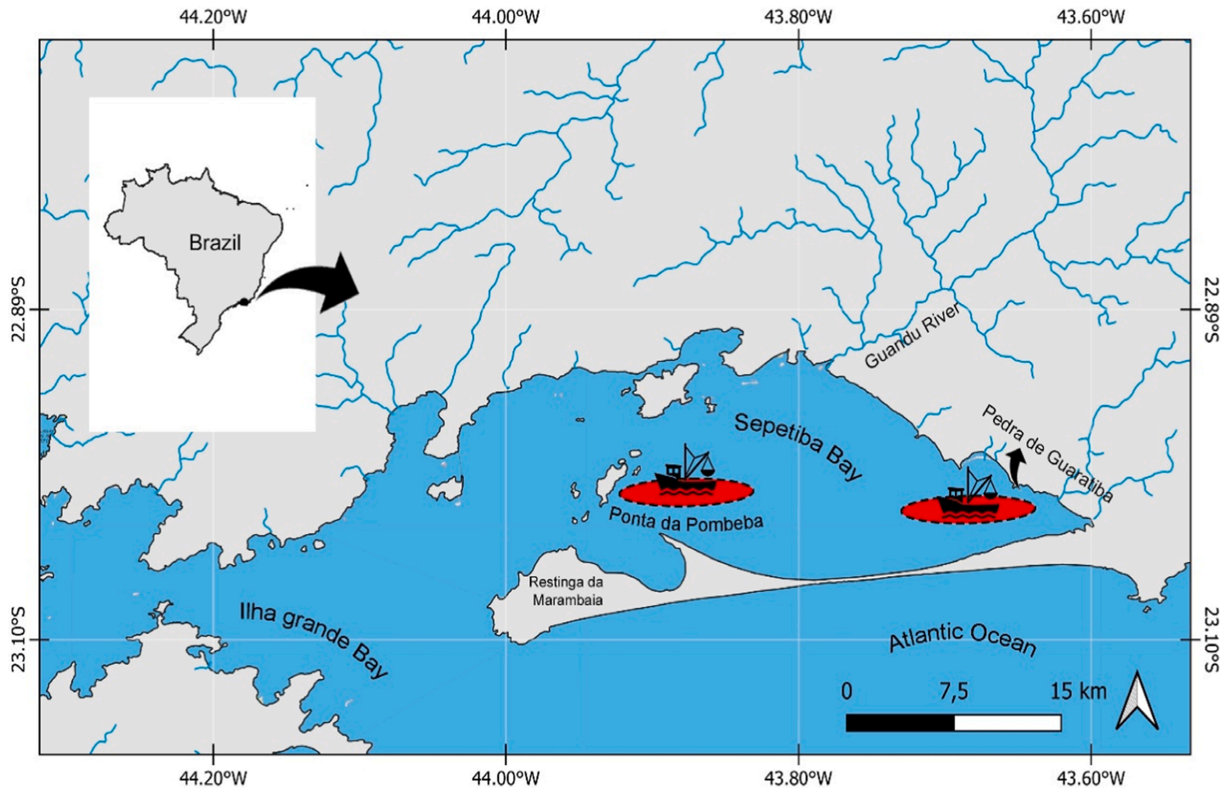

Fig. 1. Study area in Sepetiba Bay, showing fish sampling sites (red symbols): Ponta da Pombeba and Pedra de Guaratiba.

and organic matter, intensifying eutrophication and environmental degradation with direct consequences for fisheries (Lacerda and Molisani, 2006; Küttek et al., 2021).The fish analysed in this study were collected from two traditional trawling grounds in Sepetiba Bay: near Ponta da Pombeba (23°01'S, 43°53'W; depth: 15–20 m) and Pedra de Guaratiba (23°03′S, 44°40′W; depth: 4–5 m), both located in the inner bay (Fig. 1).

2.2. Sample collection and preparation

Otoliths were obtained from fish caught by trawl fisheries in Sepetiba Bay. Most individuals were subadults that use the bay as rearing grounds. Sampling was conducted weekly from September to November 2017, with occasional interruptions due to adverse weather, and trawling depended on the availability of local fishermen. After commercially valuable species were sorted, the remaining bycatch was stored by the boat crew and later transported to the laboratory for counting, sorting, and species identification. The number of individuals analyzed (at least 12 per species) was constrained by the total number of individuals captured per species during the scheduled sampling campaigns. While larger sample sizes are generally preferable for statistical robustness, fieldwork constraints, such as weather conditions, fishing logistics, and species abundance, limited the number of specimens available for analysis. However, the statistical procedures applied accounted for these limitations, ensuring that the results accurately represent the sampled population and allowing a greater number of species to be analyzed.

Each specimen was identified, and its total length (TL, cm) was measured. Otoliths were extracted via a ventral incision from the anus toward the head, a technique chosen for its practicality. After removal of the viscera and gills, the otic capsules in the posterior neurocranium were exposed. These capsules were carefully opened with scissors, and the otoliths were extracted using forceps or needles, depending on their size.

