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Different *sagitta* otolith morphotypes for the whitemouth croaker *Micropogonias furnieri* in the Southwestern Atlantic coast

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ABSTRACT

Otoliths are part of the auditory system of teleostean fishes, and are species-specific structures that may have intraspecific variation depending on the environmental conditions at a regional scale. The main aim of this study was to assess changes in otolith shape of *Micropogonias furnieri* along its geographical distribution range between the tropical and warm temperate coastal of Southwestern Atlantic. Ten otoliths from each of six areas along the South American coast were examined:1) Rio de Janeiro State (23°S); 2) São Paulo State (24°S); 3) Santa Catarina State (27°S), 4) Rio Grande do Sul State (32°S); 5) Uruguayan coast (35°S); 6) Northern coast of Argentine (39°S). The *sagitta* otolith contour and shape were characterized using the Elliptical Fourier Analysis (EFA) and the morphometric measurements of both otoliths and respective *sulcus acusticus* were performed using the software ImageJ. Variations of otolith shape were assessed by five explanatory variables (area, perimeter, width, circularity and the maximum Feret diameter) and a Principal Components Analysis (PCA) was applied to Elliptical Fourier Descriptors. We found significant differences in area, perimeter and circularity of otoliths. Clustering the range of the 30 first harmonics using Ward's hierarchical algorithm yielded three otolith morphotypes. Plots of the two first Principal Component (PCs) axes for the 60 examined otoliths did not discriminate the population distribution along the six areas. The three different otolith morphotypes do not seem to be subjected to a clinal variation for this transition area in the Southwestern Atlantic, and suggest an overlap of individuals of different stocks/populations mixing along their geographical distribution range favored by their eurythermic and euryhaline characteristics.

1. Introduction

Otoliths are paired calcified structures (earstones) used for balance and/or hearing in all teleost fishes (Popper and Coombs, 1982; Ramcharitar et al., 2006; Schulz-Mirbach et al., 2014). These structures have been employed to indicate fish age in several studies (Campana, 2005; Cavole and Haimovici, 2015; Morales-Nin, 2000). Numerous studies have demonstrated that otolith morphology is also related to swimming (Volpedo and Echeverría, 2003; Volpedo et al., 2008), feeding (Lombarte et al., 2010), spatial distribution (Gauldie and Crampton, 2002; Lombarte and Cruz, 2007; Sadighzadeh et al., 2014) and acoustic communication (Cruz and Lombarte, 2004; Popper and Lu, 2000). More recently, otolith shape has been used to describe fish species (Aguirre and Lombarte, 1999; Reichenbacher et al., 2009; Zhuang et al., 2014) and habitat use (Avigliano et al., 2014; Galley et al., 2006).

Besides these applications, analysis of growth from otolith measurement have been used for stock differentiation since shape can shift at a spatial scale (Avigliano et al., 2015; Begg and Brown, 2000; Benzinou et al., 2013; Campana and Casselman, 1993; Cañás et al., 2012; Duarte-Neto et al., 2008; Ferguson et al., 2011). Otoliths grow throughout the life of fish and are metabolically inert (Campana and Neilson, 1985). Their shape is complex, being able to present clinal variation relative to the distance and geographic location (Worthmann, 1979). Moreover, feeding activity, temperature and photoperiod are other sources of influence to provide stimuli to the macula, the sensory epithelium of the fish inner ear (Lecomte-Finiger, 1999) that control otolith growth and shape.

Morphometric analyses of otoliths that include the description of shape and contour yield morphometric indices that can be statistically

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compared (Lestrel, 1997; Tuset et al., 2013). The outline of an otolith is a closed curve, which is the result of a combination of sine and cosine functions (Doering and Ludwig, 1990). Rectangularity and circularity are among the most common morphometric indices used to compare otoliths (Avigliano et al., 2015; Cañás et al., 2012; Longmore et al., 2010; Tuset et al., 2013). Otolith morphology is influenced by both biotic and abiotic factors (Capoccioni et al., 2011) besides the genetic information and ontogenetic stage of fish (Campana, 2004, 2005; Lombarte et al., 2003). Otolith morphology and morphometry have also been used to describe the diversity of fish communities (Tuset et al., 2016) and to discriminate populations among diverse geographic areas, such as the cases for the haddock (Begg and Brown, 2000) from the Georges Bank, cod (Galley et al., 2006; Petursdottir et al., 2006; Stransky et al., 2008) along the spawning areas in Northeast Atlantic and Mediterranean, anglerfish (Cañás et al., 2012) from the Northeast Atlantic, striped red mullet (Benzinou et al., 2013) from the Northeast European Seas, swordfish (Mahé et al., 2016) from the Indian Ocean; blue whiting (Keating et al., 2014) from the Northeast Atlantic, fork-beard (Vieira et al., 2014) from the Northeast Atlantic and Croak River (Avigliano et al., 2015) from the Paraná and Paraguay rivers in South America. As the *lapillus* and the *asteriscii*, the otoliths *sagitta* are species-specific (Sadighzadeh et al., 2012) and are the largest otoliths in most teleosts, being therefore the most studied (Begg et al., 2005).

