Discriminating *Scleromystax barbatus* (Siluriformes: Callichthyidae) populations from Atlantic Rainforest streams employing otolith shape

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This study assessed the *lapillus* otolith shapes of males and females of *Scleromystax barbatus* from southern and southeastern regions in the Atlantic Rainforest biome employing Fourier and Wavelet descriptors. The utricular otoliths of *S. barbatus* are ovoid, with the *gibbus maculae* occupying almost all the ventral portion, similar to most Callichthyidae species. Otoliths of males and females of *S. barbatus* from the southeastern studied region are more elongated in the anterior-posterior direction and present larger *sulcus* and *gibbus maculae*, with heterogeneous borders. We found no sexual-based dimorphism in otolith shape within regions, however regional differences were registered and attributed to variations in fish life history mediated by differences in environmental factors (*e.g.*, climatic conditions) between the southern and southeastern regions in the Atlantic Rainforest biome. Additional studies are suggested to investigate the influence of genetic effects and their environmental interactions to better understand how these factors are related with otolith shape and influence the discrimination among *S. barbatus* populations.

Keywords: Armored-catfish, Corydoradinae, Morphology, Neotropical, Population structure.

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Este estudo avaliou as formas dos otólitos *lapillus* de machos e fêmeas de *Scleromystax barbatus* provenientes das regiões sul e sudeste do bioma Mata Atlântica, empregando os descritores de Fourier e Wavelet. Os otólitos utriculares de *S. barbatus* são ovóides, com *gibbus maculae* ocupando quase toda a porção ventral, semelhante à maioria das espécies de Callichthyidae. Os otólitos de machos e fêmeas de *S. barbatus* provenientes da região sudeste são mais alongados no sentido ântero-posterior e apresentam *sulcus* e *gibbus maculae* maiores, com bordas heterogêneas. Não encontramos dimorfismo sexual na forma dos otólitos em cada região, porém diferenças regionais foram registradas e atribuídas a variações na história de vida dos peixes mediadas por diferenças em fatores ambientais (*e.g.*, condições climáticas) entre as regiões sul e sudeste do bioma Mata Atlântica. Estudos adicionais são sugeridos para investigar a influência de efeitos genéticos e suas interações ambientais para melhor compreender como esses fatores estão relacionados com a forma dos otólitos e influenciam a discriminação entre as populações de *S. barbatus*.

Palavras-chave: Coridoras, Corydoradinae, Estrutura populacional, Morfologia, Neotropical.

INTRODUCTION

Atlantic Rainforest rivers and streams harbor a high taxonomic and functional fish fauna diversity (Abilhoa *et al*., 2011). Many fish species within this biome display restricted distributions, associated to the high number of independent hydrological drainages that arise in high altitudes and flow towards the Atlantic Ocean (Menezes *et al*., 2007). Both geological and ecological conditions have influenced the unique diversification patterns, genetic population structuring and high number of endemic fish species noted in this region (Torres, Ribeiro, 2009; Thomaz *et al*., 2015).

The Brazilian Atlantic Rainforest biome is one of the most biologically rich, albeit threatened, biodiversity hotspot (Myers *et al*., 2000). Despite their wide geographical range throughout the Brazilian coast at almost 30° of latitude, after five centuries of human occupation less than 7% of original forests remain, now comprising only fragments surrounded by open-habitat matrices, such as agricultural and urban landscapes (Ribeiro *et al*., 2009).

Due to the isolating effect of mountain ranges on the geographic distribution of fishes that occur coastal Atlantic Rainforest rivers, several dispersal-vicariance mechanisms and hypotheses based on headwater stream capture and isolation/connection due to marine transgressions and regressions have been proposed (Ribeiro, 2006; Roxo *et al*., 2014; Thomaz, Knowles, 2018). Paleodrainage connections between coastal drainages have been used to explain the current distribution and genetic diversity of many fish populations in this region (Lima *et al*., 2016; Thomaz *et al*., 2017; Pio, Carvalho, 2021; Souto-Santos *et al*., 2022).

Species belonging to the *Scleromystax* Günther, 1864 genus (Siluriformes, Callichthyidae, Corydoradinae), popularly known as banded coridoras, are geographically widespread throughout coastal Atlantic Rainforest rivers (Britto, 2003; Britto *et al*., 2016). This monophyletic genus includes six species of small armored-catfish, namely *S. barbatus* (Quoy & Gaimard, 1824), *S. macropterus* (Regan, 1913), *S. prionotos* (Nijssen & Isbrücker, 1980), *S. salmacis* Britto & Reis, 2005, *S. reisi* Britto, Fukakusa & Malabarba, 2016 and *S. virgulatus* (Nijssen & Isbrücker, 1980). The banded coridoras *S. barbatus* exhibits a relatively broad distribution area through coastal rivers basins off southern and southeastern Brazil, from the Paraíba do Sul River basin to the Itapocu River basin (Menezes *et al*., 2007). This species differs from its congeners by the presence of dark brown blotches, often coalesced, extending from almost the entire dorsolateral body plates towards the base of caudal fin, displaying irregular small dark brown blotches distributed over the head (Oyakawa *et al*., 2006). It is mostly found in small groups inhabiting shallow stream portions, feeding on benthic invertebrates while moving and probing the substrate (Aranha *et al*., 1998; Gonçalves, Cestari, 2013). Molecular genetic variations within *S. barbatus* populations in southern Brazil indicate that this species is an interesting model for phylogeographic studies (Tschá *et al*., 2017).

Studies concerning evolutionary and biogeographic history effects on Atlantic Rainforest freshwater fish distributions have revealed that many widely distributed species display high genetic divergences (Torres, Ribeiro, 2009; Thomaz *et al*., 2017; Souza *et al*., 2018), often with low morphological variations between geographically isolated populations, indicating that the ichthyofauna in this region is much more diverse than previously thought. Therefore, an ongoing need to explore additional methodological approaches to address issues such as species identification, speciation, geographic variation, structured genetic variation, and phylogeny, is paramount (Padial *et al*., 2010; Garavello *et al*., 2021; Ibañez *et al*., 2022).

