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Original Article

Identification of yellowtail snapper, *Ocyurus chrysurus* (Bloch, 1791), stocks using otolith shape analysis in the coastal waters of northeastern Brazil

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Abstract
Otoliths, which grow continuously throughout a fish's life, play a crucial role in identifying fish stocks. This study aimed to distinguish stocks of *Ocyurus chrysurus* (Bloch, 1791) in the coastal waters of northeastern Brazil using otolith morphology. Otolith morphology was examined in specimens collected from three regions (Ceará, Pernambuco and Bahia) of northeastern Brazil using morphometric parameters, shape indices, and wavelet coefficients. The results revealed significant differences in otolith morphology between Bahia and the other two regions. Classification based on morphometric parameters and shape indices correctly assigned 43%, 45%, and 87% of specimens from Ceará, Pernambuco, and Bahia, respectively. Wavelet coefficients further improved classification accuracy to 45%, 48%, and 98%, respectively. Mean otolith outlines showed marked regional differences, particularly in the rostrum (0–40° and 320–360°) and post-rostrum (145–200°) regions. These findings provide valuable information for stock discrimination and support fisheries management of *O. chrysurus* along the northeastern coast of Brazil.

Keywords: morphometrics; *Ocyurus chrysurus*; otoliths; shape index; yellowtail snapper; wavelet coefficients

1 | INTRODUCTION

Identifying fish stocks and understanding population structure are fundamental for the conservation and sustainable management of marine fish populations (Wang *et al*. 2018; Rahnama *et al*. 2023; Vaz *et al*. 2023). Several approaches have been used to discriminate fish stocks, including otolith shape analysis, genetics, otolith chemistry, demographic analyses, meristic characteristics, tagging, and acoustic telemetry (Hawkins *et al*. 2016; Vaz *et al*. 2023). Among these, otolith morphology has become a

widely used and reliable tool because it reflects both genetic influences and environmental conditions experienced during fish growth (Mounir *et al*. 2022; Qiao *et al*. 2022; Geladakis *et al*. 2023). Otoliths are metabolically inert calcified structures located in the inner ear of teleost fishes that play essential roles in hearing and balance. Unlike bones and scales, they retain a permanent chronological record of fish growth because they are not resorbed or remodeled after formation (Di Franco *et al*. 2014).

Recent advances in digital image processing have expanded the application of otolith morphology in stock discrimination studies (Park *et al*. 2023). Otolith shape can be quantified using two main approaches: traditional morphometric measurements and shape indices (Avigliano *et al*. 2015; Zhou *et al*. 2023), or contour-based methods such as wavelet transform and elliptic Fourier analysis. Wavelet transform compares otolith contours point by point, allowing regional differences in shape to be identified with high precision (Sadighzadeh *et al*. 2014; Wiff *et al*. 2020). The resulting descriptors are commonly analyzed using multivariate statistical methods, such as principal component analysis and discriminant analysis, to distinguish fish stocks (Bourel and Segura 2018; Wiff *et al*. 2020). Furthermore, the open-source package developed by Libungan and Pálsson (2015) has facilitated standardized otolith shape analyses and improved multiclass classification.

The yellowtail snapper, *Ocyurus chrysurus* (Bloch, 1791), is a reef-associated species distributed throughout the western Atlantic, where it inhabits shallow coastal waters, coral reefs, rocky reefs, and sandy substrates (da Silva *et al*. 2015). According to the IUCN Red List, *O. chrysurus* is currently classified as Data Deficient (DD), highlighting the need for additional biological and population-level studies to support future conservation assessments. Although several studies have investigated the species' biology and ecology (Araujo *et al*. 2002; Begossi *et al*. 2011), information on its stock structure remains limited, particularly along the Brazilian coast.

Therefore, the present study aimed to discriminate stocks of *O. chrysurus* from three regions of northeastern Brazil using two complementary otolith shape analysis approaches: morphometric parameters with shape indices and wavelet transform (WT). The results provide new information on the population structure of this commercially important species and contribute to future fisheries management and conservation strategies in Brazilian coastal waters.

2 | METHODOLOGY

2.1 Study area

The northeastern Brazilian (NEB) region is a crucial area for fisheries, particularly for groupers and snappers (Fonteles-Filho 1969; Rezende *et al*. 2003). Three important fishery sites of *O. chrysurus* were chosen to cover the widest possible range of the NEB coast: Ceará (CE), Pernambuco (PE), and Bahia (BA) (Figure 1). Ceará is in the North region of NEB and is historically an important fishery area of snappers (Fonteles-Filho 1969). Pernambuco is one of the most productive fishing areas, especially in the catch of snappers. South Bahia harbors the largest and richest coral reefs of the South Atlantic (Abrolhos Bank).