The development of digital techniques has offered new possibilities for the investigation based on image handling and analysis of otoliths (e.g., Gauldie and Crampton, 2002; Parisi-Baradad et al., 2005; Stransky et al., 2008). Furthermore, morphometric analyses allow to explore more complex details of otolith shape (Ponton, 2006; Ramirez-Perez et al., 2010; Vergara-Solana et al., 2013), such as the wavelet functions (Parisi-Baradad et al., 2005; Sadighzadeh et al., 2014) that supply information on the exact point in the contour where shape difference can occur. The Eliptic Fourier Analysis (EFA) has been commonly used in shape studies. According to Kuhl and Giardina (1982), this procedure decomposes the object contour through each X and Y coordinates, as a parametric function of the distance in the cumulative chord (t) along the contour. Fourier analysis, given enough terms, capture all the information required to describe shape completely (Bird et al., 1986). Fourier series are mathematically defined as a series of sinusoids and can be used to objectively and rapidly describe the outline of a shape. Each Fourier descriptor is characterized by an amplitude and a phase angle (Castonguay et al., 1991).

In Sciaenidae, the morphometry of otolith *sulcus acusticus* is unique and consistently differs among genera and species (Corrêa and Vianna, 1993). The *sulcus acusticus* shape usually expresses the physiological adaptation of the otolith and the specialization in the auditory capacity of the fish (Lombarte, 1992; Popper et al., 2005). Therefore, variability in the *sulcus acusticus* shape is a morpho-functional characteristic (Platt and Popper, 1981). The whitemouth croaker *Micropogonias furnieri* (Desmarest, 1823) is a very abundant species of the Sciaenidae family widely distributed in the Southwestern Atlantic coast. There is no scientific consensus on the number of stocks/populations along the M. *furnieri* distribution range in the Southwestern Atlantic coast. For example, Vazzoler (1971) suggested that two *M. furnieri* populations occur along the Brazilian coast, one (Population I) in the Southeast coast between Cape Frio (23 °C) and Cape Santa Marta (29°S), and another (population II) between Cape Santa Marta and the extreme southern Brazilian coast (33°S). Such two populations were determined based on meristic and morphometric studies and biological endpoints (Vazzoler, 1971) and also on the electrophoretic patterns of crystalline proteins (Phan and Vazzoler, 1976; Vazzoler et al., 1985; Vazzoler and Phan, 1989). Detecting population discontinuity of *M. furnieri* is a hard task, because of its wide geographical distribution range and the absence of biogeographic barriers along the coast. Isaac (1988) confirmed the existence of these two populations and added other two populations, one (Population III) off the La Plata River estuary (36° S; Figueroa and Astarloa, 1991, in Isaac, 1988) and the other (Population IV) off the

Bahia Blanca (39°S; Cotrina, 1986). Recently, Vasconcellos et al. (2015) used molecular markers to identify three different *M. furnieri* stocks: one off the coast of Pará State (0°), a second between Rio de Janeiro (23°S) and Santa Catarina coast (29°S), and a third between Torres (30°S) and Chui (34°S) in the coast of Rio Grande do Sul.

Although different *M. furnieri* stocks along the range of geographical distribution are not well known, there is compelling information from a population discontinuity. In this study, we analyzed *M. furnieri sagitta* otoliths from several areas along the Southwestern Atlantic coast, from Rio de Janeiro State (23°S) to the northern coast of Argentina (39°S). Our goal was to describe otolith morphology and to detect eventual differences in otolith shape in this transitional area between the tropical and warm-tropical waters. Specifically, we aimed to: (1) develop a morphometric method for combined analysis of otolith outline and *sulcus acusticus* features to detect morphotypes; (2) evaluate the spatial distribution of otolith morphotypes along the studied area to detect eventual clinal variation; and (3) analyze whether there is correspondence between otolith shape and fish body size. We expect that the intraspecific variability in otolith shape within each area is not significant in relation to between-areas variability since previous studies discriminated different populations in the area and because the wide range of geographic distribution encompasses tropical, transitional and warm temperate waters.