Otoliths, commonly known as earstones, are calcareous structures positioned in the inner ear of teleost fish, in close association with the sensorial macula, whose main functions comprise hearing and gravity perception (Platt, Popper, 1981). Three pairs of otoliths are present, the *sagittae*, *lapillus*, and *asteriscus*, which in many cases, display a species-specific morphology (Campana, 1999; Mendoza, 2006). The saccular otolith, *sagitta*, is largest in most teleosts, and the lagenar otolith, *asteriscus*, is largest in most ostariophysians, while the utricular otolith, *lapillus*, is usually the most conspicuous otolith in Siluriformes (Platt, Popper, 1981; Assis, 2003). Although otolith appearance and shape indicate high levels of morphological differentiation between species (*e.g*., Popper *et al*., 2005; Deng *et al*., 2013; Volpedo, Vaz-dos-Santos, 2015), intraspecific variations may occur according to sex (*e.g*., Bose *et al*., 2017; Maciel *et al*., 2019), growth (*e.g*., Lombarte, Castellón, 1991; Bose *et al*., 2018), geography (*e.g*., Stransky *et al*., 2008; Treinen-Crespo *et al*., 2012) and environmental factors (*e.g*., Lombarte, Lleonart, 1993; Schulz-Mirbach *et al*., 2011).

Otolith morphological, morphometric and shape analyses have increasingly been applied as tools in stock assessments (Paul *et al*., 2013; Hüssy *et al*., 2016; Morawicki *et al*., 2022), ecological studies (Schulz-Mirbach *et al*., 2011; Tuset *et al*., 2016) and species discrimination (Popper *et al*., 2005; Deng *et al*., 2013; Mereles *et al*., 2021), evaluating intra- and inter-specific variability between different populations. Thus, the specific aim of our study was to provide an in-depth appraisal of the value of a *lapillus* otolith shape analysis to examine sexual-based dimorphism and for the identification of divergences among isolated *Scleromystax barbatus* populations from coastal Atlantic Rainforest streams.

MATERIAL AND METHODS

Study area. *Scleromystax barbatus* individuals were sampled in eight clear-water streams encompassing the southern (VR, GR, DM, CU, and PE locations) and southeastern (AR, PM, and AE locations) distribution of the species in the Atlantic Rainforest biome (Fig. 1), between 2019 and 2021, employing electrofishing and seine nets. Voucher specimens were deposited in the fish collection of the Museu de História Natural Capão da Imbuia (MHNCI) (Tab. 1).

Data collection. Total lengths (TL; mm) were determined and otoliths were extracted from each specimen, washed, and stored dry before subsequent analyses. To reduce the effect of ontogenetic otolith changes, analyses were performed only for adult fish ranging from 4.0 to 8.0 cm TL. For the morphological analyses, the left *lapillus* otoliths of 93 individuals of *S. barbatus*, whereby 47 originated from females and 46 from males, were placed with the macular hump facing up (ventral surface), and with the anterior extreme pointing left. Two-dimensional digital images were recorded using a Leica® M205 C stereomicroscope using 16.0x objective coupled with a digital camera MC170 HD (Leica®) (magnification 160x), employing the LAS 4.8.0 software (Leica®) using reflected light with a dark background.

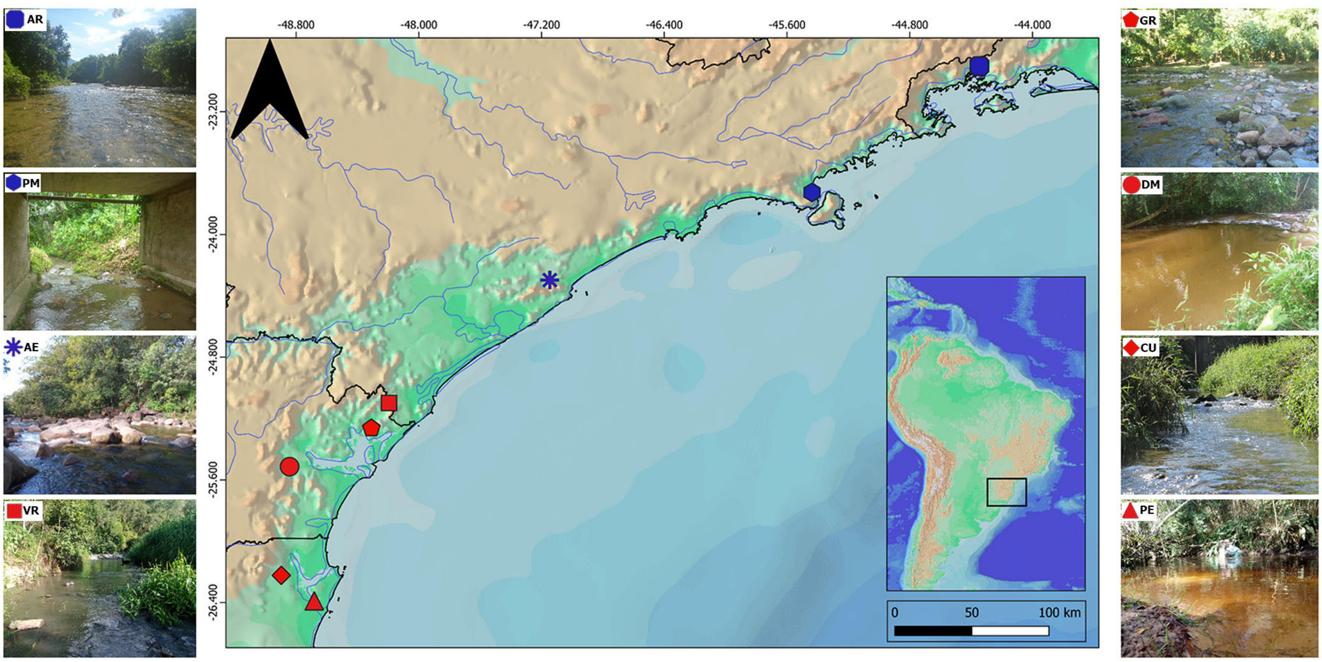

FIGURE 1 | Locations where *Scleromystax barbatus* populations were sampled in clear-water streams of the coastal Atlantic Rainforest biome. Red symbols represent the southern locations (VR, GR, DM, CU, and PE) and blue symbols indicate the southeastern locations (AR, PM, and AE).

TABLE 1 | Locations, number of females and males of *Scleromystax barbatus*, total length (mean ± SD) of individuals and vouchers of samplings conducted in the coastal Atlantic Rainforest biome.