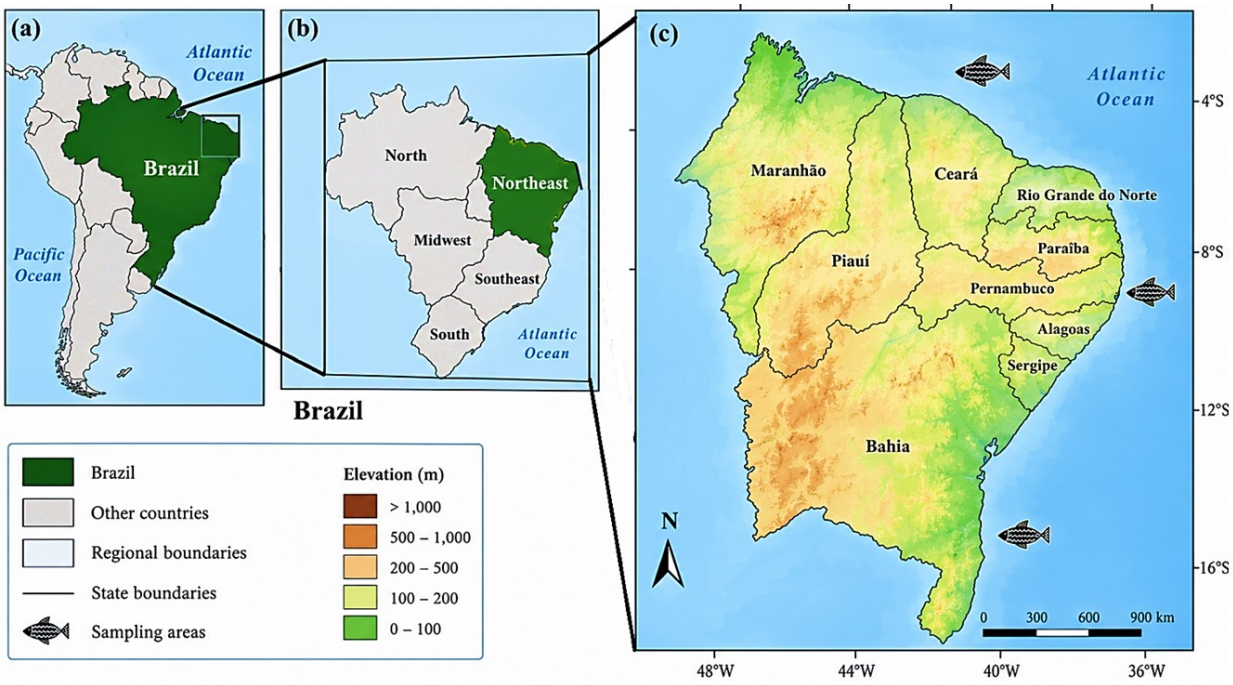


FIGURE 1 Sampling areas in northeast Brazil. Abbreviations; CE = Ceará; PE = Pernambuco and BA =Bahia.

2.2 Sample collection and otolith preparation

A total of 120 specimens of *O. chrysurus* were collected between 2019 and 2020, including 40 individuals from each study area (CE, PE, and BA). Fish were obtained from local artisanal fisheries using gillnets, traps, and hand lines. Standard biological measurements, including total weight (g), standard length (cm), and fork length (cm), were recorded before both sagittal otoliths were removed. The right sagittal otolith of each specimen was photographed against a black background using a Leica M205A stereomicroscope equipped with a Leica DFC450 digital camera. Images were calibrated using a 1-mm scale and analyzed with ImageJ software to obtain otolith measurements.

2.3 Otolith morphometry and shape indices

Otolith images were analyzed using ImageJ software. The following morphometric variables were measured (Figure 2): area (mm²), length (mm), width (mm), perimeter (mm), ostium length (mm), caudal length (mm) and sulcus length (mm).

From these measurements, six shape indices were calculated following Agüera and Brophy (2011): circularity, rectangularity, form factor, ellipticity, roundness, and aspect ratio.

Circularity = P / A^2

Rectangularity = $A / (OL \times OW)$

Form-factor = $4\pi A / P^2$

Roundness = $4A / (\pi(OL)^2)$

Ellipticity = $(OL - OW) / (OL + OW)$

Aspect ratio = OL / OW

Here, P = perimeter; A = area; OL = otolith length; OW = otolith width.

The magnification of the Leica M205A stereomicroscope was standardized for all image acquisitions to ensure measurement consistency. Otolith morphometric variables, including area (mm²), length (mm), width (mm), perimeter (mm), ostium length (mm), caudal length (mm), and sulcus length (mm), were measured from digital images using ImageJ software (Figure 2 and 3). Six shape indices—circularity, rectangularity, form factor, ellipticity, roundness, and aspect ratio—were subsequently calculated following Agüera and Brophy (2011).

