



Ontogenetic development of the sagittal otolith of the anchovy, *Anchoa tricolor*, in a subtropical estuary

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Summary: In order to characterize the ontogeny of *Anchoa tricolor*, the morphology and morphometry of sagitta otoliths were described. A total of 397 pairs of sagitta otoliths of *A. tricolor* (20 to 85 mm) were measured and analysed by means of digital images. Morphological description was done in terms of shape and features. The Huxley model was fitted to otolith length against total length (TL) and weight (TW), and residual analyses were done in order to detect the polyphasic growth. Six shape indices were calculated (otolith height/otolith length and otolith length/total length aspect ratios, rectangularity, roundness, relative sulcus surface and rostrum index) and after size effect removal, they were analysed in terms of TL. The results of these analyses allowed three distinct growth phases to be identified: (1) up to 40 mm TL and related to low swimming ability; (2) between 41 and 60-70 mm TL, when fish displacements increase but the first maturation has not yet occurred; and (3) after 71 mm TL, when fish become adults. Landmarks and semi-landmarks were used to analyse relative warps during the otolith development. The MANOVA analysis between the centroid size and class intervals showed significant differences in the interaction of all classes except between 71-80 and 81-90 mm.

Keywords: shape index; geometric; morphometry; Engraulidae; Brazil; description.

Desarrollo ontogénico del otolito sagitta de la anchoa, *Anchoa tricolor*, en un estuario subtropical

Resumen: Se describen la morfología y morfometría de los otolitos sagitta de *Anchoa tricolor* con el objetivo de caracterizar su ontogenia. Se midieron un total de 397 pares de otolitos sagitta de *A. tricolor* (20 a 85 mm) y se analizaron por medio de imágenes digitales. La descripción morfológica se hizo en términos de forma y características. Se ajustó el modelo de Huxley a la relación longitud del otolito, longitud total (TL) y peso (TW), respectivamente, y se realizaron análisis de residuos para detectar el crecimiento polifásico. Se calcularon seis índices de forma (OH/OL y OL/LT radios de aspecto, rectangularidad, redondez, superficie relativa del sulcus e índice de rostrum) y, una vez eliminado el efecto de talla, se analizaron en términos de longitud total. Los resultados de estos análisis permitieron identificar tres fases distintas de crecimiento: (1) una fase inicial, hasta 40 mm TL y relacionada con una habilidad natatoria baja; (2) entre 41 y 60-70 mm TL, cuando los desplazamientos del pez aumentan, pero aún no se ha producido la primera madurez y (3) superior a 71 mm TL, cuando el pez es ya adulto. Se utilizaron puntos homólogos y puntos equidistantes para analizar deformaciones relativas durante el desarrollo del otolito. El análisis MANOVA entre el tamaño del centroide y los intervalos de clases de talla mostraron diferencias significativas en la interacción de todas las clases, excepto entre 71-80 y 81-90 mm.

Palabras clave: índice de forma; geometría; morfometría; Engraulidae; Brasil; descripción.

Citation/Como citar este artículo: Carvalho B.M., Vaz-dos-Santos A.M., Spach H.L., Volpedo A.V. 2015. Ontogenetic development of the sagittal otolith of the anchovy, *Anchoa tricolor*, in a subtropical estuary. *Sci. Mar.* 79(4): 409-418. doi: <http://dx.doi.org/10.3989/scimar.04218.31A>

Editor: I. Palomera.

Received: January 29, 2015. **Accepted:** September 18, 2015. **Published:** November 3, 2015.

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INTRODUCTION

The otoliths of teleosts are complex polycarbonate structures composed mainly of aragonite. Three pairs (sagittae, asteriscus and lapilli) are present in the inner ear chambers. They are responsible for equilibrium and audition (Popper et al. 2005). The otolith shape make them an important tool for taxonomical purposes (Nolf 1985, Volpedo and Echeverría 1999, Tombari et al. 2005), population identification (Campana and Caselman 1993, Carvalho and Castello 2013), ecology (Waessle et al. 2003, Avigliano et al. 2014), biology (Lombarte 1992, Vignon 2012, Avigliano and Volpedo 2013), life history (Campana 2001, Elsdon et al. 2008) and growth studies (Bellido et al. 2000).

Many qualitative attributes have been used to describe otolith morphology (Smale et al. 1995, Volpedo and Echeverría 1999, Assis 2005, Tuset et al. 2008), although morphometry reduces the subjectivity of this kind of analysis. The use of bivariate morphometry to describe the relationships between fish and otolith measurements is common (Cadrin and Friedland 1999, Ponton 2006, Perin and Vaz-dos-Santos 2014), including shape indices (Tuset et al. 2003a, Leguá et al. 2013). The association of traditional morphometry and geometric studies makes it possible to expand the use of otoliths as key elements for understanding species and populations (Rohlf and Marcus 1993, Monteiro et al. 2005, Vignon 2012).

Several studies have described morphological variations during the ontogenetic development of otoliths. Volpedo and Echeverría (1999) and Waessle et al. (2003) identified a strong modification in the otolith morphology of *Micropogonias furnieri* during the growth of individuals, associated with sexual maturation. Gonzalez-Naya et al. (2012) described a change in the type and otolith tail position of sagitta in *Australoheros facetus* associated with variations in habitat

use. Vignon (2012) demonstrated the environmental influence on the morphology of the sagitta otolith during growth in *Lutjanus kasmirai*. De La Cruz-Agüero et al. (2012) showed variations in morphology and morphometry of otoliths of six species of the family Gerreidae. Carvalho and Corrêa (2014) identified a depression in the dorsal region of the sagitta otolith of *Atherinella brasiliensis* after its first maturity. These examples confirm the broad applicability of otolith morphology and morphometry.