Region	Location	Municipality/State	Stream	Coordinates	Females	Males	TL (mean ± SD)	Voucher
Southeastern (Se)	AR	Angra dos Reis/RJ	Ariró	22°54’07.33”S 44°20’46.56”W	3	9	55.0 ± 0.61	MHNCI 12735
	PM	Caraguatatuba/SP	Pereque-Mirim	23°43’33.51”S 45°26’14.10”W	4	8	50.5 ± 0.40	MHNCI 12827
	AE	Itariri/SP	Aerado	24°17’51.31”S 47°08’47.83”W	6	7	52.2 ± 0.59	MHNCI 12734
Southern (S)	VR	Jacupiranga/SP	Unamed	25°05’57.35”S 48°11’40.67”W	7	5	49.7 ± 0.30	MHNCI 12828
	GR	Guaraqueçaba/PR	Guaraqueçaba	25°15’49.11”S 48°18’35.24”W	4	8	52.0 ± 0.81	MHNCI 12829
	DM	Paranaguá/PR	Do Meio	25°24’11.27”S 48°52’23.54”W	7	3	49.9 ± 0.62	MHNCI 12830
	CU	Joinville/SC	Cubatão Norte	26°13’21.76”S 48°53’49.48”W	8	4	52.8 ± 0.33	MHNCI 12831
	PE	Barra do Sul/SC	Pernambuco	26°23’19.60”S 48°40’55.94”W	8	2	47.4 ± 0.51	MHNCI 12736

Data analysis. The *lapillus* otoliths were described according to Adams (1940) and Assis (2005). Otolith image analyses were performed in the shapeR package (Libungan, Pálsson, 2015) available in R environment (R Development Core Team, 2021). Otolith outlines and shape coefficients were assessed employing normalized elliptic Fourier transform and Wavelet functions. For all analyses, male and females of *S. barbatus* were grouped according to the location of capture: southern (VR, GR, DM, CU, and PE) and southeastern (AR, PM, and AE) distribution in the Atlantic Rainforest biome.

The Elliptic Fourier analysis provides information concerning overall differences in otolith shape, describing and characterizing outlines in a quantifiable manner (Lestrel, 1997). The method fitted a number of harmonic functions to capture crenulations and lobes on the otolith edges (Tracey *et al*., 2006). The Wavelet method is useful for detecting shape differences at specific regions, which may be located at a given angle on the otolith outline (Libungan, Pálsson, 2015). The Wavelet method fits a series of approximating functions within restricted domains to quantify the outline shapes (Graps, 1995), and has been proven an adequate method to detect inter- and intraspecific differences (Tuset *et al*., 2021). Prior to the shape analyses, the outlines of each image were smoothed to remove the high frequency of pixel noise around the otolith contour using the *smoothout* function with 100 iterations.

The Fourier/Wavelet coefficients were standardized by fish length (Libungan, Pálsson, 2015), and the otolith shape of *S. barbatus* was reconstructed using the mean Fourier/Wavelet coefficients and plotted using the ‘plotFourierShape’ and ‘plotWaveletShape’ functions, respectively (Libungan, Pálsson, 2015). Prior to the analysis concerning inter-population differences (males/females and southern/southeastern groups), five Fourier and Wavelet coefficients that did not meet normality (Shapiro-Wilk’s tests) and homoscedasticity (Levene’s tests) assumptions were removed, totaling 40 Fourier and 50 Wavelet coefficients.

The shape variations among the groups were compared by a Canonical Analysis of Principal coordinates (CAP) applied to the Fourier/Wavelet coefficients using the capscale function available in the vegan package (Oksanen *et al*., 2013)The ordination of the population's averages along the first two canonical axes was evaluated graphically with shape descriptors. An ANOVA-like permutation test for the CAP was applied to assess the significance of constraints employing 1000 permutations. A minimum of 0.01% was adopted as the significance level. All statistical analyses were performed in the R environment (R Development Core Team, 2021).

RESULTS

The lapillus otoliths of *S. barbatus* display an elongation in the superior-inferior axis, with uneven wavy bottom edges, while the *gibbus maculae* is large, prominent, occupying much of the surface of the otolith and the sulcus is short, wide and contours the inner *gibbus maculae* region (Fig. 2). The ANOVA-like permutation test for the Fourier/Wavelet coefficients indicated significant differences between otoliths of males of *Scleromystax barbatus* from southern and southeastern areas in the Atlantic Rainforest biome, and also between otoliths of males from southeastern areas and females of southern areas of the Atlantic Rainforest biome (Tab. 2).

The Fourier and Wavelet reconstructions showed differences among the mean otolith outlines between the southern and southeastern areas (Fig. 3). The highest variation was observed for the extremum anterior, the posterior region and medial regions of the *sulcus*. Otoliths from the southeastern areas are more elongated in these portions, mainly in the Wavelet reconstruction. Conversely, high overlaps in the lateral *sulcus* and in the lateral and medial *gibbus maculae* regions were observed.

The CAP assessments indicated that otolith shape was not homogeneous among the southern and southeastern regions, for both coefficients (ANOVA-like, $P < 0.01$). The first two principal coordinates axes explained 94.4% of the total Fourier coefficient variation and 94.3% of the Wavelet coefficient variation (Fig. 4).

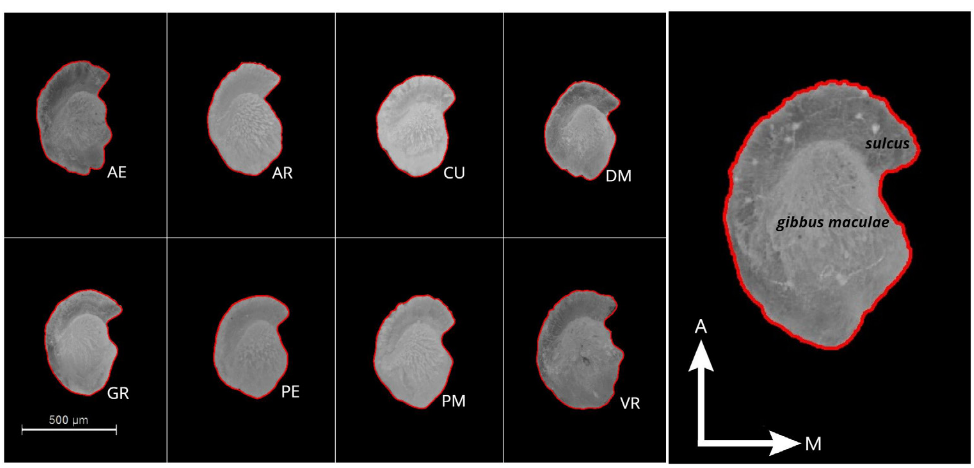

FIGURE 2 | Ventral view of the left *lapillus* otoliths of *Scleromystax barbatus* from streams in the southern (VR, GR, DM, CU, and PE) and southeastern (AR, PM, and AE) regions of the Atlantic Rainforest biome. A (Anterior), M (Medial).