Outliers in the otolith morphometric variables and shape indices were identified using Grubbs' test. Data normality and homogeneity of variances were assessed using the Kolmogorov–Smirnov and Levene's tests, respectively. When necessary, data were log (x+1) transformed to improve normality and reduce within-group variation.

A *t*-test was used to compare fork length and total length among specimens from Ceará (CE), Pernambuco (PE), and Bahia (BA). A paired *t*-test was also performed to compare measurements of the left and right otoliths. As no significant differences were detected between sides ($p > 0.05$), only the right otolith was used in subsequent analyses, while the left otolith was used only when the right otolith was damaged or unavailable.

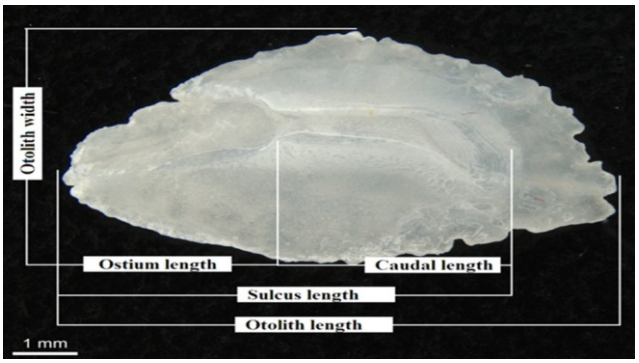


FIGURE 2 Measurements of otolith dimensions, including area, width, length, perimeter, ostium length, caudal length, and sulcus length, were taken using ImageJ software.

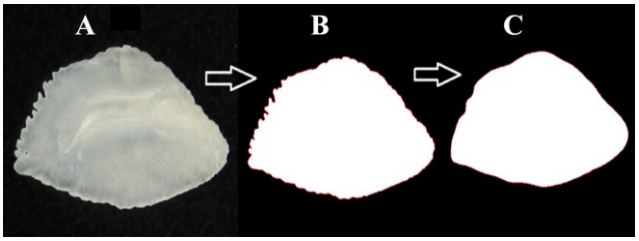


FIGURE 3 Otolith shape differences among stocks and fish populations.

Differences in otolith morphometric variables and shape indices among the three regions were evaluated using analysis of covariance (ANCOVA), with total length and sex included as covariates. Significant variables were adjusted using the within-group common slope (*b*) according to Lleonart *et al*. (2000). The adjustment was performed using the following equation:

Adjusted value = $Vi - b \times TL$

Here, Vi: Original measurement; b: Common within-group regression slope; TL: Total length of the corresponding fish.

This adjustment removes the effect of fish size on otolith measurements, allowing morphological differences among regions to be evaluated independently of body size. Where Vi is the original variable, *b* is the common within-group slope, and TL is the total length (Campana and Casselman 1993).

Principal component analysis (PCA) was used to summarize variation in otolith morphometric variables and shape indices. Before performing PCA, sampling adequacy and data suitability were assessed using the Kaiser–Meyer–Olkin (KMO) measure (Kaiser 1974) and Bartlett's test of sphericity (Bartlett 1954). KMO values were interpreted as excellent (0.90–1.00), very good (0.80–0.89), good (0.70–0.79), moderate (0.60–0.69), and unaccepta-

ble (< 0.60). Significant principal components were identified using the Jolliffe criterion (Jackson 1993).

Linear discriminant analysis (LDA) was performed to evaluate differences among sampling regions and estimate classification accuracy. Model performance was assessed using leave-one-out (jackknife) cross-validation and Wilks' λ (Correia *et al*. 2011). Lower values of Wilks' λ indicate greater discrimination among groups, whereas values approaching one indicate little or no discrimination (Everitt and Dunn 1991).

2.4 Wavelet coefficient analysis

Wavelet coefficient analysis was performed using the R package ShapeR (Libungan and Pálsson 2015) in R version 4.0.5 (R Core Team 2025). Otolith outlines were automatically extracted from calibrated digital images following grayscale conversion and image binarization. Wavelet coefficients describing the otolith contours were then generated for each specimen, and fish length was standardized to minimize the effects of allometric growth (Stransky 2014).

The resulting wavelet coefficients were used to reconstruct and compare mean otolith outlines among the three sampling regions. The accuracy of the wavelet reconstruction was assessed by comparing reconstructed contours with the original otolith outlines. Spatial variation in wavelet coefficients along the otolith outline was visualized using graphical functions implemented in the ShapeR package. Wavelet analyses were performed using the ShapeR package (version 1.0.1) implemented in R version 4.0.5. Differences among populations were quantified using the intraclass correlation coefficient (ICC), which estimates the proportion of shape variation attributable to differences among sampling regions (Libungan and Pálsson 2015).