The anchovy, *Anchoa tricolor* (Spix and Agassiz, 1829), is an important small-sized species (>110 mm) that forms shoals in coastal regions, bays and estuaries in the southwestern Atlantic (Whitehead et al. 1988). Classified as a euryhaline species (Araújo et al. 2008, Vilar et al. 2011), it grows up to 118 mm long, with a life span of 2.3 years (Whitehead et al. 1988). It has an isometric growth pattern (Vaz-dos-Santos and Rossi-Wongtschowski 2013, Franco et al. 2013) and spawns between September and February (Araújo et al. 2008). Very few studies on the relationship between the species and estuaries are extant: the exclusive presence of juveniles and young fish of the year have been reported in Sepetiba Bay and in the Paranaguá Estuarine Complex (PEC), Brazil, with the occurrence of adults in open beaches outside these ecosystems (Araújo et al. 2008, Contente et al. 2011).

In the PEC, an important subtropical estuary of the southwestern Atlantic (Lana et al. 2001), anchovy coexists with six other congeneric species (Whitehead et al. 1988). It occupies a key position in the food web (Araújo et al. 2008), constituting an important prey for marine mammals, seabirds and other fish (Santos et al. 2002, Bugoni and Vooren 2004, Bornatowski et al. 2014). Some of these piscivorous species are top predators in the southwestern Atlantic and have different levels of conservation risk (IUCN 2014). Despite its importance, little is known about the life cycle of *A.*

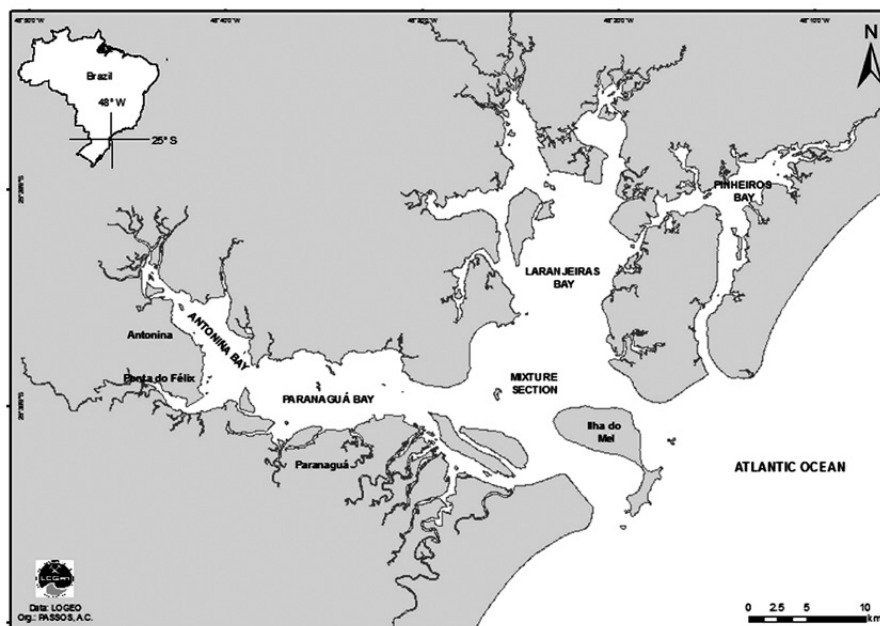


Fig. 1. – Map of the Paranaguá Estuarine Complex (Paraná State, Brazil). Source: Passos et al. (2012).

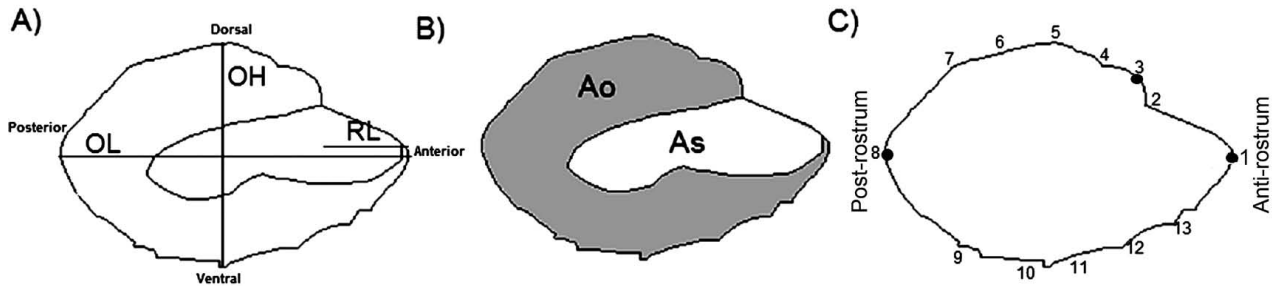


Fig. 2. – Morphometry of the sagittal otolith *Anchoa tricolor* from the Paranaguá Estuarine Complex (Paraná state, Brazil). A, longitudinal length of the otolith (OL), the greatest perpendicular height of the otolith (OH) and the length of the rostrum (RL); B, area of the otolith (Ao) and area of the sulcus acusticus (As); C, position of the landmarks (1,3,8) and semi-landmarks (2,4,5,6,7,9,10,11,12,13).

tricolor and its contribution to the ecological compartments of the estuary, due to its misidentification. In this study, the morphological features and the morphometry of sagitta otoliths during the ontogenetic development of anchovy from the PEC are described, providing a useful and precise tool for its identification. Additionally, these results are also discussed in relationship to the life cycle of anchovy and may facilitate future studies of its stock.

MATERIALS AND METHODS

Monthly samplings were conducted between October 2010 and June 2011, consisting of trawls in the PEC (25°15'–25°35'S; 48°20'–48°45'W) along the north-south axis (Fig. 1). Trawlers of 30 m were employed parallel to the coast at an average depth of 1.5 m using a beach seine net (length, 15 m; height, 2 m; mesh, 2.5 mm) pulled by two people. In the laboratory, individuals of *A. tricolor* were measured for total length (TL in mm) and weighed (TW in g), and sagitta otoliths were extracted, cleaned and stored.