Extracted *sagittal* otoliths (left and right) were cleaned and examined macroscopically and microscopically to assess potential shape differences. To evaluate bilateral symmetry, morphometric variables from each otolith pair (length, width, area, perimeter, and weight) were compared using paired Student's *t*-tests. No significant size differences were detected between left and right otoliths; therefore, only the left otolith was selected for all subsequent analyses. All otoliths were measured using a caliper or a micrometer eyepiece and photographed in proximal view. For imaging, a Moto G4 Plus smartphone (Motorola, USA) was mounted on a Nikon SMZ1000 stereomicroscope, with lighting and magnification adjusted according to otolith size.

To illustrate the structural details of the otolithic system, Fig. 2A–B provide rare visual insights. Fig. 2A depicts an intact otolithic membrane with the optic nerve still attached, demonstrating the meticulous preparation that preserved both structural and fluid integrity. In contrast, Fig. 2B shows a ruptured membrane, exposing the nerve endings of the sensory epithelium and highlighting the close interaction between the otoliths and sensory cells.

Otolith shape terminology follows the standards established by the Southeast and South Brazil Otolith Collection (COSSBrasil) at the University of São Paulo (http://www.usp.br/cossbrasil/relespecie.php?sigla=GEBA).

2.3. Image processing and morphometric analysis

Otolith photographs were converted to bitmap files and analyzed using SHAPE v1.3 software (Iwata and Ukai, 2002). Based on contour outlines, the software generated numerical shape descriptors (harmonics, *Ha*), which were used in an Elliptic Fourier Analysis (EFA). Principal components (PCs) were then derived from the variance–covariance matrix, allowing variables to be compared on a common scale and ensuring that shape variation was accurately represented across harmonics. The first 20 harmonics were retained because they contain the most relevant shape information while reducing noise and

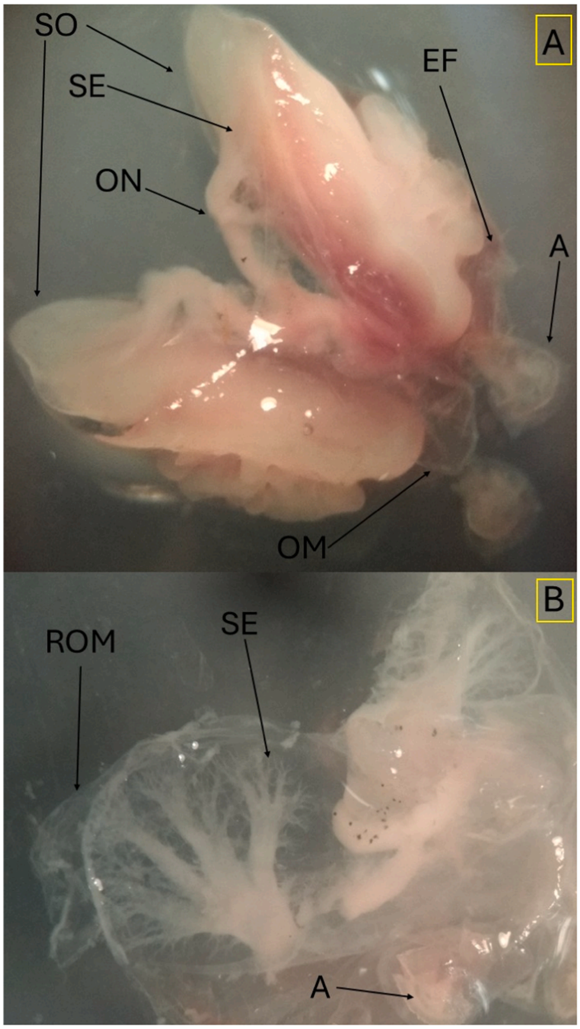

Fig. 2. A-B. A. Pairs of *sagitta* (SO) and *asteriscus* (A) otoliths within the otolithic membrane (OM), immersed in endolymphatic fluid (EF) and connected to the sensory epithelium (SE) via the optic nerve (ON) in *Paralonchurus brasiliensis*. **B.** Ruptured otolithic membrane (ROM) showing nerve endings of the sensory epithelium (SE) and the *asteriscus* otolith (A) still enclosed within the membrane. Photos: personal archive.

dimensionality, thereby achieving a balance between morphological detail and statistical robustness. These data were subsequently subjected to cluster analysis using a Euclidean distance matrix and Ward's linkage method in STATISTICA 10 (StatSoft, 2011) to identify potential groups based on otolith shape among the studied species. Euclidean distance was selected for its ability to preserve the geometric relationships among observations in morphometric space and for its compatibility with Ward's method, which minimizes within-cluster variance and promotes the formation of compact, spherical clusters.

Subsequently, the images were saved as JPEG files and imported into FIJI/ImageJ2 to extract the following otolith morphometric parameters: total length (TL, mm), width (mm), area (mm^2), perimeter (mm), circularity, and Feret diameter (mm). The effect of fish length on each morphometric variable was evaluated using ANCOVA in STATISTICA 10 (StatSoft, 2011). Variables significantly correlated with fish length were size-corrected using the common-slope method described by Lleonart et al. (2000):

$$Y_i^* = Y_i \left(\frac{X_o}{X_i}\right)^b$$

where Y_i is the i-th observation of the morphometric variable, X_o is the mean fish length, X_i is the individual fish length, and b is the common slope across groups. After size correction, ANOVA was performed to compare morphometric measurements among groups. The dataset supporting the findings of this study will be deposited in Zenodo and made publicly accessible upon publication.