TABLE 2 | Analysis of variance (ANOVA-like) permutation tests for Fourier and Wavelet coefficients of otolith shape among males and females of *Scleromystax barbatus* from southern and southeastern areas in the Atlantic Rainforest biome. Bold numbers denote significant differences (considering F and p-values). DF = Degree of freedom, SS = Sum of squares. SeFe (Females from the southeastern region), SeMa (Males from the southeastern region), SFe (Females from the southern region), SMa (Males from the southern region).

Variables	DF	Fourier			Wavelet		
		SS	F	P	SS	F	P
All groups	3	0.027	1.9393	**0.024**	5.178	1.8634	**0.027**
Southeastern (Se) *vs.* Southern (S)	1	0.008	2.3115	**0.039**	3.908	4.5173	**0.003**
Female (Fe) *vs.* Male (Ma)	1	0.002	0.4975	0.765	0.332	0.3593	0.944
SeFe *vs.* SMa	1	0.002	0.3212	0.902	0.451	0.4431	0.862
SeFe *vs.* SFe	1	0.009	2.2639	0.061	1.890	2.0973	0.059
SeFe *vs.* SMa	1	0.013	2.0781	0.073	2.293	2.0503	0.063
SeMa *vs.* SFe	1	0.011	3.1020	**0.014**	2.281	2.8054	**0.014**
SeMa *vs.* SMa	1	0.015	2.9842	**0.015**	2.954	3.1036	**0.010**
SFe *vs.* SMa	1	0.003	0.7711	0.544	0.492	0.5679	0.780
Residual	89	0.406			82.432		

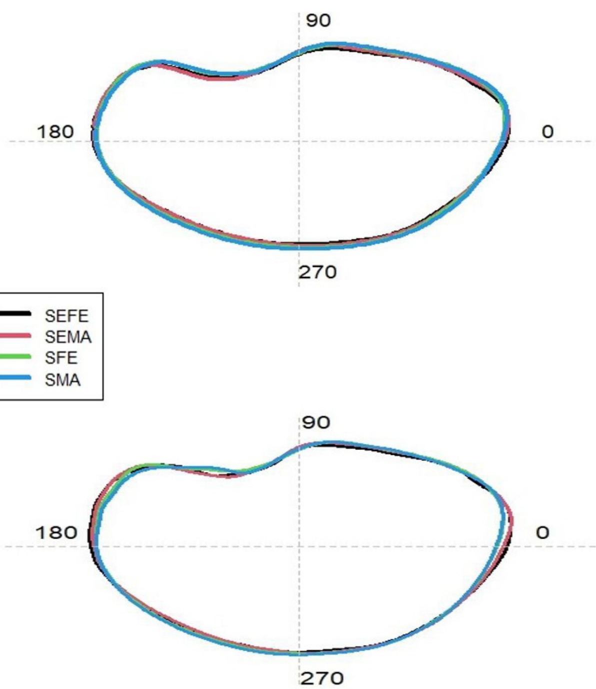

FIGURE 3 | Mean otolith shape based on the Fourier (above) and Wavelet (below) reconstructions for males and females of *Scleromystax barbatus* from southern and southeastern regions in the Atlantic Rainforest biome. Lines represent the groups: SeFe (Females from the southeastern region), SeMa (Males from the southeastern region), SFe (Females from the southern region), SMa (Males from the southeastern region).

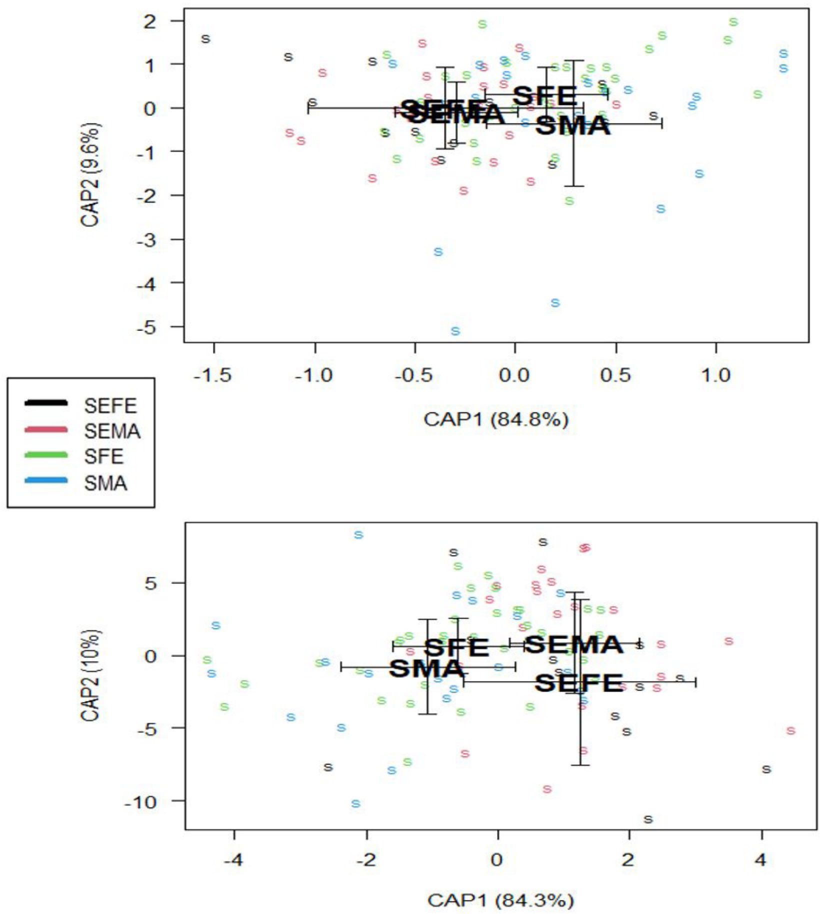

FIGURE 4 | Otolith shapes of males and females of *Scleromystax barbatus* from southern and southeastern regions in the Atlantic Rainforest biome employing a Canonical Analysis of Principal Coordinates (CAP) applied to Fourier (above) and Wavelet (below) coefficients. Bold letters represent the mean canonical coordinates surrounding the standard error for each population. Lines represent the groups: SeFe (Females from the southeastern region), SeMa (Males from the southeastern region), SFe (Females from the southern region), SMa (Males from the southeastern region).

