Differences in the otoliths support the distinction of the genus *Macrodon* into two species in the south-western Atlantic Ocean

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*Morphological differences in the otoliths of Macrodon atricauda from Rio Grande do Sul State (southern Brazil) and of M. ancylodon from Pará State (northern Brazil) were examined. Until recently, these were considered two populations of the same species. Although relatively similar in their general shape, those of large *M. atricauda* (>300 mm total length) were shorter and thinner and their outer surface was convex (those of the smallest specimens had a more lobulated antero-dorsal margin) whereas those of similarly sized *M. ancylodon* were larger, thicker and less lobulated. These features of the otoliths allow these morphologically similar species to be discriminated. The pattern of translucent and opaque bands in sectioned otoliths also differed. The otoliths of *M. atricauda* showed clear annual banding which allowed ageing, while those of *M. ancylodon* had no distinct alternate bands.*

**Keywords:** otoliths, species differentiation, *Macrodon*, south-western Atlantic

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**INTRODUCTION**

Until recently, due to their morphological similarity, it was thought that the genus *Macrodon* in the south-western Atlantic was composed of a single species (Casatti & Menezes et al., 2003). However, recent studies by Santos et al. (2006) and Carvalho-Filho et al. (2010) found genetic and morphological evidence, respectively, that suggested two distinct species: *M. ancylodon* (Bloch & Schneider, 1801), which occurs from Venezuela to Bahia State (Brazil) and *M. atricauda* (Günther, 1880), which occurs from Espírito Santo State (Brazil) to northern Argentina.

*Macrodon atricauda* (local names include *pescadinha, pescada-foguete* and *pescadinha real*) is a commercial species for which the fisheries biology is relatively well known (Yamaguti, 1967, 1968; Haimovici, 1998). It is exploited in an industrial trawl fishery in the coastal waters of southern and south-eastern Brazil. Its otoliths are used for ageing the species, since they show alternate opaque and translucent bands, which are evident in both whole and sliced otoliths (Yamaguti & Santos, 1966; Haimovici, 1988; Cardoso & Haimovici, 2011). *Macrodon ancylodon* (local name *pescada-go*) also sustains fishing in Pará State (Isaac et al., 2009). Although some aspects of its life cycle have been studied (Camargo & Isaac, 2005), its ageing was not consistent when sectioned otoliths were used.

*Macrodon ancylodon* and *M. atricauda* are morphologically very similar, although they are genetically distinct (Santos et al., 2006). Reported morphological differences are subtle: the size of the largest canine teeth, the number of soft rays in the fins and the number of lateral line scales with pores (Carvalho-Filho et al., 2010). Otolith shape can also be a species-specific (see examples quoted in Campagna & Casselman, 1993) and has been used to discriminate marine fish species (Härkönen, 1986; Malcom et al., 1995). The present study investigated the possibility of using the shape of the otoliths as a diagnostic taxonomic character. Our secondary goal was to analyse the suitability of *M. ancylodon* otoliths for ageing.

**MATERIALS AND METHODS**

The sagittal otoliths of 44 specimens were examined, including 22 that were collected from artisanal fisheries in Bragança, Pará (00°51’S), in northern Brazil and 22 that were collected from commercial landings in Rio Grande, Rio Grande do Sul (32°12’S) in southern Brazil (Table 1). Total length (TL) was measured between the beginning of the snout and the end of the tail. Otolith weight (OW, g), length (OL, mm), width (OW) and thickness (OT) were measured for comparisons, as shown in Figure 1.

The right otoliths were sectioned through the nucleus with a Buehler® Isomet® low-speed saw with a high concentration diamond blade, as described by Cardoso & Haimovici (2011), and the annual growth rings were identified when viewed.

A discriminant function analysis (DFA) was performed with the measures on otoliths (OL, OW, OT) expressed as ratios of total length of fish, and the weight of the otoliths,
as ratios of total weight. Multidimensional scaling (MDS: Clarke & Warwick, 2001) was used to visually discriminate the otoliths of each species.

Changes in the shape of the otoliths with growth of both species were analysed through the linear regressions between total length and the length (OL), width (OW) and weight (OW, g) of the otoliths. These regressions were compared with a covariance analysis (Zar, 1996), using both species as covariate.

### RESULTS

The specimens from northern Brazil measured 195–400 mm and the specimens from southern Brazil measured 182–410 mm (Table 1).

Otolith descriptions: the inner surface of the otoliths of both species are slightly convex; the sulcus, which ranges along most of the inner surface, is open at the anterior end. The ostium is large and rectangular in shape; the tail is deep and curved ventrally with a rounded tip. The dorsal crista is well developed in the central portion of the larger specimens and margins are lobulated in specimens which are smaller than 250 to 300 mm TL. The outer surface was slightly concave in *M. atricauda* and straighter in *M. ancylodon*. Lobulation of the dorsal-anterior margin was more pronounced in *M. ancylodon*.

### Table 1. Total length (TL), total weight (TW) and otolith measurements of *Macrodon ancylodon* and *M. atricauda* used in growth and shape comparisons.