3. Results

The ichthyofauna analyzed in this study comprised 15 species from nine families, representing a broad taxonomic spectrum of the class *Actinopteri*. These species were distributed across five orders: Clupeiformes (1 species), Scorpaeniformes (1), Acanthuriformes (4), Perciformes (4) and Pleuronectiformes (4), reflecting substantial ecological and morphological diversity within the sampled assemblage (Table S1).

3.1. Rostrum and antirostrum

Among the fifteen species analyzed, eight exhibited an absent or poorly developed *rostrum*, whereas seven showed a developed *rostrum* to varying degrees (Table 1). Species with a developed *rostrum* included *Cetengraulis edentulus*, *Diplectrum radiale*, *Eucinostomus argenteus*, *E. gula*, *Orthopristis ruber*, *Prionotus punctatus*, and *Trichiurus lepturus*. Species with an absent or poorly developed *rostrum* included *Achirus lineatus*, *Citharichthys spilopterus*, *Etropus crossotus*, *Menticirrhus americanus*, *Micropogonias furnieri*, *Paralonchurus brasiliensis*, *Stellifer rastrifer*, and *Symphurus tessellatus*. Otoliths displayed either medial or supramedial positions of the *sulcus acusticus*, and four distinct *sulcus* opening types were identified (Table 1, Fig. S1).

3.2. Harmonic analysis

Cluster analysis based on numerical descriptors of otolith contour shape (first 20 harmonics) divided the samples into nine distinct groups using a Euclidean distance cutoff of 1.0 (Fig. 3).

The variation in otolith shapes and the corresponding groupings are shown in Figs. 4A–B, 5A–D and 6A–C. These figures illustrate, respectively, otoliths with narrow and elongated shapes, otoliths with anterior extensions, and otoliths with shapes ranging from oval to round.

Group 1: *Menticirrhus americanus* and *Paralonchurus brasiliensis* (both Sciaenidae), characterized by elliptical contours (Fig. 4A)

Group 2: *Trichiurus lepturus* (Trichiuridae) and *Diplectrum radiale* (Serranidae), exhibiting fusiform contours with well-developed *rostra* (Fig. 4B).

Group 3: *Eucinostomus argenteus* and *E. gula* (Gerreidae), with elliptical contours and a broader median region (Fig. 5A).

Group 4: *Prionotus punctatus* (Triglidae) and *Orthopristis ruber* (Haemulidae), characterized by oval-shaped otoliths with a developed *rostrum*, a practically absent *antirostrum*, and a well-defined *sulcus acusticus* with smooth ostial openings (Fig. 5B).

Group 5: *Etropus crossotus* and *Citharichthys spilopterus* (both Paralichthyidae), exhibiting pentagonal contours and relatively small sizes (Fig. 5C).

Group 6: *Cetengraulis edentulus* (Engraulidae), elliptical in shape with a prominent *rostrum* and serrated ventral margins (Fig. 5D).

Group 7: *Stellifer rastrifer* (Sciaenidae), rectangular in shape, lacking a *rostrum*, and featuring two distinct dorsal peaks (Fig. 6A).

Group 8: *Symphurus tesselatus* (Cynoglossidae), discoidal in shape with smooth edges (Fig. 6B).

Group 9: *Micropogonias furnieri* (Sciaenidae) and *Achirus lineatus* (Achiridae), with pear-shaped oval contours, respectively (Fig. 6C).

3.3. Principal component analysis - PCA

Elliptical Fourier Analysis (EFA) identified seven significant principal components (PCs), accounting for 95.31% of the total shape variation. PC1 alone explained 71.23% of the variation, while PC2

Table 1
Morphological characteristics of the *sulcus acusticus, rostrum*, and *antirostrum* for each species.

Species	*Sulcus* Position	Opening type	*Rostrum*	*Antirostrum*	Ecological guild
Cetengraulis edentulus	Medial	Ostial	Developed	Developed	Pelagic
Diplectrum radiale	Medial	Ostial	Developed	Slightly developed	Demersal
Eucinostomus argenteus	Medial	Ostial	Developed	Slightly developed	Demersal
Eucinostomus gula	Supramedial	Ostial	Developed	Absent	Demersal
Orthopristis ruber	Supramedial	Ostial	Developed	Slightly developed	Demersal
Prionotus punctatus	Medial	Ostial	Developed	Absent	Demersal
Trichiurus lepturus	Medial	Ostial	Developed	Slightly developed	Pelagic
Stellifer rastrifer	Medial	Pseudo-ostiocaudal	Slightly developed	Developed	Demersal
Achirus lineatus	Medial	Mesial	Absent	Absent	Benthic
Citharichthys spilopterus	Medial	Mesial	Absent	Absent	Benthic
Etropus crossotus	Medial	Mesial	Absent	Absent	Benthic
Menticirrhus americanus	Medial	Pseudo-ostial	Absent	Absent	Demersal
Micropogonias furnieri	Medial	Pseudo-ostial	Absent	Absent	Demersal
Paralonchurus brasiliensis	Medial	Pseudo-ostial	Absent	Absent	Demersal
Symphurus tesselatus	Medial	Mesial	Absent	Absent	Benthic