DISCUSSION

The morphologies of *Scleromystax barbatus lapillus* otolith are very characteristic and can be effectively assign to *lapillus* otolith of most Callichthyidae family (Volpedo, Fuchs, 2010). Although it is not possible to establish a morphological pattern for the otoliths of Siluriformes (Cutrim, Batista, 2005; Volpedo, Fuchs, 2010; Sánchez, Martínez, 2017), due to the great diversity of this monophyletic group (Malabarba, Malabarba, 2019) and phenotypic plasticity (Alexandrou *et al*., 2011; Lujan, Armbruster, 2012), the *lapillus* otoliths analyzed in the present study correspond to the non-clupeiform type proposed by Assis (2005). Furthermore, the prominent and large *gibbus maculae* and the elongation noted in its posterior-anterior axis correspond to the ovoid model proposed by Sánchez, Martínez (2017) for the Loricariidae *lapillus*.

Among the three pairs of otoliths, the *lapillus* is the one that presents the most regular edge shape and the most homogeneous structure constitution, indicating that geometric morphometry and shape indices are less efficient, due to the difficulty in finding comparable (homologous) structures (Assis, 2005; Adams *et al*., 2009; Tuset *et al*., 2021). For this reason, the use of contour analyses is more indicated for this type of otolith, as these methodologies are more efficient to capture all small otolith silhouette variations (Mereles *et al*., 2021; Tuset *et al*., 2021). In fact, Fourier and Wavelet analyzes were proven efficient in identifying different *S. barbatus* groups, with Wavelet descriptors proving slightly more sensitive to capture these variations through canonical and ANOVA analyses. Similar results have been reported by other studies for *lapillus* using shape analyses (Pavlov, 2022; Qiao *et al*., 2022; D'Iglio *et al*., 2023).

Reconstruction of otolith shapes employing Fourier and Wavelet analyses indicates that changes in *S. barbatus* otolith shapes occur mainly in the anterior-posterior direction and at the edges of the *sulcus* and *gibbus maculae* regions. The otoliths of males and females of *S. barbatus* from the southeastern areas in the Atlantic Rainforest biome are more elongated in the anterior-posterior direction and present larger *sulcus* and *gibbus maculae* regions, and their borders are more heterogeneous when compared to males and females from the southern areas.

Otolith shapes have been proven an efficient tool to identify marine and estuarine fish populations (Cardinale *et al*., 2004; Boudinar *et al*., 2016; Bose *et al*., 2017; Adelir-Alves *et al*., 2018; Zarei *et al*., 2023), assisting in the management of species used as fishing resources (Avigliano *et al*., 2014; Moreira *et al*., 2019; Kikuchi *et al*., 2021; Neves *et al*., 2021; Ibañez *et al*., 2022; Morawicki *et al*., 2022; Soeth *et al*., 2022; Barnuevo *et al*., 2023; Franco *et al*., 2023), and also used to explain the current distribution of many species, although few studies on neotropical fish employing otolith shape to distinguish populations are available (da Costa *et al*., 2018; Mereles *et al*., 2021). As for marine and estuarine fish, our results indicate that assessments of otolith shape also constitute an interesting tool to differentiate isolated populations of freshwater fish.

Although the factors that determine otolith shapes are not fully understood, as they can be generated and influenced by ontogeny, adaptations, sexual dimorphism, phylogenetic and biogeographical processes (Tuset *et al*., 2016), the combination of genetic and environmental causes may be responsible for the differentiation of the otolith shapes in geographically isolated populations (Cardinale *et al*., 2004; Vignon, Morat, 2010; Berg *et al*., 2018; Santos, Vaz-dos-Santos, 2023). Differences observed in the otolith shapes of *S. barbatus* can therefore be attributed to regional variations in fish life history mediated by differences in environmental factors between the southern and southeastern areas in the Atlantic Rainforest biome, for instance. The combination of these factors may explain otolith shape differences among the investigated *S. barbatus* populations, which despite being restricted to coastal Atlantic Rainforest streams, is widely distributed (Reis, 2003; Oyakawa *et al*., 2006), inhabiting regions presenting different climatic conditions. Our results also indicate that sex may not be the determining factor for differentiation between otoliths of *S. barbatus*, although this species presents marked sexual dimorphism (Oyakawa *et al*., 2006).

The otolith shape analysis applied herein was efficient in geographically discriminating the sampled groups and may contribute to a better understanding of the current *S. barbatus* distribution in the Atlantic Forest. However, additional studies are required to investigate the influence of genetic effects and their environmental interactions to better understand how these factors can affect otolith shape among different *S. barbatus* populations.

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REFERENCES

- **Abilhoa V, Braga RR, Bornatowski H, Vitule JRS.** Fishes of the Atlantic Rain Forest streams: ecological patterns and conservation. In: Grillo O, Venora G, editors. Changing diversity in changing environment. Croatia; 2011. p.259–82.
- **Adams DC, Rohlf FJ, Slice DE.** Geometric morphometrics: Ten years of progress following the 'revolution'. Italian J Zool. 2009; 71(1):5–16. https://doi.org/10.1080/11250000409356545
- **Adams LA.** Some characteristic otoliths of American Ostariophysi. J Morphol. 1940; 66(3):497–527. https://doi.org/10.1002/jmor.1050660307
- **Adelir-Alves J, Daros FALM, Spach HL, Soeth M, Correia AT**. Otoliths as a tool to study reef fish population structure from coastal islands of South Brazil. Mar Biol Res. 2018; 14(9–10):973–88. https://doi.org/10.1080/17451000.2019.1572194
- **Alexandrou MA, Oliveira C, Maillard M, Mcgill RAR, Newton J, Creer S *et al*.** Competition and phylogeny determine community structure in Müllerian co-mimics. Nature. 2011; 469:84–88. https://doi.org/10.1038/nature09660
- **Aranha JMR, Takeuti DF, Yoshimura TM.** Habitat use and food partitioning of the fishes in a coastal stream of Atlantic Forest, Brazil. Rev Biol Trop. 1998; 46(4):951–59. Available from: http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0034-77441998000400008&lng=en&nrm=iso
- **Assis CA.** The lagenar otoliths of teleosts: their morphology and its application in species identification, phylogeny and systematics. J Fish Biol. 2003; 62(6):1268–95. https://doi.org/10.1046/J.1095-8649.2003.00106.X
- **Assis CA.** The utricular otoliths lapilli of teleosts: Their morphology and relevance for species identification and systematics studies. Sci Mar. 2005; 69(2):259–73. https://doi.org/10.3989/scimar.2005.69n2259
- **Avigliano E, Martinez CFR, Volpedo AV.** Combined use of otolith microchemistry and morphometry as indicators of the habitat of the silverside (*Odontesthes bonariensis*) in a freshwater-estuarine environment. Fish Res. 2014; 149:55–60. https://doi.org/10.1016/j.fishres.2013.09.013
- **Barnuevo KDE, Morales CJC, Calizo JKS, Delloro ES, Añasco CP, Babaran RP *et al*.** Distinct stocks of the redtail scad *Decapterus kurroides* Bleeker, 1855 (Perciformes: Carangidae) from the Northern Sulu and Southern Sibuyan Seas, Philippines revealed from otolith morphometry and shape analysis. Fishes. 2023; 8(1):12. https://doi.org/10.3390/fishes8010012
- **Berg F, Almeland OW, Skadal J, Slotte A, Andersson L, Folkvord A.** Genetic factors have a major effect on growth, number of vertebrae and otolith shape in Atlantic herring (*Clupea harengus*). PLoS ONE. 2018; 13(1):1–16. https://doi.org/10.1371/journal.pone.0190995