2.5 Data analysis

Normality and homogeneity of variance of the wavelet coefficients were assessed using the Kolmogorov–Smirnov and Levene's tests, respectively. When assumptions of normality were not met, appropriate data transformations ($\log_{10}$ or reciprocal transformation) were applied. No wavelet descriptors were excluded after normalization. Fish length was standardized to reduce the influence of allometric growth following Tuset *et al*. (2006).

Principal component analysis (PCA) was used to summarize variation in wavelet coefficients and to visualize differences among sampling regions along the first two principal components (PC1 and PC2). Linear discriminant analysis (LDA) was subsequently applied to evaluate population discrimination and estimate classification accuracy among the three study regions (Ripley *et al*. 2020). The discriminative performance of the model was assessed using Wilks' λ, where lower values indicate stronger separation among groups.

3 | RESULTS

3.1 Morphometrics

No significant differences were observed in either total length or fork length among specimens collected from Ceará (CE), Pernambuco (PE), and Bahia (BA) (*t*-test, p = 0.31 and p = 0.490, respectively; Figure 4), indicating that fish size was comparable among the three sampling regions.

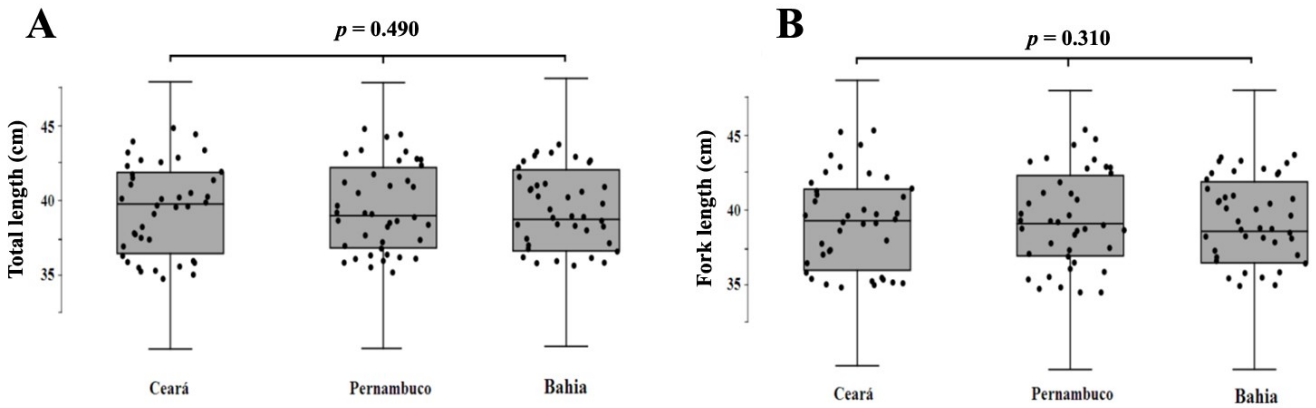


FIGURE 4 Comparison of the total length (A) and fork length (B) of O. chrysurus between Ceará, Pernambuco, and Bahia with a *t*-test plot.

The morphometric variables and shape indices satisfied the assumptions of normality and homogeneity of variance, as assessed by the Kolmogorov–Smirnov and Levene's tests (p > 0.05). ANCOVA revealed significant regional differences in three shape indices (form factor, aspect ratio, and ellipticity) and two morphometric variables (perimeter and area), all of which were significantly associated with fish size (Table 1). Prior to ANCOVA, the assumptions of linear relationships between the covariates and dependent variables, as well as the homogeneity of regression slopes, were tested and met (p > 0.05). Variables showing significant relationships with fish size were

subsequently adjusted using the within-group common slope (*b*). No significant differences were detected between male and female otoliths (ANOVA: $p > 0.05$).

Principal component analysis (PCA) showed that the first two principal components explained 56.78% of the total variation in otolith morphometric variables and shape indices (Figure 5). PC1 and PC2 accounted for 33.50% and 23.27% of the total variance, respectively. PC1 was mainly associated with otolith width and weight, whereas PC2 was primarily influenced by area and form factor. Bartlett's test of sphericity ($p = 0.012$) and the Kaiser–Meyer–Olkin (KMO) value (0.71) confirmed that the dataset was suitable for PCA. The remaining variation likely reflects individual biological variability, environmental influences, and morphological characteristics not captured by the first two principal components.

TABLE 1 Analyses of covariance (ANCOVA) for each otolith shape indices and morphometric parameter between three regions.