Only left otoliths from fish between 20 and 85 mm TL were selected for the analysis. The morphological features of the sagittae were described according to Tuset et al. (2008). For morphometric analysis, micrographs of otoliths of fish smaller than 30 mm TL were processed using a scanning electron microscope for a better resolution. Otoliths of fish larger than 30 mm TL were photographed using an Olympus DP71 coupled to a stereomicroscope. Otolith images were measured with the Image Tool 3.0 software (Wilcox et al. 2002). Data of otolith length (OL, maximum longitudinal length, mm), otolith height (OH, maximum perpendicular length, mm), rostrum length (RL, the horizontal distance between the rostrum and the antirostrum, mm) and the areas of the otolith (A, mm²) and of the sulcus acusticus (mm²) were obtained (Fig. 2).

Three different analyses were used to characterize otolith morphometry of *A. tricolor*: regression analysis (Casselmann 1990, Huxley 1993), shape indices (Tuset et al. 2003a, b, Volpedo and Echeverría 2003) and morphometric geometric analysis (Rohlf and Marcus 1993, Zelditch et al. 2004).

The Huxley model ($y=ax^b$) was fitted between the TL and the total weight (TW) of fish and the OL (Huxley 1993). Data were previously transformed (ln) and the models were estimated by the linear least-squares

method (Zar 2010). In order to detect changes in the growth pattern, analysis were performed following Bervian et al. (2006). The predictive variable (OL in size class of 0.1 mm) was plotted against the average x values (TL and TW, respectively). The exponential coefficient of each size class (b_{sc}) was estimated with the inverse Huxley model. Outliers ($-1.96 < Z_{\text{residual}} < +1.96$) were removed. Then, the b_{sc} values were plotted against the average x values and polynomial models were fitted. The first-order derivatives were used to estimate b_{sc} , allowing the detection of the stanza changing points of TL and TW, by solving the equations. The stanza changing points of TW were converted into TL through the inversion of the formulae $TW = 1 \times 10^{-6} TL^{3.3623}$ ($r^2 = 0.9908$, $p < 0.001$).

Six shape indices were calculated to describe otolith variation over ontogenetic development of anchovy: aspect ratios (OL/TL, OH/OL), rectangularity [$Rc = A / (OL \times OH)$], roundness [$Ro = (4A) / (\pi \times OL^2)$], relative sulcus surface [$Rss = SA / A$] and rostrum index ($Ri = RL / OL$). Average values of each index were calculated by TL, minimizing the effect of different quantities of data (Bervian et al. 2006). The Huxley model was fitted to TL and each index by the linear least-squares method, with the outliers being disregarded (Zar 2010). The size effect of all individual data was removed by applying the formula proposed by Lombarte and Leonart (1993) in which a corrected predictive variable (y') is estimated from the equation $y' = y_i \times (x_0 / x_i)^b$, where y_i is the original value (shape index), x_0 is the referential value ($TL_{\text{minimum}} = 22$ mm) and x_i is the original TL value. With the corrected values, interval plots (means and their confidence intervals) were used to visualize ontogenetic variations of each shape index in relation to TL class (Tuset et al. 2003b, Volpedo and Echeverría 2003). After verification of assumptions, a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations using raw data (ln-transformed) and the similarity matrix (Bray-Curtis index) was used to ascertain differences between all shape indices and TL class (Anderson et al. 2008), followed by a post-hoc Mann-Whitney test with the Bonferroni correction (Zar 2010).

In order to characterize the geometric morphometry of *A. tricolor*, 274 sagitta of fish between 30 and 90 mm TL were analysed. Landmarks were positioned on structures with biological references in all class intervals, namely landmarks 1, 3 and 8, which represented the rostrum, the