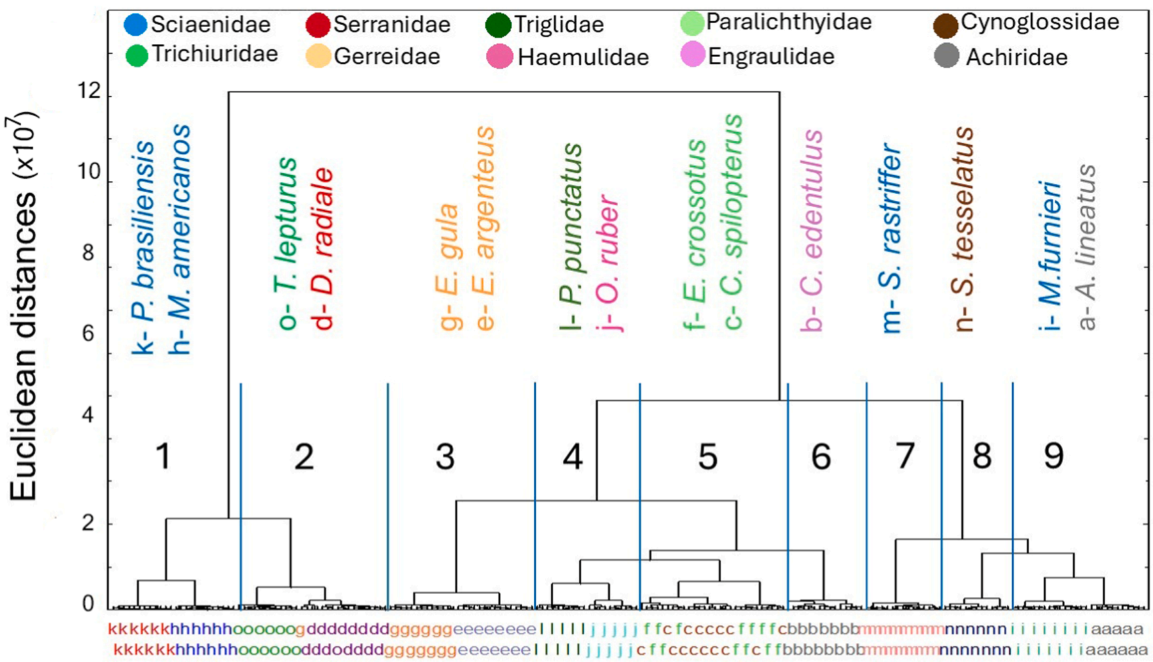

Fig. 3. Dendrogram from the cluster analysis (Ward's method, Euclidean distance) based on the first 20 harmonics from the Fourier analysis. Species codes (letters **a** to **o**) and names indicate by their respective colours: *Achirus lineatus* (a); *Cetengraulis edentulus* (b); *Citharichthys spilopterus* (c); *Diplectrum radiale* (d); *Eucinostomus argenteus* (e); *Eucinostomus gula* (g); *Etropus crossotus* (f); *Menticirrhus americanus* (h); *Micropogonias furnieri* (i); *Orthopristis ruber* (j); *Paralonchurus brasiliensis* (k); *Prionotus punctatus* (l); *Stellifer rastrifer* (m); *Symphurus tessellatus* (n); *Trichiurus lepturus* (o). Blue vertical bars indicate separation among the nine groups using a Euclidean distance cutoff of 1.0.

accounted for 9.33% (Fig. S2).

Symphurus tesselatus (group 8) was clearly separated from the sciaenid species *Paralonchurus brasiliensis* and *Menticirrhus americanus* (group 1), as well as from *Trichiurus lepturus* and *Diplectrum radiale* (group 2), along axis 1 of the PCA ordination plot (Fig. 7). This separation reflects the discoidal otolith shape of *S. tesselatus*, which contrasts markedly with the elongated and narrow otoliths of the other four species (Figs. 4A–B, 6B).

Cetengraulis edentulus (group 6) was clearly separated along axis 2 of the PCA from the sciaenid species *Menticirrhus americanus* and *Paralonchurus brasiliensis* (group 1), *Micropogonias furnieri* (group 9), and *Stellifer rastrifer* (group 7) (Fig. 7). This separation is driven by a distinctive otolith morphology of *C. edentulus*, characterized by a serrated ventral margin and well-developed *rostrum* and *antirostrum*. In contrast, the other four species exhibit smooth-edged otoliths with a slight rightward tilt and poorly developed or absent rostral structures. Additional morphological differentiation was observed between *P. brasiliensis* and *M. americanus*, which have elongated, elliptical otoliths, and *M. furnieri* and *S. rastrifer*, whose otoliths are more rounded.

The two *Eucinostomus* species (group 3) stand out due to their narrow, elongated otoliths with irregular edges, in contrast to the flatfish species *Citharichthys spilopterus* and *Etropus crossotus* (group 5), which possess shorter, broader otoliths with smoother margins. *Achirus lineatus* (Achiridae) is characterized by a rounded otolith with an irregular margin and no evident inclination.