- **Bose APH, Adragna JB, Balshine S.** Otolith morphology varies between populations, sexes and male alternative reproductive tactics in a vocal toadfish *Porichthys notatus*. J Fish Biol. 2017; 90(1):311–25. https://doi.org/10.1111/jfb.13187
- **Bose APH, McCallum ES, Raymond K, Marentette JR, Balshine S.** Growth and otolith morphology vary with alternative reproductive tactics and contaminant exposure in the round goby *Neogobius melanostomus*. J Fish Biol. 2018; 93(4):674–84. https://doi.org/10.1111/jfb.13756
- **Boudinar AS, Chaoui L, Quignard JP, Aurelle D, Kara MH.** Otolith shape analysis and mitochondrial DNA markers distinguish three sand smelt species in the *Atherina boyeri* species complex in western Mediterranean. Estuar Coast Shelf Sci. 2016; 182:202–10. https://doi.org/10.1016/J.ECSS.2016.09.019
- **Britto MR.** Phylogeny of the subfamily Corydoradinae Hoedeman, 1952 (Siluriformes: Callichthyidae), with a definition of its genera. Proc Acad Nat Sci Phila. 2003; 153(1):119–54. https://doi.org/10.1635/0097-3157(2003)153[0119:POTSCH]2.0.CO;2
- **Britto MR, Fukakusa CK, Malabarba LR.** New species of *Scleromystax* Günther, 1864 (Siluriformes: Callichthyidae) - extending the meridional distribution of genera endemic to the Atlantic Forest. Neotrop Ichthyol. 2016; 14(3):e150158. https://doi.org/10.1590/1982-0224-20150158
- **Campana SE.** Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Prog Ser. 1999; 188:263–97. https://doi.org/10.3354/meps188263
- **Cardinale M, Doering-Arjes P, Kastowsky M, Mosegaard H.** Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. Can J Fish Aquat Sci. 2004; 61(2):158–67. https://doi.org/10.1139/f03-151
- **da Costa RMR, Fabré NN, Amadio SA, Tuset VM.** Plasticity in the shape and growth pattern of asteriscus otolith of black prochilodus *Prochilodus nigricans* (Teleostei: Characiformes: Prochilodontidae) freshwater neotropical migratory fish. Neotrop Ichthyol. 2018; 16(4):e180051. https://doi.org/10.1590/1982-0224-20180051
- **Cutrim L, Batista VS.** Determinação de idade e crescimento do mapará (*Hypophthalmus marginatus*) na Amazônia Central. Acta Amaz. 2005; 35:85–92. https://doi.org/10.1590/S0044-59672005000100013
- **D'Iglio C, Famulari S, Albano M, Carnevale A, Di Fresco D, Costanzo M *et al*.** Intraspecific variability of the saccular and utricular otoliths of the hatchetfish *Argyropelecus hemigymnus* (Cocco, 1829) from the Strait of Messina (Central Mediterranean Sea). PLoS ONE. 2023; 18(2):e0281621. https://doi.org/10.1371/journal.pone.0281621
- **Deng X, Wagner H-J, Popper AN.** Interspecific variations of inner ear structure in the deep-sea fish family Melamphaidae. Anat Rec. 2013; 296(7):1064–82. https://doi.org/10.1002/ar.22703
- **Franco TP, Vilasboa A, Araújo FG, Gama JM, Correia AT.** Identifying whitemouth croaker (*Micropogonias furnieri*) populations along the Rio de Janeiro Coast, Brazil, through microsatellite and otolith analyses. Biology. 2023; 12(3):360. https://doi.org/10.3390/biology12030360
- **Garavello JC, Ramirez JL, Oliveira AK, Britski HA, Birindelli JLO, Galetti Jr. PM.** Integrative taxonomy reveals a new species of Neotropical headstanding fish in genus *Schizodon* (Characiformes: Anostomidae). Neotrop Ichthyol. 2021; 19(4):e210016. https://doi.org/10.1590/1982-0224-2021-0016
- **Gonçalves C, Cestari C.** Uso de riacho costeiro da mata atlântica pelo limpa-fundo *Scleromystax barbatus* (Quoy & Gaimard, 1824). Neotrop Biol Conserv. 2013; 8(3):115–20. https://doi.org/10.4013/nbc.2013.83.01