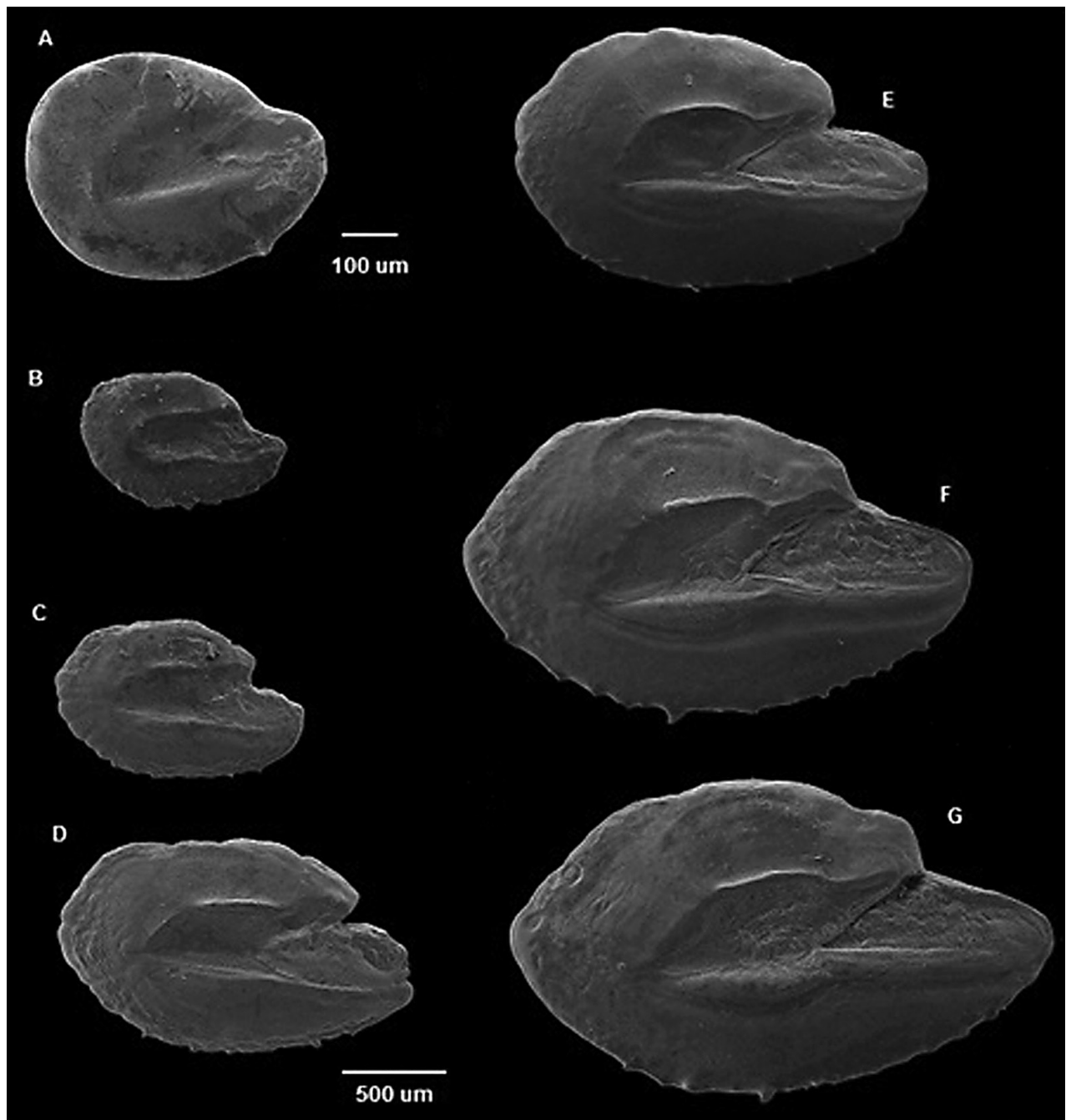


Fig. 3. – Micrographs of the inner face of the left sagitta otolith of *Anchoa tricolor*, with sizes of 22 mm (A), 34 mm (B), 40 mm (C), 55 mm (D), 65 mm (E), 75 mm (F) and 81 mm (G). Images A (120 \times ; scale 100 μ m) and B-G (45 \times ; scale 500 μ m).

antirostrum and post-rostrum, respectively (Fig. 2). The remaining points selected, five dorsal and five ventral, were semi-landmarks (2,4,5,6,7,9,10,11,12,13) (Fig. 2). An equidistant distance was maintained between the semi-landmarks, assisting in the interpretation of the otolith outline (Monteiro et al. 2005, Ponton 2006, Vignon and Morat 2010).

Landmarks and semi-landmarks were inserted in each photograph using the tpsUtil and tpsDig2 software. In the tpsRelw software, 3 landmarks and 10 semi-landmarks were assigned and fitted using the sliding method for semi-landmarks (Rohlf and Marcus 1993). The images were saved in TPS format in

TpsUtil and in TpsRelw we extracted the centroid size and plotted data in relative warps to identify possible variations in shape (Monteiro and Reis 1999, Ponton 2006). The relationship between the centroid size (otolith shape) and the fish length (TL) were checked using a linear regression. Subsequently, multivariate analysis of variance (MANOVA with Hotelling for significant test) was applied to test the difference between otolith shape and size class intervals in the Rmorph package, a geometric and multivariate morphometrics library for R (Baylac 2008). All analyses were performed in spreadsheet software and in PAST 3.06. In all statistical procedures $\alpha=0.05$.