Orthopristis ruber (Haemulidae) and *Prionotus punctatus* (Triglidae) share otolith contours that are intermediate between rounded and elongated forms but differ in edge texture, smooth in *O. ruber* and serrated in *P. punctatus*. Both species exhibit a developed *rostrum*, while the *antirostrum* is weakly developed in *O. ruber* and absent in *P. punctatus*.

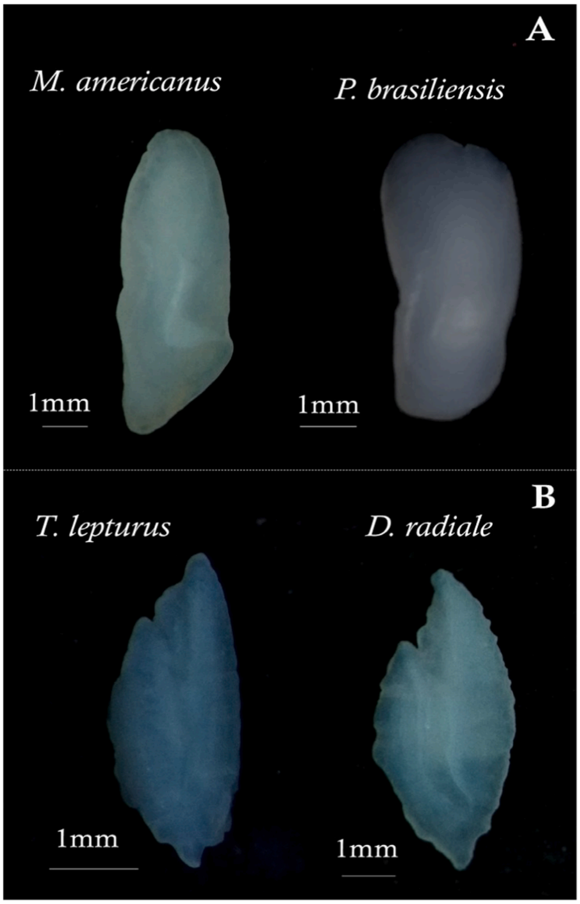

Fig. 4. A–B. Illustrations of otolith morphological groups 1 and 2, showing narrow and elongated otoliths. Scale bars represent 1 mm.

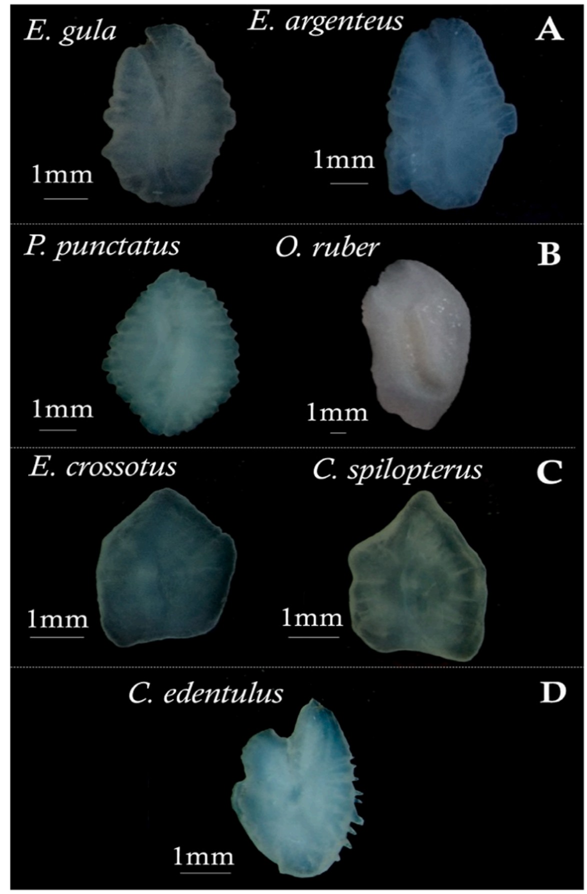

Fig. 5. A–D. Illustrations of otolith morphological groups 3 through 6, showing otoliths with anteriorly extended *rostra*. Scale bars represent 1 mm.

3.4. Morphometric analysis

Otolith biometric measurements (length, width, circularity, perimeter, area, and Feret diameter) were generally similar across the cluster-defined groups, except for circularity. The highest values for length, perimeter, area, and Feret diameter were observed in group 9, followed by groups 4 and 1. In contrast, circularity exhibited a distinct pattern, with the highest values recorded in groups 5 and 8. These differences were statistically significant (one-way ANOVA; Fig. 8).

4. Discussion

The morphometric analysis of otoliths from Sepetiba Bay, based on Fourier harmonics and Principal Component Analysis (PCA), revealed a more complex pattern than initially anticipated. Rather than being driver for a single dominant factor, otolith shape variation reflects a dynamic interplay between evolutionary history and ecological pressures, in which strong phylogenetic signals coexist with clear cases of ecomorphological convergence. This pattern challenges our initial hypothesis of environmental dominance and highlights evolutionary heritage as a major factor structuring otolith morphology. At the same time, recurrent habitat-associated patterns indicate that ecological factors continue to modulate shape within phylogenetically cohesive lineages. Overall, these findings suggest that otolith morphology is shaped by both evolutionary constraints and functional adaptations, refining current interpretations in ecomorphological studies. Examination of individual morphogroups further clarifies how specific shapes align with either phylogenetic relationships or ecological pressures, revealing the selective forces underlying the observed morphological diversity.