2. Materials and methods

2.1. Study area

The study area cover an heterogeneous region along the Southwestern Atlantic coast, from the limit of tropical region at the coast of Rio de Janeiro State (latitude 23°S) to the northern Argentinean coast (39°S), This area encompasses a transition zone from the tropical to temperate region, between Rio de Janeiro and the Cape Santa Marta Cape (29°S), also known as South American Bight, and the warm temperate region, including the Rio Grande do Sul State coast (33°S, the Uruguayan coast (35°S) and the northern Argentine coast (39°S) (Fig. 1). The region is influenced by the Brazil (warm) and Falklands (cold) currents (BC and FC, respectively), and by the South Atlantic Subtropical Convergence Zone (SASCZ). The BC (tropical and subtropical waters) is a narrow, well defined and flows southward along the continental margin (Silveira et al., 2000). Temperatures are higher than 20 °C and salinity higher than 36 in a depth between surface and 100 m between latitude 13° S to 25°S (Campos et al., 2000). The Falklands Current, on the other hand, is formed by sub-Antarctic subsurface waters of South Atlantic, with temperature of approximately 6 °C, low salinity (33.5–34.5) and moves from the South of Argentinean coast toward the north. The South Atlantic Subtropical Convergence Zone (SASCZ) is formed by the convergence of BC and FC, has temperatures ranging from 6 °C to 20 °C and salinities from 34.6 to 36 (Silveira et al., 2000) between the Argentinean and the southern Brazilian Coasts, sometimes reaching up to Cape São Tome (22°S) (Campos et al., 2000).

Otoliths from fish collected in six areas along the Southwestern Atlantic coast (Fig. 1) were examined: 1) Rio de Janeiro State (23°S); 2) São Paulo State (24°S); 3) Santa Catarina State (27°S), 4) Rio Grande do Sul State (32°S); 5) Uruguayan coast (35°S); 6) Northern coast of Argentine (39°S).

2.2. Data collection and analysis

The fishes were collected from the artisanal fishery landings from each one of the six areas (Fig. 1). Specimens from Uruguay and Argentina were provided by local contributors to cover a wider range of the species distribution, which limited our sampling size. We choose 10 individuals of each area ranging between 46 cm and 59 cm Total Length (all adults) and that are likely to have similar age, thus avoiding effects
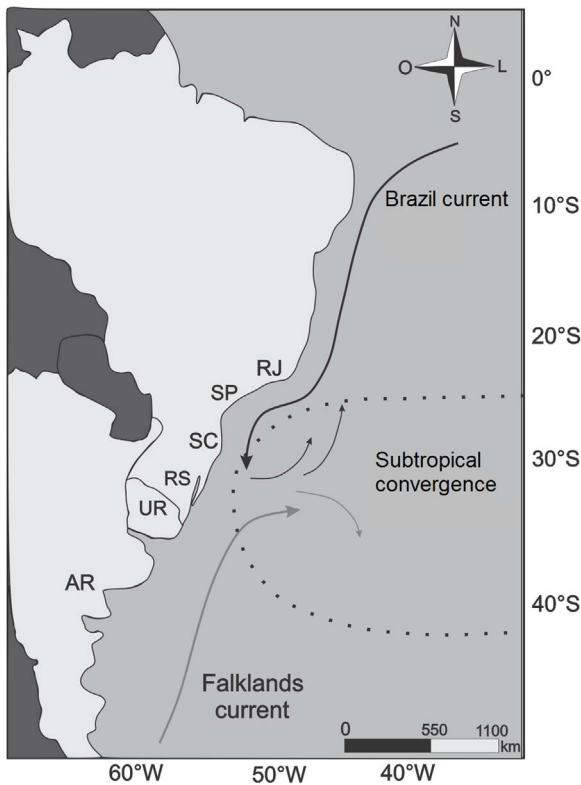
Fig. 1. Study area in Southwester Atlantic coast, with indication of the six coastal areas where the fish samples were obtained: RJ, Rio de Janeiro; SP, São Paulo; SC, Santa Catarina, RS, Rio Grande do Sul; UR, Uruguay; AR, Argentine.

Fig. 3. Shape average and range (means ± 2 standard deviation- SD) for the first six principal components.

of eventual ontogenetic changes in otolith shape. The studied area covers the whole distribution range where the species has commercial importance.

The *sagitta* otoliths were extracted through the opening of the otic capsule in the ventral part of the neurocranium, washed and stored dry. The otoliths were described morphologically according to the terminology proposed by Tuset et al. (2003). We used the Levene test for homogeneity of variance and the Shapiro-Wilks W test for normality, and since the null hypothesis was not rejected, parametric statistical tests were applied in the analyses. A t-Student test was used to compare otolith size between the right and left side. As no significant difference

was found, we chose the right otolith for all analyses.

The photographs were taken with the otoliths oriented with the *sulcus* up. A Sony-Optical Steady Shot model DSC-W570 digital camera was used with a 4 × zoom in macrophotography mode aiming to catch details of the image structure. The photos were converted to Bitmap files and implemented in SHAPE v. 1.3 software (Iwata and Ukai, 2002). This software was used to perform the Elliptic Fourier Analysis (EFA) and to produce the principal components (PC) axes and the Fourier series, named harmonics (Ha), and the numeric shape descriptors.