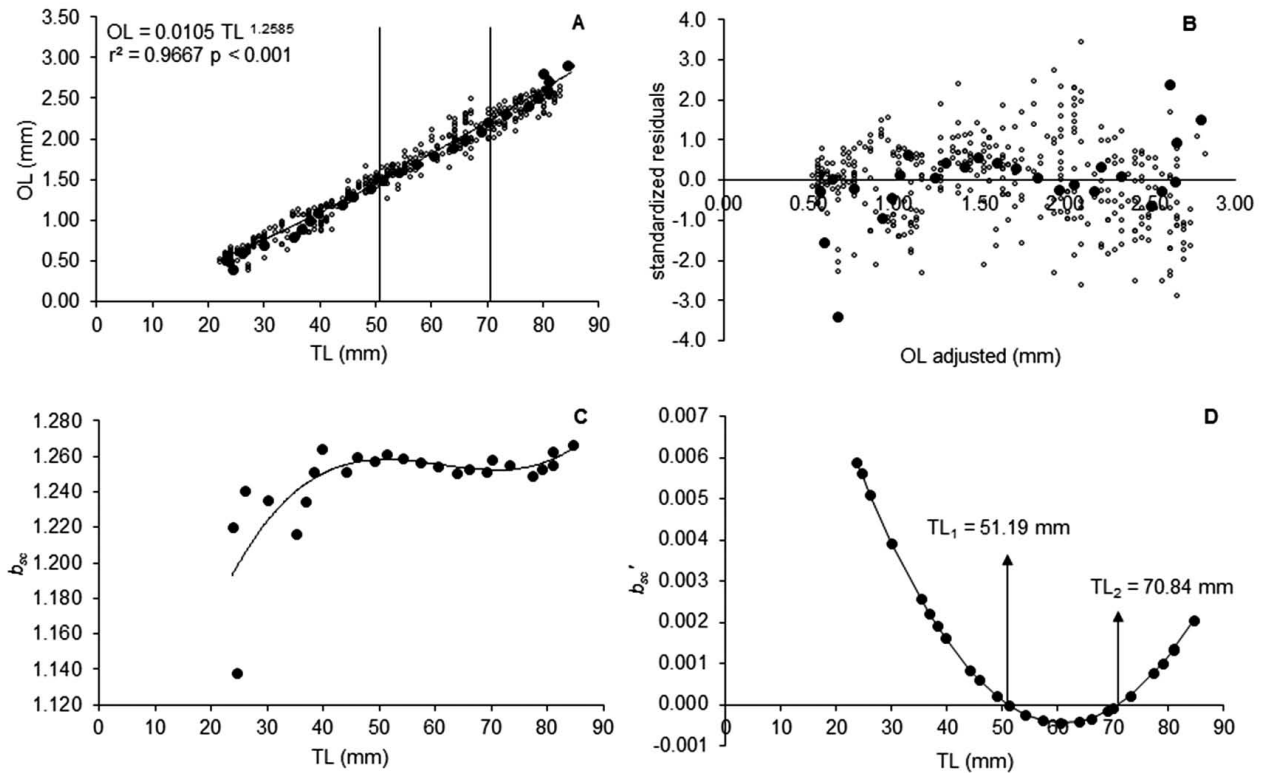


Fig. 4. – A, power regression between total length (TL) and otolith length (OL) of *Anchoa tricolor* of the Paranaguá Estuarine Complex (Paraná State, Brazil) (open circle = individual measurements; filled circle = average values for 0.1 mm OL class). B, standardized residuals adjusted from power regression. C, exponential coefficient (b_{sc}) for each total length (line = polynomial function). D, first derivative of polynomial function showing the stanza changing points of total length (TL_1 and TL_2).

RESULTS

A total of 397 specimens of *A. tricolor* (TL range: 22 to 85 mm; TW range: 0.04 to 3.76 g) were analysed. The general morphological pattern of otolith was the following: elliptic shape; round anterior-posterior region; margins varying between serrated (ventral) and smooth (dorsal) over ontogenetic development; hetero-sulcoid and ostial sulcus acusticus, deep and located in the middle region of the otolith; well-developed rostrum, antirostrum, rostrum and antirostrum in agreement, excisural notch shallow and acute in the otolith (Fig. 3). Some morphological attributes varied during the ontogenetic development. In smaller fish (21–25 mm TL), shape was circular (Fig. 3A). In fish of 35–45 mm TL, the excisura intensified and was maintained (Fig. 3D). At 55–75 mm TL, the dorsal margin of the otolith started to crenate and at 75–85 mm TL it reached the general pattern.

The regressions involving otolith length were $OL = 0.0105 TL^{1.2585}$ ($r^2 = 0.9667$, $p < 0.001$) (Fig. 4A) and $OL = 1.6862 TW^{0.3732}$ ($r^2 = 0.9669$, $p < 0.001$) (Fig. 5A). The residual analysis evidenced more than one growth phase (Fig. 4B and 5B). The polynomial equations fitted were $b_{sc} = 1.515 \times 10^{-6} TL^3 - 2.773 \times 10^{-4} TL^2 + 0.01648 TL + 0.938$ (Fig. 4C) and $b_{sc} = -0.02011 TW^3 + 0.1534 TW^2 - 0.3145 TL + 0.4734$ (Fig. 5C). The first derivatives from these equations were used to detect the stanza changing points. Three growth phases (two stanza changing points) were identified, related to 51.19 and 70.84 mm TL (Fig. 4D) and to 1.42 and

3.66 g (Fig. 5D), respectively. These TWs correspond to 67.6 mm and 89.6 mm TL. In relation to the relative sulcus surface index, its maximum development occurred in the main phase of longitudinal otolith development, corresponding to the second growth phase (41–60 mm TL) of *A. tricolor*.

Shape indices of *A. tricolor* otoliths showed different patterns of variation (Table 1): the aspect ratios, relative sulcus surface index and rostrum index were positive related to the TL, but the rectangularity and the roundness showed a negative pattern. After size effect removal, the shape indices also showed tendencies related to TL, both in the separate analysis of each index (Fig. 7) and in the joint analysis (PERMANOVA pseudo- $F = 13.6$, $p < 0.001$) (see Table 2 for the results of pairwise comparisons). Joining these results and also taking into consideration the otolith morphology (Fig. 3), at least three distinct growth phases of *A. tricolor* were identified, as follows. The first growth phase was related to individuals of less than 40 mm TL, in which the distinct pattern of shape indices reflected the conspicuous morphological changes in the otolith. In this phase, the body growth was greater than the otolith growth in length (lower values of the OL/TL aspect ratio), although otoliths showed a reduction in their circularity. This was revealed by decreasing values of OH/OL and roundness and a gradual increase in the rectangularity and rostrum index (Fig. 6). The second growth phase clearly occurred between 41 and 60 mm TL, although in some indices up to 70 mm TL. Due to the pronounced growth in the longitudinal axis,