Phylogenetic conservatism emerged as the main force structuring the morphogroups. Morphological coherence in groups composed exclusively of congeneric or confamilial species, such as morphogroups 1 (*Menticirrhus americanus* and *Paralonchurus brasiliensis*, Sciaenidae), 3 (*Eucinostomus argenteus* and *E. gula*, Gerreidae), and 5 (*Etropus crossotus* and *Citharichthys spilopterus*, Paralichthyidae), indicates that shared lineage is a strong predictor of otolith shape. Fundamental traits, such as overall outline, proportions, and the presence of prominent structures tend to be conserved over evolutionary time, reflecting both genetic inheritance and developmental constraints. This pattern supports the idea that morphological plasticity operates within phylogenetically imposed limits (Parsons et al., 2011). The recurrence of this pattern across families highlights phylogenetic conservatism as a widespread structuring force. Even subtle differences among closely related species typically occur in secondary features, preserving each lineagés morphological signature. Conversely, the isolated placement of species in morphogroups 6 (*Cetengraulis edentulus*, Engraulidae) and 8 (*Symphurus tessellatus*, Cynoglossidae) indicates that otolith morphology can also reflect a balance between phylogenetic heritage and habitat-specific ecological adaptations (Assis et al., 2020). This coexistence of strong phylogenetic signals with targeted ecological adjustments demonstrates that, despite deep evolutionary conservation, otolith morphology remains responsive to contemporary selective pressures.

The interplay between phylogeny and habitat is evident in singular cases within the assemblage, where otolith morphology retains the evolutionary signature while reflecting functional adaptations. Morphogroup 7, represented solely by *Stellifer rastrifer* (Sciaenidae), exemplifies this pattern. Although it did not cluster with its benthic congeners in morphogroups 1 and 9, this divergence does not weaken the phylogenetic signal; rather, it highlights a lineage-specific morphology shaped

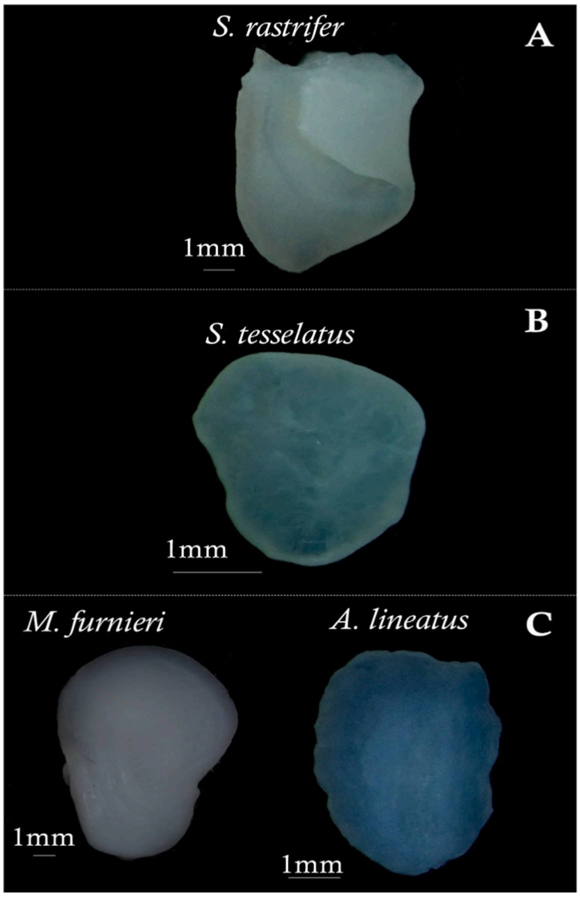

Fig. 6. **A–C**. Illustrations of otolith morphological groups 7 through 9, showing shapes ranging from oval to round. Scale bars represent 1 mm.

by ecological requirements. The separation likely reflects its distinct foraging strategy: whereas species in morphogroups 1 and 9 are specialized benthic forms (Haluch et al., 2007; Denadai et al., 2015; Sedrez et al., 2021), *S. rastrifer* is a generalist benthopelagic predator that forages just above the substrate (Chaves and Vendel, 1998). Its otolith morphology, characterized by a slightly developed *rostrum* and a prominent *antirostrum*, may supports this behavior by enhancing vibration detection and stability near the bottom (Volpedo and Echeverría, 2003; Tuset et al., 2008). In contrast, morphogroup 2, comprising *Trichiurus lepturus* (Trichiuridae) and *Diplectrum radiale* (Serranidae), illustrates the extent to which environmental pressures can override phylogenetic differences. These taxonomically distant species converged on a fusiform otolith shape with a pronounced *rostrum*, a feature typical of active open-water swimming that enhances directional hearing and hydrodynamic perception (Tuset et al., 2010; Schulz-Mirbach et al., 2019). This finding expands upon Vignon and Morat (2010), demonstrating that environmental drivers can promote morphological convergence even among species from different families and emphasizing that the relative influence of phylogeny and environment varies across systems and taxa.