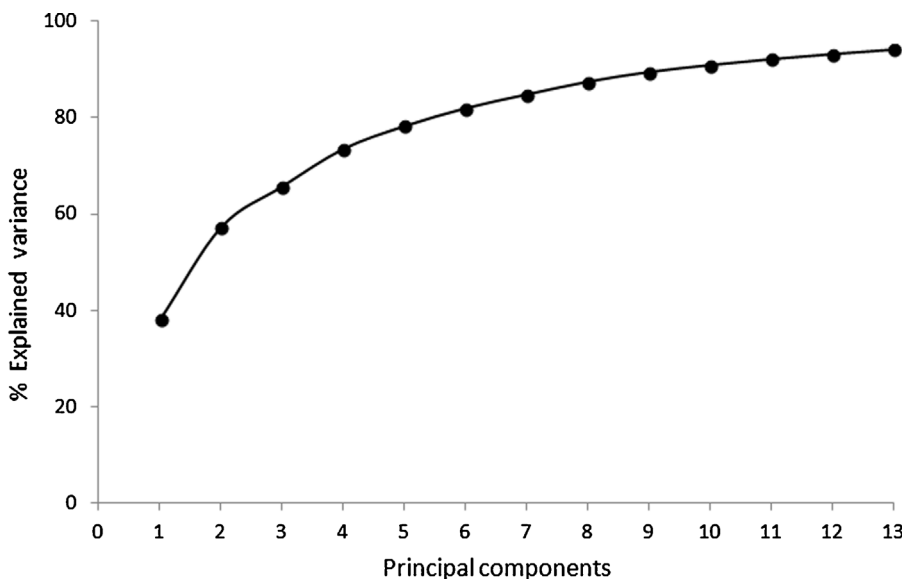Otolith shape was characterized through perimeter (PE), area (AR),

Fig. 2. Graphic representation of the data percent of explanation by the 13 eigenvalues from Principal Component Analysis. Percent cumulative of explanation of data variation indicated for the first six axes shown.
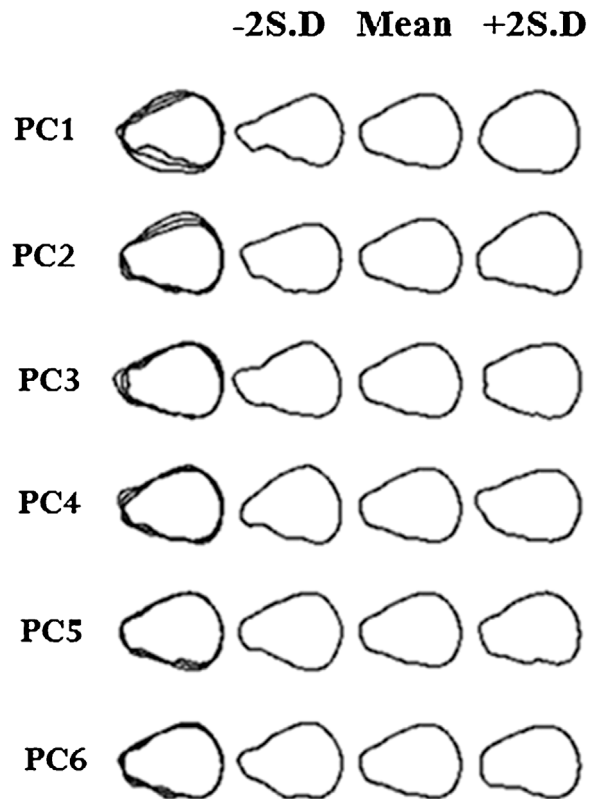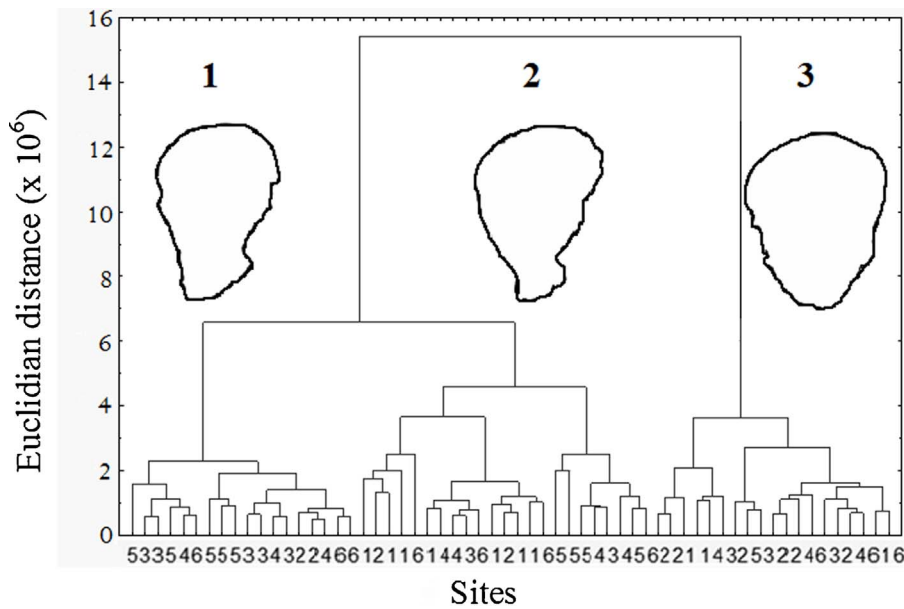
Fig. 4. Dendrogram from cluster analysis on the Euclidian distance of the ranges of the 30 first harmonics.
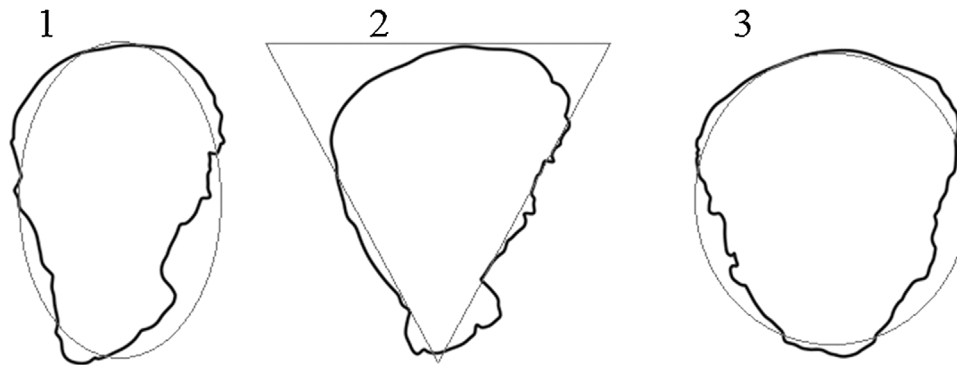