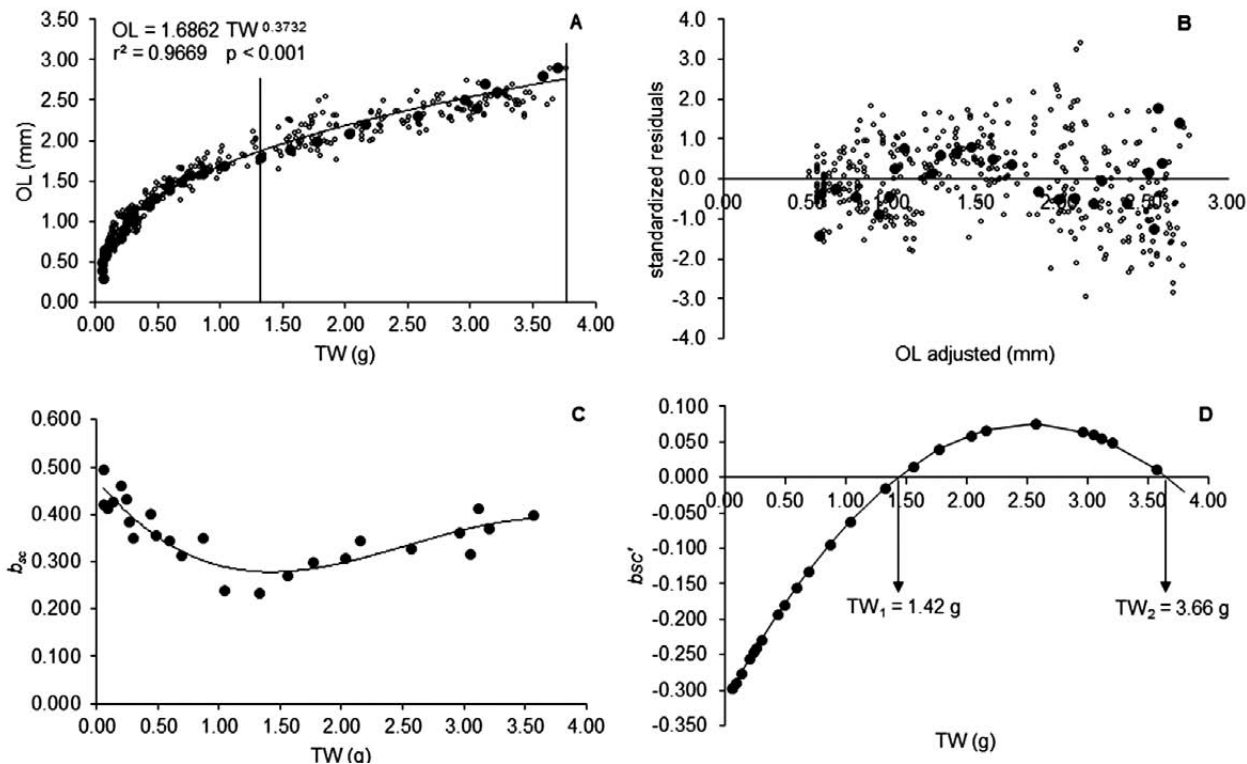


Fig. 5. – A, power regression between total weight (TW) and otolith length (OL) of *Anchoa tricolor* of the Paranaguá Estuarine Complex (Paraná State, Brazil) (open circle = individual measurements; filled circle = average values for 0.1 mm OL class. B, standardized residuals adjusted from power regression. C, exponential coefficient (b_{sc}) for each total weight (line = polynomial function). D, first derivative of polynomial function showing the stanza changing points of total weight (TW_1 and TW_2).

the rectangular shape of otoliths became evident (highest values of OL/TL aspect ratio and rectangularity and lowest values of OH/OL aspect ratio and roundness). In this phase, the rostrum reached its maximum proportional development (highest values of rostrum index) (Fig. 6). From 61 to 71 mm TL, otoliths remained constant in their patterns. Aspects ratios indicated an OL and OH with proportionally low rectangularity and more circularity (high roundness). The rostrum index also remained constant (Fig. 6).

The scatterplot of relative warps illustrated a set of dots arranged closer to RW1, which showed more elongated otoliths, different from the group closer to RW2, which was more associated with more rounded otoliths (Fig. 7). Based on the total length and the centroid size, regression analyses were run (TL:Centroid Size: $y = -3.11 \times 10^{15} + 1.30 \times 10^{15}x$; $r^2 = 0.9707$, $p < 0.001$). Moreover, for all Cartesian coordinates, the MANOVA revealed significant differences between all size classes (Wilk's $\lambda = 0.002$; $F = 24.44$, $p < 0.001$). A comparison between the centroid size and class intervals by MANOVA showed significant differences in the interaction of all class intervals ($p < 0.001$), except for the interaction between 71-80 mm and 81-90 ($p = 0.089$).

DISCUSSION

In the present study, the objectives were achieved and concise morphologic and morphometric descriptions of *A. tricolor* otolith have been presented. Although the identification of smaller specimens from otoliths is more difficult, due to their unfinished de-

Table 1. – Huxley model (power regressions, $y = ax^b$) between total length (TL) and shape indices (see the text for details) of *Anchoa tricolor* in the Paranaguá Estuarine Complex (Paraná State, Brazil) (n = 64; r^2 , coefficient of determination; p, probability value).

x vs. y	a	b	r^2	p
TL vs. OL/TL	0.0113	0.242	0.7683	2.36×10^{-21}
TL vs. OH/OL	1.6125	0.219	0.8195	9.95×10^{-25}
TL vs. Rectangularity	0.8475	-0.036	0.4429	1.97×10^{-9}
TL vs. Roundness	1.7437	-0.255	0.8721	2.23×10^{-29}
TL vs. Relative sulcus surface	0.2168	0.069	0.1417	0.00217
TL vs. Rostrum index	0.1229	0.169	0.3694	1.00×10^{-7}

Table 2. – Probability values (p) obtained from a post-hoc Mann-Whitney test (with Bonferroni correction) in order to detect differences in shape indices related to total length (TL) class (mm) of *Anchoa tricolor* in the Paranaguá Estuarine Complex (Paraná State, Brazil) (bold italics emphasize significant differences, $p < 0.05$).

TL class (mm)	21-30	31-40	41-50	51-60	61-70	71-80
31-40	0.032					
41-50	0.002	0.004				
51-60	0.002	0.002	1.000			
61-70	0.004	0.139	0.002	0.002		
71-80	0.063	0.056	0.002	0.002	0.002	
81-90	1.000	1.000	0.002	0.002	0.002	1.000

velopment, in larger (and/or adult) specimens otolith features differentiate among genera of the same family, allowing them to be differentiated (Campana 2001, Ponton 2006, Tuset et al. 2008).