Analysis of *rostrum* and *antirostrum* development revealed a clear morphological gradient aligned with habitat use, as captured by the second PCA axis. At one extreme, the pelagic *Cetengraulis edentulus* (morphogroup 6, Engraulidae) exhibits fully developed *rostrum* and *antirostrum*. At the benthic extreme, species in morphogroups 1, 5, 8, and 9 (e.g., *Menticirrhus americanus*, *Etropus crossotus*, *Symphurus tessellatus*, *Achirus lineatus*) show these structures absent or weakly developed. Intermediate morphologies occur in morphogroup 4 (*Prionotus punctatus* and *Orthopristis ruber*), and morphogroup 3 (*Eucinostomus* spp.). The former includes species from different families with an almost absent *rostrum*, suggesting adaptive convergence toward a nekton-benthic lifestyle. In the latter, *E. argenteus* exhibits a slightly developed *antirostrum* whereas *E. gula* lacks it, consistent with fine-scale adjustments to shallow, sandy habits where balance and orientation are essential.

A comparable pattern of habitat-related divergence has been reported for sea breams of the genus *Pagellus* (Sparidae): the pelagic *P. acarne* exhibits elongated otoliths with a pronounced *rostrum*, whereas the demersal *P. erythrinus* has broader, more rounded shapes (D'Iglio et al., 2021). Variation in *rostrum, antirostrum*, and *sulcus*

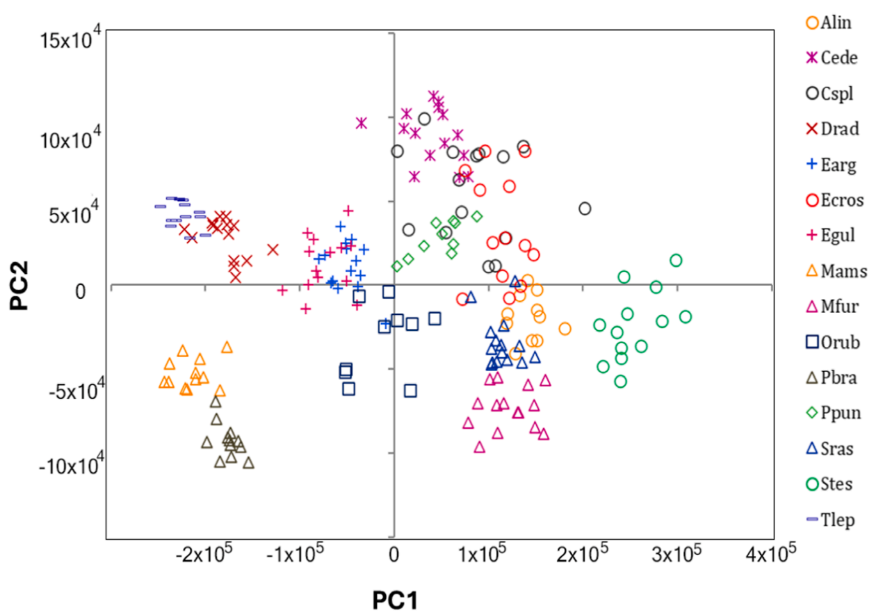

Fig. 7. Ordination plot of the first two principal component (PC) axes of otolith contours, derived from harmonics generated by elliptical Fourier analysis (EFA). Species codes: Mfur, *Micropogonias furnieri*; Egul, *Eucinostomus gula*; Earg, *Eucinostomus argenteus*; Drad, *Diplectrum radiale*; Orub, *Orthopristis ruber*; Sras, *Stellifer rastrifer*; Cede, *Cetengraulis edentulus*; Cspl, *Citharichthys spilopterus*; Ecro, *Etropus crossotus*; Alin, *Achirus lineatus*; Mams, *Menticirrhus americanus*; Pbra, *Paralonchurus brasiliensis*; Ppu, *Prionotus punctatus*; Stes, *Symphurus tessellatus*; Tlep, *Trichiurus lepturus*.