Parameters	Ceará(CE)	Pernambuco (PE)	Bahia(BA)	ANCOVA
Otolith weight (g)	0.099±0.0534	0.120±0.0522	0.259±0.0488	ns
Area (m^2)	45.681±7.801	42.171±6.655	48.581±7.831	*
Perimeter (m)	32.828±3.987	31.154±2.703	33.828±3.987	*
otolith length (mm)	11.822±1.821	10.592±1.832	11.992±1.821	ns
otolith width (mm)	6.858±0.903	5.775±0.892	6.990±0.903	ns
Sulcus length (mm)	8.510±0.441	8.524±0.424	8.610±0.451	ns
Ostium length (mm)	4.239±0.392	4.194±0.376	4.250±0.393	ns
Caudal length (mm)	4.454±0.763	4.485±0.761	4.554±0.773	ns
Shape indices				
Aspect ratio	0.768±0.019	0.573±0.014	0.789±0.019	*
Circularity	19.092±0.609	19.903±0.672	19.098±0.659	ns
Ellipticity	0.581±0.017	0.389±0.018	0.594±0.017	*
Form factor	0.780±0.045	0.711±0.042	0.789±0.046	*
Rectangularity	0.851±0.014	0.877±0.014	0.855±0.015	ns
Roundness	3.688±0.222	3.755±0.191	3.688±0.236	ns
Age (year)	4.537±2.621	4.278±2.325	4.647±2.681	ns

Values are given in Mean ± Standard-Deviation. Abbreviations: ns = not significant; * = significant ($p < 0.05$)

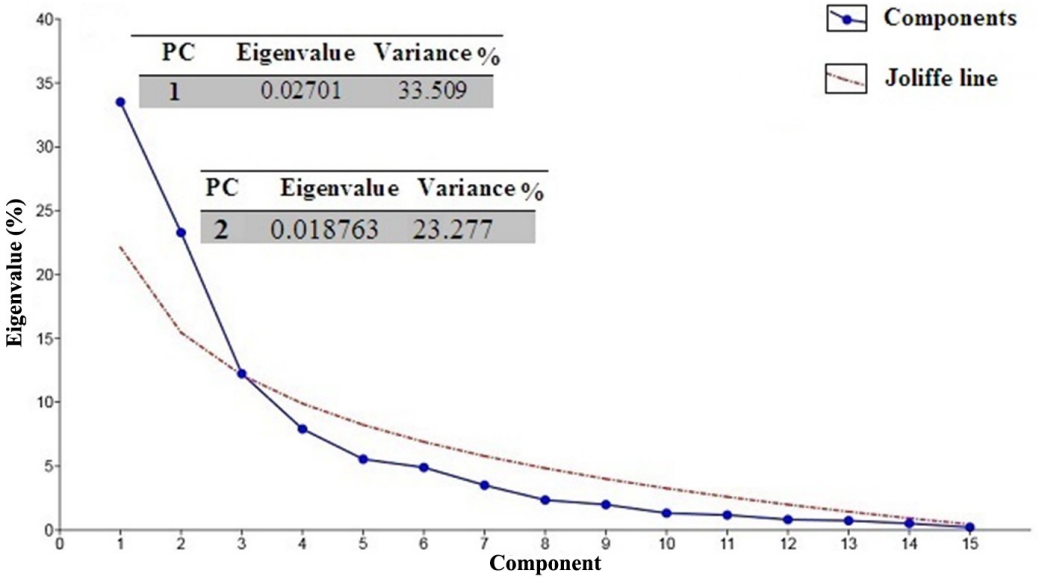


FIGURE 5 Principal component axes, eigenvalues, and percent variation for the significant otolith morphometric parameter and shape indices.

Linear discriminant analysis (LDA) based on otolith morphometric variables and shape indices correctly classified 56.2% of the specimens using leave-one-out cross-validation. The first two discriminant functions explained 78.5% (LD1) and 21.5% (LD2) of the total variation, respectively. LD1 primarily separated specimens from Bahia from those collected in Ceará and Pernambuco, whereas LD2 discriminated between Ceará and Pernambuco (Figure 6). Classification success was highest for Bahia (87%), while Ceará and Pernambuco showed lower classification accuracies (43% and 45%, respectively). Most classification errors occurred between Ceará and Pernambuco,

indicating substantial overlap in otolith morphology. The relatively low classification success for these two regions suggests limited discriminatory power of otolith morphometric variables and shape indices for separating Ceará and Pernambuco. The Wilks' λ value (0.854) indicated a moderate discriminatory ability of the model.

3.2 Wavelet coefficients

Wavelet analysis revealed subtle but distinct regional differences in otolith shape among specimens from Ceará, Pernambuco, and Bahia (Figure 7). The greatest variation in wavelet coefficients (ICC) occurred in the rostrum region (0–40° and 320–360°), whereas additional differences were observed in the post-rostrum region (145–200°). These results indicate that the rostrum contributed most to regional differences in otolith morphology.