<table>
<thead>
<tr>
<th></th>
<th><em>M. ancylodon</em></th>
<th><em>M. atricauda</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Mean (N=22)</td>
</tr>
<tr>
<td>TL (mm)</td>
<td>195</td>
<td>303.1</td>
</tr>
<tr>
<td>TW (g)</td>
<td>46.3</td>
<td>273.4</td>
</tr>
<tr>
<td>Otolith weight (g)</td>
<td>0.06</td>
<td>0.17</td>
</tr>
<tr>
<td>Otolith width (mm)</td>
<td>3.81</td>
<td>5.82</td>
</tr>
<tr>
<td>Otolith thickness (mm)</td>
<td>1.22</td>
<td>1.94</td>
</tr>
<tr>
<td>Otolith length (mm)</td>
<td>9.96</td>
<td>13.34</td>
</tr>
</tbody>
</table>

Min., minimum; Max., maximum.

### Table 2. Wilks’ lambda, F-remove scores and probability of the null hypothesis of differences among the otoliths of *Macrodon ancylodon* and *M. atricauda*.

<table>
<thead>
<tr>
<th></th>
<th>Wilks’ lambda</th>
<th>F-remove (df: 1.39)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>0.371</td>
<td>9.120</td>
<td>0.004</td>
</tr>
<tr>
<td>Otolith width (OW)</td>
<td>0.345</td>
<td>5.725</td>
<td>0.022</td>
</tr>
<tr>
<td>Otolith thickness (OT)</td>
<td>0.450</td>
<td>19.433</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Otolith length (OL)</td>
<td>0.307</td>
<td>0.782</td>
<td>0.382</td>
</tr>
</tbody>
</table>

### Fig. 1.

Inner surface (left), outer surface (middle) and dorsal view (right) of the left otoliths of male specimens of *Macrodon ancylodon* (A) length (L) 222 mm, weight (W) 89 g and of *M. atricauda* (B) L 220 mm, W 89.3 g; the same images of the right otoliths of male specimens of *M. ancylodon* (C) L 360 mm, W 372.5 g and *M. atricauda* (D) L 361 mm, W 384 g. OL, otolith length; OW, otolith width; OT, otolith thickness.
Otoliths in the larger specimens (>300 mm) of *Macrodon atricauda* were smaller (OL average of 3.7% of TL) and thinner (OL 1.7% of TL) than those of *M. ancyldon* (OL 4.2% of TL).

Wilks' lambda and F-remove discrimination coefficients showed that the otoliths of *M. ancyldon* and *M. atricauda* were significantly different regarding their weight, width and thickness, while no significant differences were observed in their length (Table 2). The classification matrix of the DFA classified 90.9% of the northern *Macrodon* otoliths and 100% of the southern *Macrodon* otoliths accurately.

A MDS showed a clear separation between the northern and the southern *Macrodon* otoliths in two main coordinates (Figure 2).

Otoliths of *M. ancyldon* grew significantly more in weight (*P* < 0.01), length (*P* < 0.01), width (*P* = 0.04) and thickness (*P* < 0.01) than those of *M. atricauda* (Figure 3). However, differences among specimens less than 250 mm were not so evident, as shown in Figure 1A, B.

The 22 specimens of *M. atricauda* from southern Brazil had between one and three alternate opaque bands that were easily observed in the sectioned otoliths (Figure 4B). In contrast, the sectioned otoliths of the 22 *M. ancyldon* specimens did not show alternation of opaque and translucent bands that could be associated with age (Figure 4A).

**DISCUSSION**

The otoliths of both species grow at different rates: those of *Macrodon ancyldon* become heavier, wider and thicker than the otoliths of *M. atricauda* at increasing total length. Differences were more conspicuous in larger specimens (>300 mm), and more subtle in the smaller ones. Although the otoliths of the smaller specimens were more difficult to differentiate by morphometry, the larger concavity of the outer surface and the less pronounced lobulation of the dorsal anterior margin differentiated the otoliths of *M. atricauda* from those of *M. ancyldon*.

The sectioned otoliths of *M. ancyldon* did not show annual rings comparable to those of *M. atricauda* from southern Brazil. Annual band formation has been traditionally associated with seasonal environmental rhythms and with significant ontogenetic events, such as feeding and reproduction (Green et al., 2009). Both species have a defined seasonal reproductive cycle (Juras & Yamaguti, 1989; Militelli & Macchi, 2004; Camargo & Isaac, 2005), which, apparently, does not result in the formation of alternate annual bands on the *M. ancyldon* otoliths. However, annual temperature fluctuations are very different in the environments of both
species: about 10°C in the coastal waters of southern Brazil (Haimovici et al., 1997) and only about 2°C in northern Brazil (Camargo & Isaac, 2005). The lack of strong cyclic environmental differences appears to be the explanation for the absence of growth increments in the otoliths of *Macrodon*.

Differences in otolith shape strongly corroborated both genetic and morphological differentiation previously reported and are additional evidence that support the existence of two species of the genus *Macrodon*: *M. ancyldon* on the north and *M. atricauda* on the south of the Atlantic coast in South America.

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REFERENCES


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