Fig. 5. Outline of the three *sagitta* otoliths morphotypes of *Micropogonias furnieri* in the Southeastern Atlantic coast.

width (WD), circularity (CI) and Feret diameter (FE), which were measured using JPEG images and analyzed in the software ImageJ v. 1.47. We used MANOVA to compare multiple variables among the six areas. Each variable was also compared among the areas using One-Way Analysis of Variance ANOVA.

The effect of fish size on the morphometric measurements, except the Fourier Elliptic Descriptors, was determined by an Analysis of Covariance ANCOVA using the software STATISTICA 7. The variables that had significant correlation with the fish size were corrected using the angular coefficient (*b*) between the groups.

Multivariate techniques were used to determine the otolith shape patterns from a data matrix of the Fourier components. To test for morphotype groups along the sampling area, a cluster analysis using Euclidian distances was employed. Then, a principal component analysis (PCA) was used on the first 30 harmonics of the Fourier series to reduce the data dimensionality. The principal component scores that accounted for the maximum data variability were determined. Morphometric variables were compared among the six areas with the Multivariate Analysis of Variance MANOVA. PCA and cluster analyses were performed using the software STATISTICA 7.

Four measurements (perimeter, area, height and width) were also taken from both the *ostium* and the tail of the *sulcus acusticus* to assess potential differences in otoliths among the sampling areas. Each of these measurements was regressed against fish total length to investigate the effect of fish size on these measurements. An ANCOVA was used to assess eventual differences in the regression parameters (y-

intersept and *b*-slope) between the fish size and the four *ostium* measurements for each area.

3. Results

3.1. Contour analyses

The Elliptic Fourier Analysis yielded 13 significant principal components. Only the first two axes explained 57.30% of the total data variance, whereas the first six axes accounted for 81.93%. The remaining axes explained an irrelevant amount of data variation (Fig. 2).

The mean variation of the shape explained by the principal components depicts differences in the anterior and posterior regions of the otoliths, suggesting the possibility of defining three morphotypes from wider and rounder to narrower and triangular otoliths (Fig. 3).

Principal component analyses yielded a wide individual shape variation. However, three morphotypes were identified by the cluster analysis on the range of the 30 first harmonics (Fig. 4).