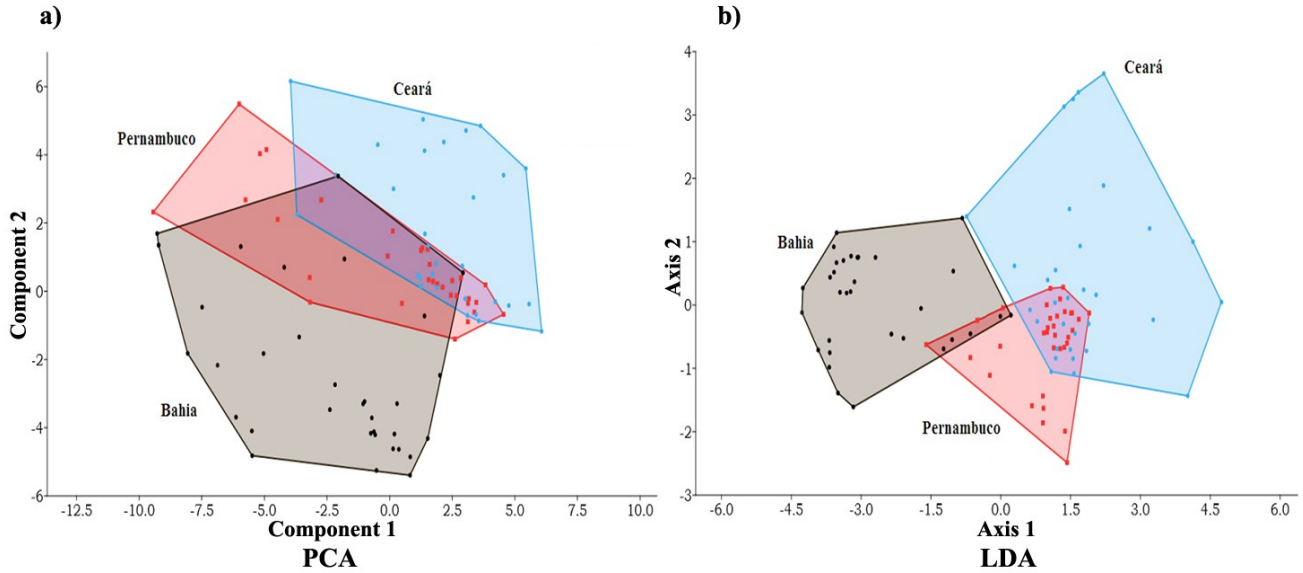


FIGURE 6 a) Principal Component Analysis (PCA), b) Linear Discriminant Analysis were conducted on Otolith morphometric parameters and shape indices of *Ocyurus chrysurus* collected from Ceará, Pernambuco, and Bahia.

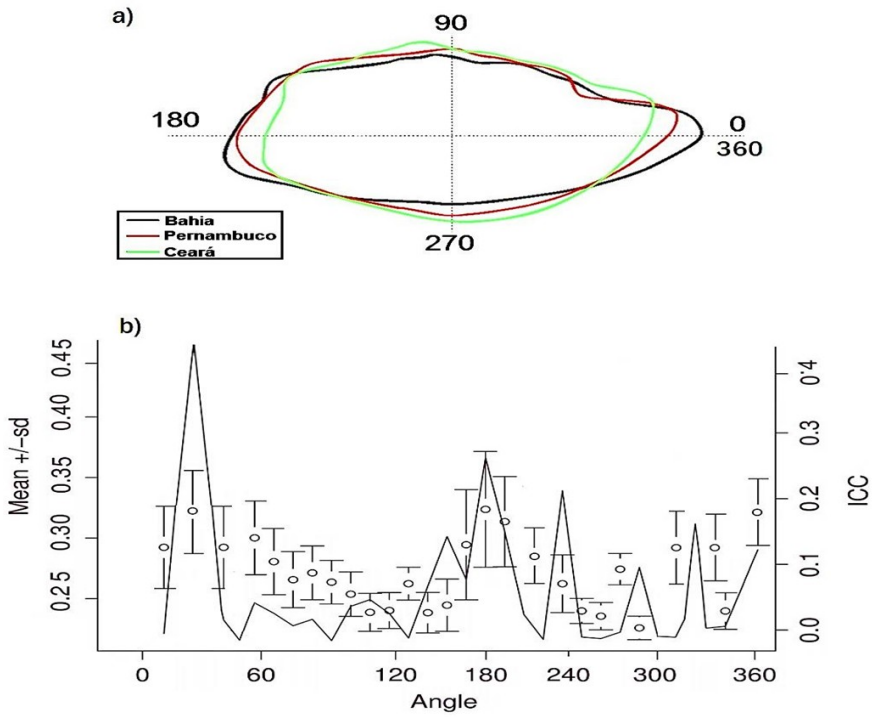


FIGURE 7 a) Average otolith shape based on Wavelet coefficients for three discrete fish populations of *Ocyurus chrysurus* from Ceará, Pernambuco, and Bahia. Numbers represent angles in degrees (°) based on polar coordinates (180, 90, 0, 270, and 360): b) Mean (±SD) of the variation in shape outline for all combined Otolith along the horizontal axis based on polar coordinates (Wavelet coefficients-ICC).