Sagitta otolith morphology has been described for diverse species of engraulids (Smale et al. 1995) and for *A. tricolor* there was a previous description (Lemos et al. 1995). The limitations due to the methodologies

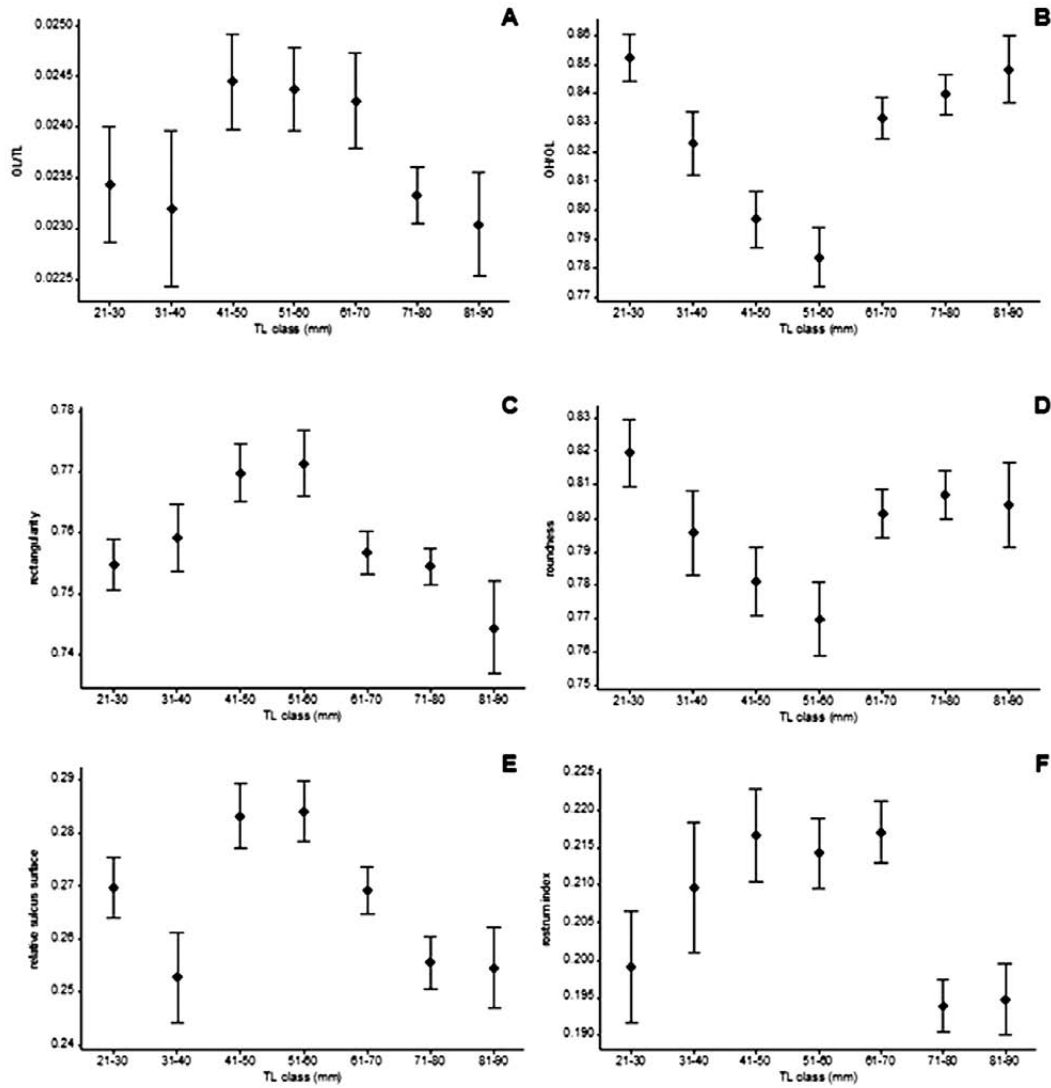


Fig. 6. – Mean and confidence interval (95%) of the otolith shape indices of *Anchoa tricolor* by total length (TL) class in the Paranaguá Estuarine Complex (Paraná State, Brazil). A, aspect ratio of the otolith length (OL) and total length (TL). B, aspect ratio of the otolith height (OH) and length (OL). C, rectangularity index. D, roundness index. E, relative sulcus acusticus surface index. F, rostrum index.

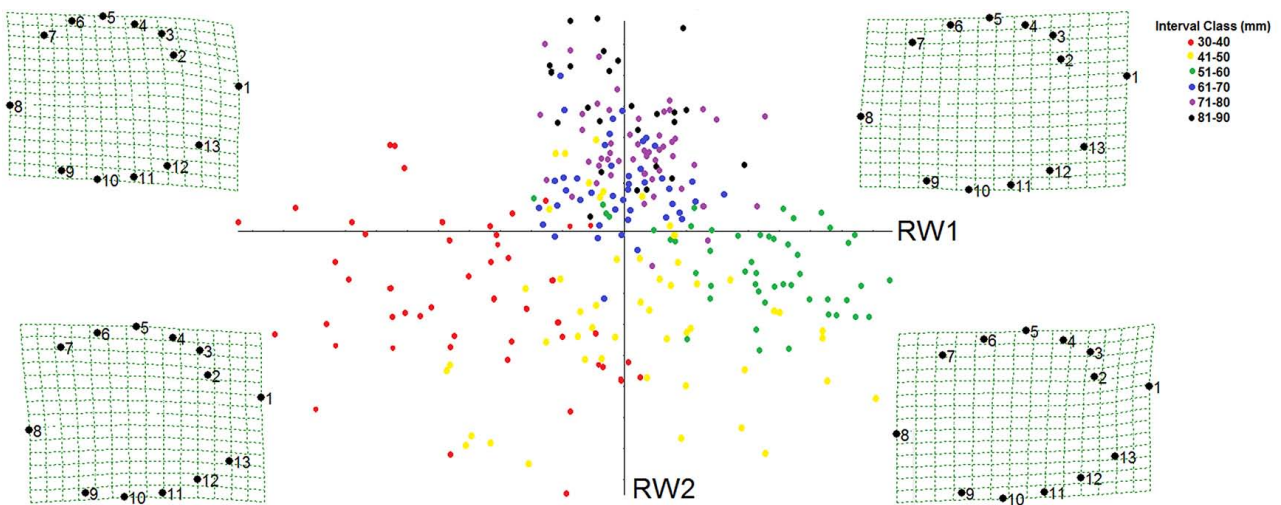


Fig. 7. – Thin-plate splines are represented near the position of the groups on the relative warps 1-2 ordination plot of the otolith shape of *Anchoa tricolor*.