These three morphotypes (Fig. 5) were not associated to the studied areas, since different morphotypes occurred irrespective of the area. Morphotype I has a close to elliptical shape with a rounded anterior region, a tapering posterior region and a small indenting in the posterior dorsal margin. This morphotype seems to be the most frequent in Santa Catarina and Uruguay. Morphotype II has a close to triangular shape, with a wide anterior region (not rounded) and the posterior region much more in tapering and a sharp recess on the posterior dorsal
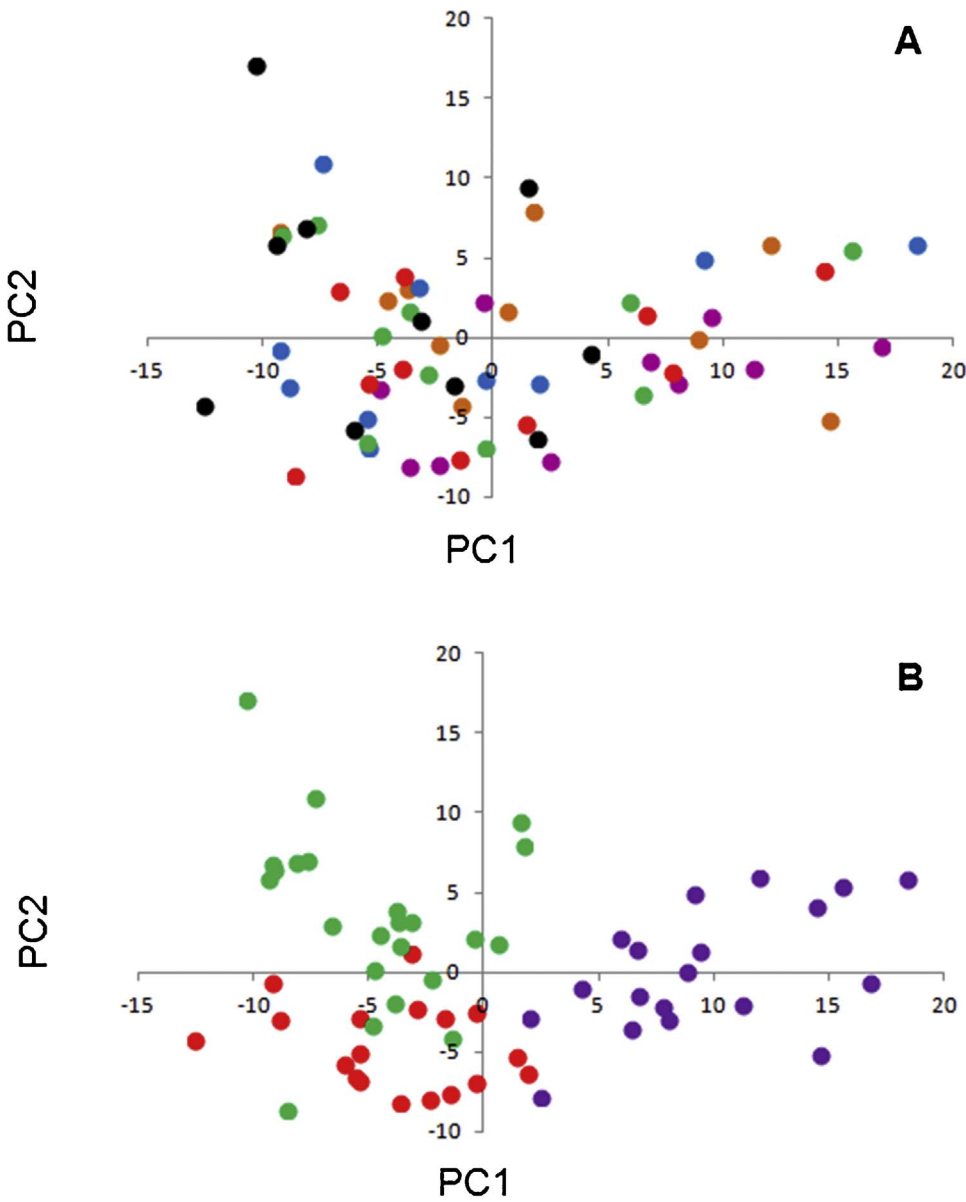
Fig. 6. Ordination plots from principal component analysis on the Fourier variables. Samples coded by areas (A) and morphotypes (B).
A- ● Rio de Janeiro; ● São Paulo; ● Santa Catarina; ● Rio Grande do Sul; ● Uruguay; ● Argentina.
B- ● morphotype I; ● morphotype II; ● morphotype III.

Table 1
Morphometric variables form *sagitta* otoliths of *M. furnieri* and their respective angular coefficients (*b*) among the sites and morphotypes. * $p < 0.05$; ** $p < 0.01$; *F* and *p*-values from ANCOVA also shown. ns, non-significant.

Variables	Sites			Morphotypes		
	F	*p*	*b*	*F*	*p*	*b*
Area	2.6	0.036*	0.12	16.6	0.0009**	0.12
Perimeter	3.1	0.015*	0.15	9.3	0.004**	0.15
Width	1.6	0.161 ns	0.03	13.5	0.001**	0.03
Circularity	2.9	0.019*	−0.003	0.3	0.511 ns	−0.003
Feret diameter	1.4	0.251 ns	0.05	14.9	0.001**	0.05

margin. This shape predominated in otoliths from the Rio de Janeiro coast. Morphotype III showed a more close to oval shape, wider and lacking the posterior dorsal indent. This shape was more common in fish from the São Paulo coast.

Scatterplots of the first two axes (PC1 and PC2) separated the three morphotypes. The first axis separates morphotype III from morphotypes I and II that overlapped on the left side of the diagram (Fig. 6). When we consider the samples coded by the areas, no discernible pattern was detected, indicating different otolith shapes throughout all studied areas.