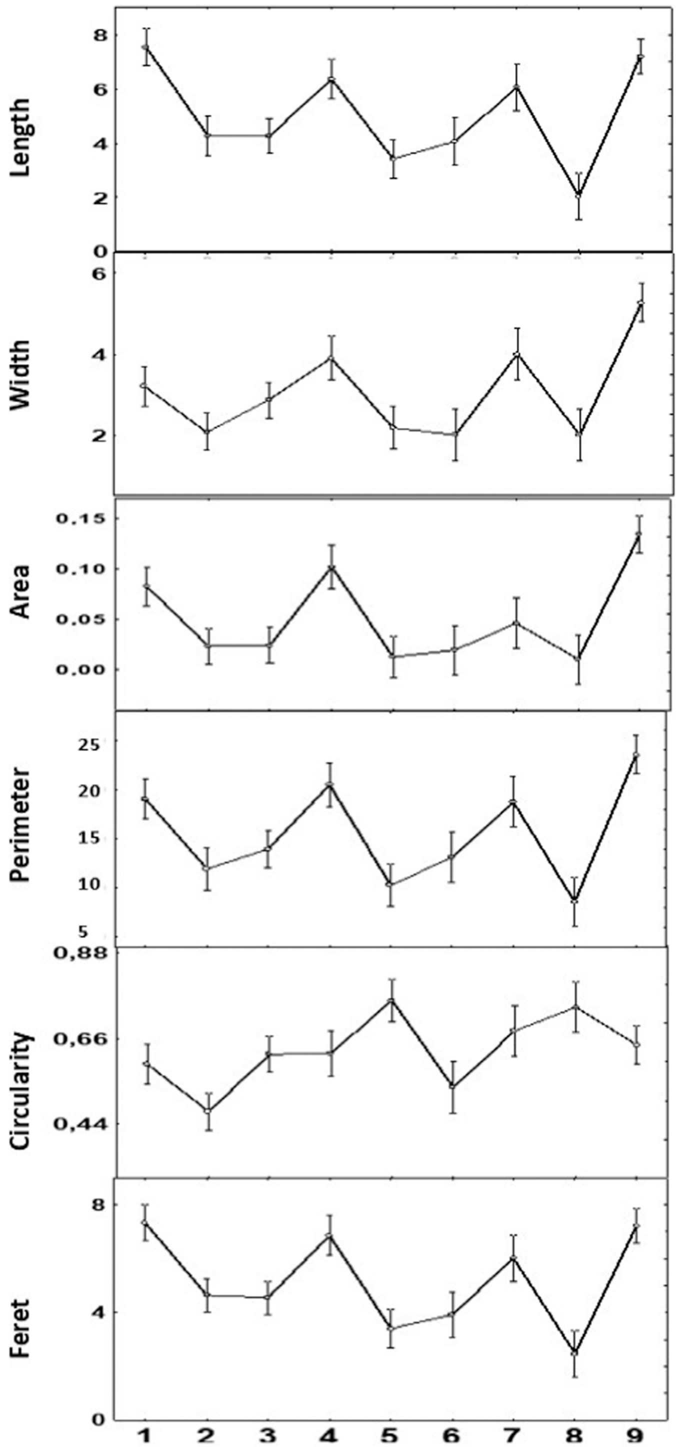

Fig. 8. Line plot of otolith morphometric measurements across groups identified by cluster analysis, showing mean values ± standard deviation. Species groups according to otolith shape (1−9) as defined in Fig. 3.

morphology therefore reflects an integrated response to ecological demands operating within phylogenetic constraints.

Complementing the evidence from shape analysis and PCA, traditional biometric metrics provided quantitative support and important nuances to the observed patterns. These analyses showed that the highest size values (area and perimeter) in morphogroups 1, 4, and 9, which include species such as *Diplectrum radiale*, *Micropogonias furnieri*, and *Menticirrhus americanus*, reflect great investment in sensory structures associated with larger body size or occupation of structurally complex environments (Lombarte and Lleonart, 1993). Circularity was particularly informative, with higher values in morphogroups 5 and 8 (benthic species) and lower values in pelagic or more active species, quantitatively reinforcing classical ecomorphological syndromes (Volpedo and Echeverría, 2003; Vignon and Morat, 2010). Overall, the interplay between phylogenetic heritage and environmental adaptation shapes the assemblagés morphological diversity, producing a continuum from rostrate otoliths in pelagic species to more rounded forms in benthic species.

Beyond their academic relevance, these findings highlight the applied value of otolith morphology in fisheries science and ecosystem monitoring. Otolith traits can aid species identification and support stock assessments, bycatch control, and biodiversity surveys, particularly when traditional approaches are limited. Because otoliths record ecological strategies, growth conditions, and habitat use, they serve as natural archives of environmental history (Campana, 2005; Park et al., 2023). Otolith shape descriptors also retain species-specific and population-level signatures, enabling stock discrimination, connectivity assessments, and informed management decisions where genetic or tagging methods are not feasible (Lombarte and Lleonart, 1993; Almeida et al., 2023). Integrating otolith morphology into monitoring frameworks therefore enhances the detection of habitat-driven shifts in fish assemblages and strengthens spatially informed management strategies.

5. Conclusion

The classification of otoliths into nine morphotypes underscores the combined influence of evolutionary history and environmental conditions in shaping otolith morphology. Although our initial hypothesis emphasized the primacy of ecological pressures, the results indicate that phylogenetic constraints exert a more dominant and consistent effect, as reflected in the cohesive clustering of species within taxonomic families.

Nevertheless, habitat-related factors appear to modulate specific morphological traits, such as *rostrum* development, *sulcus* orientation, and otolith contour, suggesting functional adjustments to habitat complexity, acoustic properties, and sensory demands. These environmentally influenced features reveal a degree of phenotypic plasticity operating within the boundaries imposed by phylogenetic heritage.

Our findings indicate that otolith morphology in tropical estuarine fishes is primarily structured by phylogenetic lineage, with ecological factors acting as modulators that fine-tune specific traits. This highlights the value of otoliths as integrative structures for exploring both evolutionary patterns and ecological adaptations in fish assemblages.

CRediT authorship contribution statement

RSS and FGA equally contributed to the completion of the study, from conceptualization and database analyses to statistical treatment and manuscript writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fishres.2026.107715.

Data availability

Data will be made available on request.

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