The principal component analysis (PCA) of the wavelet coefficients clearly distinguished the three sampling regions. The first principal component (PC1) explained 84.0% of the total variation and primarily separated spec-

imens from Bahia from those collected in Ceará and Pernambuco. The second principal component (PC2) accounted for a much smaller proportion of the variation and reflected slight differences between Ceará and Pernambuco. The suitability of the dataset for PCA was confirmed by a KMO value of 0.94 and a significant Bartlett's test of sphericity ($p < 0.05$).

Linear discriminant analysis (LDA) produced results consistent with the PCA. The first two discriminant functions explained 88.5% (LD1) and 11.5% (LD2) of the total variation, respectively. LD1 clearly separated Bahia from Ceará and Pernambuco, whereas LD2 discriminated between Ceará and Pernambuco (Figure 8). Overall classification accuracy reached 74.5% using leave-one-out cross-validation. Classification success was highest for Bahia (98%), while Ceará (45%) and Pernambuco (48%) showed lower classification accuracies, reflecting the greater similarity in otolith shape between these two regions. The Wilks' λ value (0.301) indicated strong discrimination among the three populations.

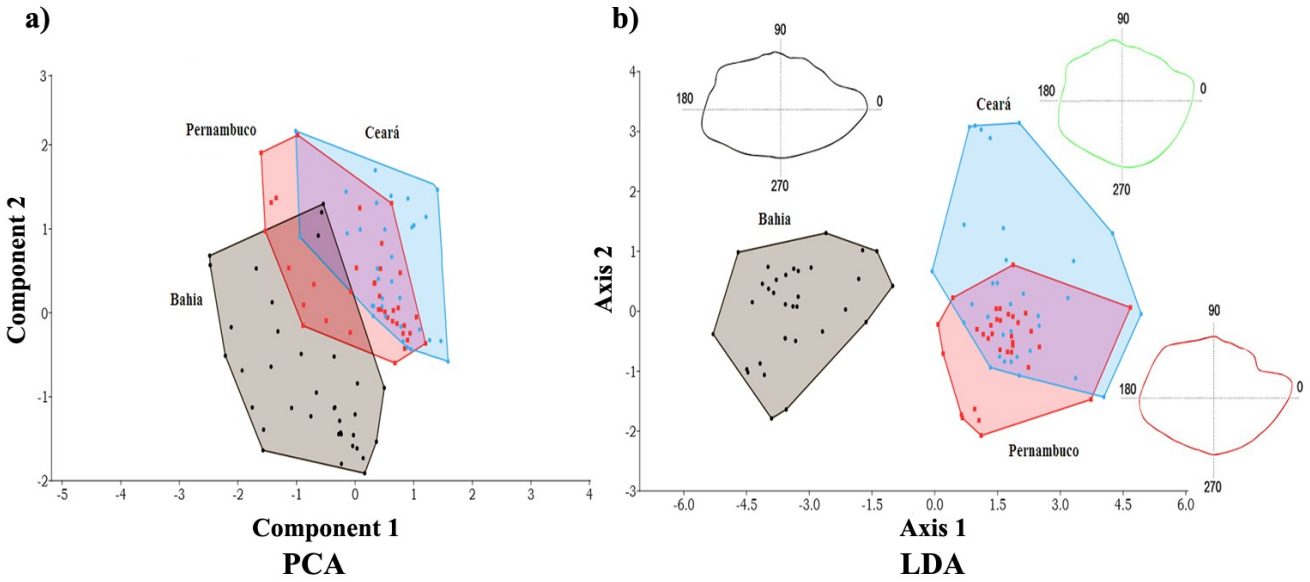


FIGURE 8 (a) Principal component analysis plot (PC1 versus PC2) of otolith contours computed for the analyzed regions. The PCA utilized Wavelet coefficients, with 95% probability ellipses depicted. (b) Linear Discriminant Analysis of otolith Wavelet coefficients (WL) counters, with a 95% confidence interval for *Ocyurus chrysurus* among Ceará, Pernambuco, and Bahia.

4 | DISCUSSION

Recent studies have demonstrated that wavelet-based analyses provide a reliable and sensitive approach for quantifying otolith shape variation and discriminating fish stocks (Wiff *et al.* 2020; Rahnama *et al*. 2023; Ozpicak *et al*. 2024). Compared with traditional morphometric approaches, wavelet analysis captures subtle contour differences more effectively and has shown high classification accuracy in several fish species. In the present study, wavelet analysis outperformed conventional morphometric variables and shape indices, resulting in higher classification success and providing stronger evidence of population differentiation. Nevertheless, morphometric parameters and shape indices complemented the wavelet analysis by supporting the observed regional patterns.