3.2. Otoliths morphometric analyses

Three variables (perimeter, area and circularity) had significant correlation with fish size ($P < 0.05$) among the areas according to ANCOVA (Table 1) and suffered correction using their common slope (regression analyses). Four variables (perimeter, area, width and Feret diameter) were correlated significantly with fish size among the morphotypes (Table 1). Otoliths from Rio de Janeiro had higher indices for area, perimeter and width, whereas the highest Feret diameter was found for Santa Catarina otoliths, and the highest circularity for otoliths from São Paulo. Moreover, morphotypes I and II had significantly higher area ($F = 3.9$; $p = 0.02$), perimeter ($F = 7.8$; $p = 0.001$) and Feret diameter ($F = 10.7$; $p = 0.001$) compared with morphotype III, whereas this latter morphotype had the highest circularity ($F = 8.74$; $p = 0.001$) according to ANOVA.

A MANOVA test detected multiple differences (Wilḱs lambda = 0.49; $F = 1.60$; $p = 0.042$) for the analyzed variables among the areas. According to ANOVA, significant differences were detected
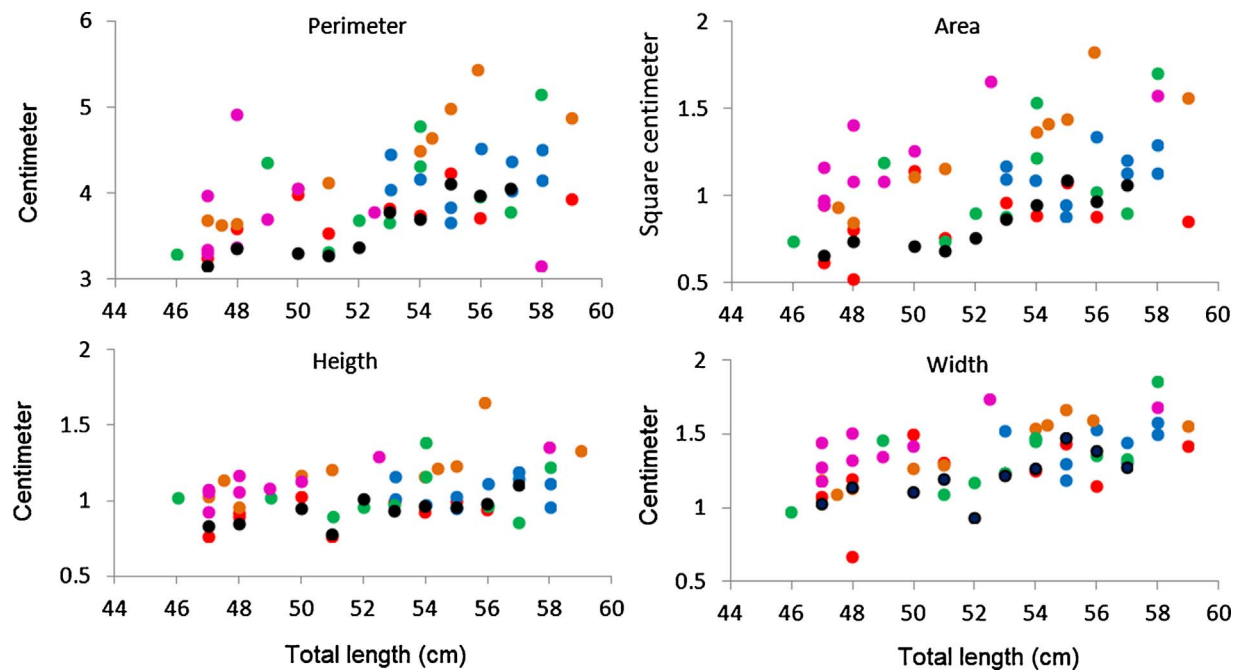
Fig. 7. Scatterplot of morphometric measures of the *ostiuḿs sulcus acusticus* of *sagitta* otoliths against total length of *M. furnieri*.
● Rio de Janeiro; ● São Paulo; ● Santa Catarina; ● Rio Grande do Sul; ● Uruguay; ● Argentina.

for the otolith perimeter ($F = 2.68$; $p = 0.03^*$) among the areas, with individuals from Rio de Janeiro and Santa Catarina having higher values than those from São Paulo. Circularity was significantly higher ($F = 3.80$; $p = 0.005$) in individuals from São Paulo compared with those from Argentina, Uruguay and Rio de Janeiro.

3.3. Sulcus acusticus morphometric analysis

The regression between otolith length (Lo) and the morphometric variables of the *ostium* showed a slight tendency to discriminate otoliths of São Paulo from Uruguay, with the São Paulo otoliths showing larger *sulcus acusticus* than those from Uruguay, for a given fish size. Similar regressions for the *sulcus acusticus* tails did not show any discrimination among the areas.