- **Graps A.** An introduction to wavelets. IEEE Computational Science and Engineering. 1995; 2(2):50–61. https://doi.org/10.1109/99.388960
- **Hüssy K, Mosegaard H, Albertsen CM, Nielsen EE, Hemmer-Hansen J, Eero M.** Evaluation of otolith shape as a tool for stock discrimination in marine fishes using Baltic Sea cod as a case study. Fish Res. 2016; 174:210–18. https://doi.org/10.1016/J.FISHRES.2015.10.010
- **Ibañez A, Rangely J, Ávila-Herrera L, Silva VEL, Pacheco-Almanzar E, Neves JMM *et al*.** Unraveling the *Mugil curema* complex of American coasts integrating genetic variations and otolith shapes. Estuar Coast Shelf Sci. 2022; 273:e107914. https://doi.org/10.1016/j.ecss.2022.107914
- **Kikuchi E, García S, Costa PAS, Cardoso LG, Haimovici M.** Discrimination of red porgy *Pagrus pagrus* (Sparidae) potential stocks in the south-western Atlantic by otolith shape analysis. J Fish Biol. 2021; 98(2):548–56. https://doi.org/10.1111/jfb.14598
- **Lestrel PE.** Fourier descriptors and their applications in biology. Cambridge University Press; 1997.
- **Libungan LA, Pálsson S.** ShapeR: An R package to study otolith shape variation among fish populations. PLoS ONE. 2015; 10(3):1–12. https://doi.org/10.1371/journal.pone.0121102
- **Lima SMQ, Vasconcellos A V, Berbel-Filho WM, Lazoski C, Russo CAM, Sazima I *et al*.** Effects of Pleistocene climatic and geomorphological changes on the population structure of the restricted-range catfish *Trichogenes longipinnis* (Siluriformes: Trichomycteridae). Syst Biodivers. 2016; 14(2):155–70. https://doi.org/10.1080/14772000.2015.1104398
- **Lombarte A, Castellón A.** Interspecific and intraspecific otolith variability in the genus *Merluccius* as determined by image analysis. Can J Zool. 1991; 69(9):2442–49. https://doi.org/10.1139/z91-343
- **Lombarte A, Lleonart J.** Otolith size changes related with body growth, habitat depth and temperature. Environ Biol Fishes. 1993; 37(3):297–306. https://doi.org/10.1007/BF00004637
- **Lujan NK, Armbruster JW.** Morphological and functional diversity of the mandible in suckermouth armored catfishes (Siluriformes: Loricariidae). J Morphol. 2012; 273(1):24–39. https://doi.org/10.1002/jmor.11003
- **Maciel TR, Vaz-dos-Santos AM, Barradas JRS, Vianna M.** Sexual dimorphism in the catfish *Genidens genidens* (Siluriformes: Ariidae) based on otolith morphometry and relative growth. Neotrop Ichthyol. 2019; 17(1):e180101. https://doi.org/10.1590/1982-0224-20180101
- **Malabarba LR, Malabarba MC.** Phylogeny and classification of neotropical fish. In: Baldisserotto B, Urbinati EC, Cyrino JEP, editors. Elsevier Inc.; 2019. p.1–19. https://doi.org/10.1016/B978-0-12-815872-2.00001-4
- **Mendoza RPR.** Otoliths and their applications in fishery science. Ribar Croat J Fish. 2006; 64(3):89–102. https://hrcak.srce.hr/8984
- **Menezes NA, Weitzman SH, Oyakawa OT, Lima FCT, Correa e Castro RM, Weitzman MJ.** Peixes de água doce da Mata Atlântica: lista preliminar das espécies e comentários sobre conservação de peixes de água doce neotropicais. São Paulo: Museu de Zoologia da Universidade de São Paulo; 2007.
- **Mereles MA, Sousa RGC, Barroco LSA, Campos CP, Pouilly M, Freitas CEC.** Discrimination of species and populations of the genus *Cichla* (Cichliformes: Cichlidae) in rivers of the Amazon basin using otolithic morphometry. Neotrop Ichthyol. 2021; 19(4):e200149. https://doi.org/10.1590/1982-0224-2020-0149
- **Morawicki S, Solimano PJ, Volpedo AV.** Unravelling stock spatial structure of silverside *Odontesthes argentinensis* (Valenciennes, 1835) from the North Argentinian Coast by otoliths shape analysis. Fishes. 2022; 7(4):e155. https://doi.org/10.3390/fishes7040155
- **Moreira C, Froufe E, Vaz-Pires P, Correia AT.** Otolith shape analysis as a tool to infer the population structure of the blue jack mackerel, *Trachurus picturatus*, in the NE Atlantic. Fish Res. 2019; 209:40–48. https://doi.org/10.1016/j.fishres.2018.09.010

- **Myers N, Mittermeler RA, Mittermeler CG, Fonseca GAB, Kent J.** Biodiversity hotspots for conservation priorities. Nature. 2000; 403(6772):853–58. https://doi.org/10.1038/35002501
- **Neves J, Silva AA, Moreno A, Veríssimo A, Santos AM, Garrido S.** Population structure of the European sardine *Sardina pilchardus* from Atlantic and Mediterranean waters based on otolith shape analysis. Fish Res. 2021; 243:106050. https://doi.org/10.1016/j.fishres.2021.106050
- **Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB *et al*.** Package 'vegan.' Community Ecology Package, Version. 2013; 2(9):1–295.
- **Oyakawa OT, Akama A, Mautari KC, Nolasco JC.** Peixes de riachos da Mata Atlântica: nas unidades de conservação do Vale do Rio Ribeira de Iguape no Estado de São Paulo. São Paulo: Neotropica; 2006.
- **Padial JM, Miralles A, De la Riva I, Vences M.** The integrative future of taxonomy. Front Zool. 2010; 7(16). https://doi.org/10.1186/1742-9994-7-16
- **Paul K, Oeberst R, Hammer C.** Evaluation of otolith shape analysis as a tool for discriminating adults of Baltic cod stocks. J Appl Ichthyol. 2013; 29(4):743–50. https://doi.org/10.1111/jai.12145
- **Pavlov DA.** Otolith morphology in gibel carp *Carassius gibelio* and crucian carp *C. carassius* (Cyprinidae). J Ichthyol. 2022; 62(6):1067–80. https://doi.org/10.1134/S0032945222060200
- **Pio NL, Carvalho TP.** Evidence on the paleodrainage connectivity during Pleistocene: Phylogeography of a hypoptopomatine endemic to southeastern Brazilian coastal drainages. Neotrop Ichthyol. 2021; 19(2):e200128. https://doi.org/10.1590/1982-0224-2020-0128
- **Platt C, Popper AN.** Fine structure and function of the ear. In: Tavolga WN, Popper AN, Fay RR, editors. Hearing and sound communication in fishes. New York: Springer; 1981. p.3–38. https://doi.org/10.1007/978-1-4615-7186-5_1
- **Popper AN, Ramcharitar J, Campana SE.** Why otoliths? Insights from inner ear physiology and fisheries biology. Mar Freshw Res. 2005; 56(5):497–504. https://doi.org/10.1071/MF04267
- **Qiao J, Zhu R, Chen K, Zhang D, Yan Y, He D.** Comparative otolith morphology of two morphs of *Schizopygopsis thermalis* Herzenstein, 1891 (Pisces, Cyprinidae) in a headwater lake on the Qinghai-Tibet Plateau. Fishes. 2022; 7(3):9. https://doi.org/10.3390/fishes7030099
- **R Development Core Team.** R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2021. Available from: https://www.r-project.org/
- **Reis RE.** Check list of the freshwater fishes of South and Central America. Porto Alegre: Edipucrs; 2003.
- **Ribeiro AC.** Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil: An example of faunal evolution associated with a divergent continental margin. Neotrop Ichthyol. 2006; 4(2):225–46. https://doi.org/10.1590/s1679-62252006000200009
- **Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM.** The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biol Conserv. 2009; 142(6):1141–53. https://doi.org/10.1016/j.biocon.2009.02.021
- **Roxo FF, Albert JS, Silva GSC, Zawadzki CH, Foresti F, Oliveira C.** Molecular phylogeny and biogeographic history of the armored Neotropical catfish subfamilies Hypoptopomatinae, Neoplecostominae and Otothyrinae (Siluriformes: Loricariidae). PLoS ONE. 2014; 9(8):e105564. https://doi.org/10.1371/journal.pone.0105564
- **Sánchez RO, Martínez VH.** Morphological variations of the three otoliths of some species of the family Loricariidae (Ostariophysi: Siluriformes). Neotrop Ichthyol. 2017; 15(1):e160058. https://doi.org/10.1590/1982-0224-20160058