and technologies of that time restricted comparisons and the current use of these earlier results (Lemos et al. 1995), but it was possible to identify some characteristics of the sagitta of anchovies from other studies. The most common characteristics among the species of engraulids are small to medium otolith, heterosulcoid and ostial sulcus acusticus. In *A. tricolor*, the otoliths have an elliptic shape, whereas in *Thryssa sitirostris*, *Thryssa vitrirostris* and *Engraulis japonicas* it is oval, elliptic and fusiform (Smale et al. 1995). In *Engraulis australis*, the shape varies from oval to fusiform (Furlani et al. 2007), being elliptic in *Engraulis encrasicolus* (Tuset et al. 2008). As is well known, otolith patterns vary in accordance with genera. What, then, is the utility of the description presented in the current study? Although it is not possible to identify the other congeneric species, in the study area *A. tricolor* is the most abundant and important engraulid in the PEC (Contente et al. 2011) and other coastal areas, including the inner continental shelf (<50m depth) (Rossi-Wongtschowski et al. 2014). This fact per se highlights the importance of an otolith description of this species, even disregarding other congenics.

Morphological and morphometric evidences indicate that the sagitta otolith represent three ontogenetic development stages of *A. tricolor*. Although no perfect coincidence was found in terms of TL values among the analyses performed (nor was it expected), it was possible to associate these stages with the biology of the species and habitat (Vignon and Morat 2010, Avigliano and Volpedo 2013).

The specimens of *A. tricolor* up to 40 mm TL perform short displacements, given the incomplete development of the dorsal and anal fins (Hofstaetter et al. 2004). The species inhabits the estuary at this stage (Araújo et al. 2008) and still has more rounded otoliths with a small rostrum, which corresponds to fish with low swimming ability (Volpedo and Echeverría 2003, Volpedo et al. 2008). The circular shape is inherited from the larval phase (Joh et al. 2015), which will be progressively reduced. From 41 to 60-70 mm TL, the swimming capacity of the anchovy increases, enabling its migration out of the estuary. This characteristic is reflected in the morphology and morphometry of the otoliths, with greater development of the excisura and higher growth of the rostrum. In this second growth phase, most of the morphological attributes are attained. Also during this stage, the species moves up to the inner continental shelf (Araújo et al. 2008), where it forms large shoals (Rossi-Wongtschowski et al. 2014). The third growth phase is related to the first maturation in the anchovy, close to 61-70 mm TL, both quoted in Whitehead et al. (1988) and diagnosed from analysis of the current data, still unpublished. The morphological and morphometric patterns of adults are achieved, when the otolith finally can be characterized as an *A. tricolor* otolith. Nevertheless, the descriptions presented for the earlier phases are useful, considering the morphological differences of other species and genera (Lemos et al. 1995).

The identification of these three growth phases of *A. tricolor*, the first two related to juveniles and the

third one to adults, gains support in view of the holistic approach adopted here, i.e. the use of different and independent methodologies that converge to the same result. The only result that partially disagreed with the general pattern was the regression between OL and total weight of the fish (TW vs. OL). TW of fish is influenced on different temporal scales by feeding (Zavala-Camin 1996) and particularly by reproduction (Froese 2006). In the anchovy, the first maturation created a conspicuous stanza changing point (the first) in the TW-OL relationship; the second one was so close to the end of the regression that it must be disregarded.

Despite the subjectivity of morphological evaluations (Rondon et al. 2014), the morphometry is quantitative and tends to be more accurate (Monteiro and Reis 1999). Traditional morphometry (the Huxley model) has a broad use related to fish studies (Casselman 1990, Hunt 1992, Volpedo and Echeverría 1997). Bervian et al. (2006) brought to it an important new approach, using the size effect as a tool for detecting changes related to different growth phases. This tool cannot always be functional: if the residual analysis follows their assumptions (Zar 2010), then this technique will be inappropriate (Bervian et al. 2006).

The initial use of geometric morphometrics applied to ontogeny was controversial (Rohlf 1998, Zelditch et al. 1998). In the early 2000s, the emergence of the geometric morphometrics applied to fishes brought a new precise and accurate tool to bioecology, fisheries research and related areas (Loy et al. 1998, 2000, Torres et al. 2000). Ponton (2006) stated its use in otolithology and its reference has quickly become a classical citation.

In the case of *A. tricolor*, geometric morphometrics showed clear patterns of change of shape during ontogenetic development, which would be related to the polyphase growth for the species found in this study. As with *Anchoa tricolor*, geometric morphometry was also efficient in the description of ontogenetic development of otoliths of Clupeiformes (Ponton 2006, Libungan et al. 2015). The lack of significance between the two major class intervals shown by geometric morphometrics indicates that there is a stability in the shape of the anchovy otoliths after first maturity.

The morphological and morphometric characterization of the sagitta of *A. tricolor*, more than another descriptive study about otoliths, reflected most of its life cycle inside an estuary. There are few references focusing on this kind of analysis (Hare and Cowen 1994, Hüsey 2008, Vignon 2012), but the use adopted here proved to be useful and efficient. Considering that *A. tricolor* is an important species both in coastal areas and on the inner continental shelf of the southwestern Atlantic, the present results are valuable.

ACKNOWLEDGEMENTS

The authors are grateful for funding from the project CAFB-BA/SPU. They also thank CONICET and UBACYT, CAPES for the master's degree scholarship to the first author, LAFMA(CEM-UFPR) for the stereomicroscope and the Olympus DP71 camera, and the

Centro de Microscopia Eletrônica (UFPR) for the micrographs. The editors and anonymous reviewers are thanked for constructive comments which improved our manuscript.

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