Regression analyses between otolith *ostium* measurements and fish total length showed differences among some areas (Fig. 7). For the relationship between each variable (perimeter, area, height and width) and the fish size, significant difference for the y-intercept ($p < 0.01$) were found among the areas, but no differences ($p > 0.05$) were detected for the b-slopes according to ANCOVA. The *ostium* perimeter ($F_{5,52} = 11.4$; $p = 0.0001$) and area ($F_{5,52} = 12.6$; $p = 0.0001$) were significantly higher in individuals from Rio de Janeiro, São Paulo and Santa Catarina (range: 4.2–4.4 mm) compared with those from Uruguay and Argentina (3.6–3.7 mm). The *ostium* height also differed significantly ($F_{5,52} = 16.1$; $p = 0.0001$) being higher in individuals from Rio de Janeiro, São Paulo and Santa Catarina (1.1–1.2 mm) compared with individuals from Rio Grande do Sul, Uruguay and Argentina (0.9–1.0 mm). Width was comparatively higher ($F_{5,52} = 6.0$; $p = 0.0001$) for individuals from São Paulo and Santa Catarina (1.4 mm) than those from Uruguay and Argentina (1.1–1.2 mm).

4. Discussion

We found three morphotypes for *sagitta* otoliths of *M. furnieri* along the Southwestern Atlantic coast, which overlap by mixing along of the six studied areas between the Coast of Rio de Janeiro (23°S) and the northern Argentina coast (39°S). These different morphotypes were spread along the whole studied area. If we assume that different

populations are indicated by different otolith shapes, our findings suggest a mixture of different *M. furnieri* populations that overlap spatially along the Brazilian, Uruguayan and Argentinean coasts. According to Marr (1957), a fish stock is a part of a given population where all members have hereditary similarities but behave as different subpopulations in response to environmental constraints. In this sense, members of different subpopulations/stocks of *M. furnieri* may coexist in the Southwestern Atlantic.

Vazzoler and Phan (1989), studying proteins in the crystalline of *M. furnieri* reported that the population I (Southeast Brazilian coast) is a homogeneous group whereas population II (Southern Brazilian coast) is a heterogeneous group that receives genic influences from both, individuals of population I, and individuals of other populations from Uruguay and Argentina coast. However, the lack of patterns in otolith shapes along the entire studied area and the presence of three morphotypes re-inforce the hypothesis that populations in southeastern and south Brazilian coast are influenced by other populations, suggesting the existence of a heterogeneous group.

Other studies also detected different morphotypes in fish species along the South America coastal areas. It was the case of Duarte-Neto et al. (2008) studying stocks of *Coryphaena hippurus* in Northeast Brazil. They also found three morphotypes, but did not discriminate spatial differences in otolith shape between two different areas, reporting that variation in morphotypes can be attributed to temporal equilibrium selection. According to Gauldie and Crampton (2002), this selection follows a basic pattern, where a given type is maintained for one year, and the other in the following year, and so on. Thus, the effects of selection lead to a persistent polymorphism.

The otolith contour analysis has been used and recognized as an important tool for discriminating fish stocks (Campana and Casselman, 1993). Similarly to our findings, other studies have used basic shape descriptors associated to the Fourier elliptic to describe shape of otoliths of several species (e.g., Begg and Brown, 2000; Begg et al., 2005; Campana and Casselman, 1993; Fergusson et al., 2011; Legua et al., 2013; Merigot et al., 2007). In this study, only adult individuals were examined and this procedure was followed to avoid confounding factors that could be caused by alometric growth. According to Volpedo and Echeverria (1999), there are morphometric changes in otoliths *sagitta* of

M. furnieri from larval stage until the adult phase when the shape is stabilized. Campana and Casselman (1993) also found that the last changes in otolith contour occur when fish reach the sexual maturity. Therefore, changes in otolith shape in this study are unlikely to have errors or bias due to allometric processes since all fish were adults.

Species of *Sciaenidae* have otoliths with a well-defined and conspicuous *sulcus acusticus* (Corrêa and Vianna, 1993; Volpedo and Echeverria, 1999), and our findings evidenced differences in the *ostium* among different areas, although no differences were found when comparing the *sulcus acusticus* cauda, since all individuals have similar *sulcus acusticus* caudal shape irrespective of the area of occurrence. The size of the *sulcus acusticus* has also been associated to fish mobility (Arellano et al., 1995; Avigliano et al., 2014; Lombarte and Popper, 1994; Tuset et al., 2003). The shape of the *sulcus acusticus* in species of the genus *Merluccius* were associated to the use of the water column, and in species of the genus *Mullus* were associated to feeding behavior (Aguirre and Lombarte, 1999). In our study, morphometric measurements of the *ostium* of the *sulcus acusticus* contributed to discriminate the morphotypes.

In conclusion, the absence of a clear pattern of otolith shape that could be associated to the different studied areas is probably associated to overlap of individuals of different populations/stocks. The three different otolith morphotypes do not seem to be subjected to a clinal variation for this transition area. Therefore, it is likely that there is a mixture of different stocks along the studied area due to the oceanographic variability present along the Southwestern Atlantic, namely the influences of Brazil and Falklands currents and the subtropical convergence. Moreover, increasing the number of samples in each area of study could bring light to this issue that yet causes concerns for fish biologists.

Acknowledgments

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