- **Santos L, Vaz-dos-Santos AM.** Insights of otoliths morphology to reveal patterns of teleostean fishes in the Southern Atlantic. Fishes. 2023; 8(1). https://doi.org/10.3390/fishes8010021
- **Schulz-Mirbach T, Heß M, Plath M.** Inner ear morphology in the Atlantic molly *Poecilia mexicana* - first detailed microanatomical study of the inner ear of a cyprinodontiform species. PLoS ONE. 2011; 6(11):e27734. https://doi.org/10.1371/journal.pone.0027734
- **Soeth M, Daros FA, Correia AT, Fabré NN, Medeiros R, Feitosa CV *et al.*** Otolith phenotypic variation as an indicator of stock structure of *Scomberomorus brasiliensis* from the southwestern Atlantic Ocean. Fish Res. 2022; 252:106357. https://doi.org/10.1016/J.FISHRES.2022.106357
- **Souto-Santos ICA, Jennings WB, Buckup PA.** Testing palaeodrainage hypotheses in south-eastern Brazil: phylogeography of the sinistral livebearer fish of the genus *Phalloceros* (Cyprinodontiformes: Poeciliidae). Zool J Linn Soc. 2022; 197(2):514–31. https://doi.org/10.1093/zoolinnean/zlac030
- **Souza CS, Costa-Silva GJ, Roxo FF, Foresti F, Oliveira C**. Genetic and morphological analyses demonstrate that *Schizolecis guntheri* (Siluriformes: Loricariidae) is likely to be a species complex. Front Genet. 2018; 9:69. https://doi.org/10.3389/fgene.2018.00069
- **Stransky C, Baumann H, Fevolden S-E, Harbitz A, Høie H, Nedreaas KH *et al*.** Separation of Norwegian coastal cod and Northeast Arctic cod by outer otolith shape analysis. Fish Res. 2008; 90(1–3):26–35. https://doi.org/10.1016/j.fishres.2007.09.009
- **Thomaz AT, Knowles LL.** Flowing into the unknown: inferred paleodrainages for studying the ichthyofauna of Brazilian coastal rivers. Neotrop Ichthyol. 2018; 16(3):e180019. https://doi.org/10.1590/1982-0224-20180019
- **Thomaz AT, Malabarba LR, Bonatto SL, Knowles LL.** Testing the effect of palaeodrainages *versus* habitat stability on genetic divergence in riverine systems: Study of a Neotropical fish of the Brazilian coastal Atlantic Forest. J Biogeogr. 2015; 42(12):2389–401. https://doi.org/10.1111/jbi.12597
- **Thomaz AT, Malabarba LR, Knowles LL.** Genomic signatures of paleodrainages in a freshwater fish along the southeastern coast of Brazil: genetic structure reflects past riverine properties. Heredity. 2017; 119(4):287–94. https://doi.org/10.1038/hdy.2017.46
- **Torres RA, Ribeiro J.** The remarkable species complex *Mimagoniates microlepis* (Characiformes: Glandulocaudinae) from the Southern Atlantic Rain Forest (Brazil) as revealed by molecular systematic and population genetic analyses. Hydrobiologia. 2009; 617(1):157–70. https://doi.org/10.1007/s10750-008-9543-5
- **Tracey SR, Lyle JM, Duhamel G.** Application of elliptical Fourier analysis of otolith form as a tool for stock identification. Fish Res. 2006; 77(2):138–47. https://doi.org/10.1016/J.FISHRES.2005.10.013
- **Treinen-Crespo C, Villegas-Hernández H, Guillén-Hernández S, Ruiz-Zárate MÁ, González-Salas C.** Otolith shape analysis as a tool for population discrimination of the white grunt (*Haemulon plumieri*) stock in the northern coast of the Yucatán Peninsula, Mexico. REVMAR. 2012; 4:157–68.
- **Tschá MK, Bachmann L, Abilhoa V, Boeger WA.** Past connection and isolation of catchments: The sea-level changes affect the distribution and genetic variability of coastal freshwater fishes. Estuar Coast Shelf Sci. 2017; 190:31–39. https://doi.org/10.1016/j.ecss.2017.02.030
- **Tuset VM, Otero-Ferrer JL, Gómez-Zurita J, Venerus LA, Stransky C, Imondi R *et al*.** Otolith shape lends support to the sensory drive hypothesis in rockfishes. J Evol Biol. 2016; 29(10):2083–97. https://doi.org/10.1111/JEB.12932

- **Tuset VM, Otero-Ferrer JL, Siliprandi C, Manjabacas A, Marti-Puig P, Lombarte A.** Paradox of otolith shape indices: routine but overestimated use. Can J Fish Aquat Sci. 2021; 78(6):681–92. https://doi.org/10.1139/cjfas-2020-0369
- **Vignon M, Morat F.** Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. Mar Ecol Prog Ser. 2010; 411:231–41. https://doi.org/10.3354/MEPS08651
- **Volpedo AV, Fuchs DV.** Ecomorphological patterns of the *lapilli* of Paranoplatense Siluriforms (South America). Fish Res. 2010; 102(1–2):160–65. https://doi.org/10.1016/j.fishres.2009.11.007
- **Volpedo AV, Vaz-dos-Santos AM.** Métodos de estudios con otolitos: principios y aplicaciones. Buenos Aires: CAFP-BA-PIESCI; 2015.
- **Zarei F, Esmaeili HR, Sadeghi R, Reichenbacher B, Schliewen UK, Abbasi K *et al*.** Phylogeography and population structure of *Ponticola gorlap* (Teleostei: Gobiidae) in an evolutionary distinctive and ecologically threatened Caspian Sea sub-basin. Aquat Sci. 2023; 85(15):1–13. https://doi.org/10.1007/s00027-022-00913-z

AUTHORS' CONTRIBUTION

Roger Henrique Dalcin: Conceptualization, Formal analysis, Methodology, Writing-original draft.

Vinícius Abilhoa: Conceptualization, Formal analysis, Methodology, Supervision, Writing-review and editing.

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COMPETING INTERESTS

The author declares no competing interests.

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