Both morphometric and wavelet analyses indicated a high degree of similarity in otolith shape between specimens from Ceará and Pernambuco, whereas fish from Bahia formed a clearly distinct group. The lower classification accuracy observed for Ceará and Pernambuco, together with the substantial overlap in the discriminant analyses, suggests a greater degree of connectivity between these regions than with Bahia. Similar regional variation has been reported in other marine fishes, where otolith shape reflects differences in environmental conditions, habitat characteristics, and population connectivity (Soeth *et al*. 2019).

Several ecological factors may explain the observed similarity between Ceará and Pernambuco. These regions are separated by approximately 800 km but share extensive reef and estuarine habitats that provide important nursery, feeding, and spawning environments for *O. chrysurus* (Leão and Dominguez 2000; Moura *et al*. 2011; Aschenbrenner *et al*. 2016). Coral reef systems may facilitate movement and gene flow among local populations, thereby reducing morphological differentiation in otolith shape (Feitoza *et al*. 2005; Ortiz-Lozano *et al*. 2013). In addition, offshore islands such as Rocas Atoll and the Fernando de Noronha Archipelago have been identified as important habitats for adult snappers and may function as stepping stones or spawning sites that enhance connectivity among northeastern Brazilian populations (Krajewski and Bonaldo 2005; Longo *et al*. 2015).

Although the specimens analyzed in this study were

collected during 2019–2020, recent studies indicate that *O. chrysurus* remains widely distributed throughout the northeastern Brazilian coast, with no evidence of major changes in its regional distribution or habitat use. Because otolith morphology reflects long-term growth and environmental history rather than short-term fluctuations, the observed stock structure is expected to remain representative of the populations inhabiting the study area. Nevertheless, continued monitoring using contemporary samples and complementary approaches such as genetics and otolith chemistry would further improve our understanding of population connectivity and stock dynamics.

During the reproductive season, *O. chrysurus* forms spawning aggregations and migrates to offshore reef habitats, where mature individuals may travel long distances before spawning (Domeier and Colin 1997; Claro and Lindeman 2003). Together with its relatively long pelagic larval duration (approximately 25–45 days; Claro and Lindeman 2008), these life-history traits likely enhance dispersal and connectivity among populations, helping to explain the strong overlap in otolith morphology observed between Ceará and Pernambuco.

This interpretation is consistent with previous genetic studies that reported high connectivity and limited population structure among Brazilian snapper populations. High genetic homogeneity has been documented for *O. chrysurus* (da Silva *et al*. 2015), *Lutjanus purpureus* (da Silva *et al*. 2016), and *L. synagris* (Silva *et al*. 2018). Likewise, Souza *et al*. (2019) found no significant geographic structure in mitochondrial DNA lineages of *L. jocu* and *L. analis* along the northeastern Brazilian coast, supporting the existence of extensive gene flow among regional populations.

In contrast, both morphometric and wavelet analyses consistently identified Bahia as a distinct stock. The separation of Bahia from Ceará and Pernambuco may be associated with the unique oceanographic and ecological characteristics of the Abrolhos Bank, which influences habitat availability, migration routes, and reproductive dynamics of reef-associated fishes. Similar patterns have been reported for other Brazilian marine fishes. For example, Soeth *et al*. (2019) identified a distinct stock of Chaetodipterus faber associated with the Abrolhos region, while Duarte-Neto *et al*. (2008) detected separate stocks of *Coryphaena hippurus* along the northeastern Brazilian coast based on otolith shape.

The apparent differences between otolith-based and genetic studies are expected because these approaches reflect different biological processes and temporal scales. Genetic markers describe historical gene flow accumulated over multiple generations, whereas otolith morphology reflects environmental conditions and population structure experienced during the lifetime of individual fish (Taillebois *et al*. 2017). Consequently, the two approaches should be considered complementary rather than contradictory.

Overall, the present findings provide preliminary evidence of stock structure in *O. chrysurus* along the northeastern Brazilian coast. The observed differences suggest that Bahia may represent a distinct stock, whereas Ceará and Pernambuco appear to comprise a mixed stock. However, because stock discrimination in the present study was based solely on otolith morphology, these findings should be interpreted with caution. Future studies integrating complementary approaches, such as genetics, otolith chemistry, tagging, and population genomics, are needed to validate the stock structure identified here. If confirmed, these findings could contribute to the development of region-specific fisheries management strategies. Given the ecological importance of Bahia and the increasing environmental pressures affecting the region, including the impacts associated with the Rio Doce mining tailings disaster (Pereira-Filho *et al*. 2019; Previero and Gasalla 2020), further investigation of population structure is warranted.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Behzad Rahnama: Conceptualization, Methodology, Formal analysis, Investigation, Software, Project administration, Writing – original draft; Eisa Hajiradkouchak: Investigation, Data curation, Writing – original draft; Mehdi Khoshnamvand: Writing – review & editing; R. Souza Rosa: Investigation, Supervision, Writing – review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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