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# Evaluation of periotic–timpanic bone complex of *Sotalia guianensis* (Cetacea: Delphinidae) as tool in identification of geographic variations

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*Morphometric characteristics of the periotic–timpanic bone complex in the middle ear of cetaceans, are effective characteristics in evaluating systematics. However, they have not been used for studies of geographic variation regarding dolphins of the genus* Sotalia. *This study aimed to compare the periotic–timpanic of* Sotalia guianensis *from four distinct locations, considered here as different operational taxonomic units, Amapá/Pará (AM/PA), Maranhão/Piauí (MA/PI), Ceará (CE), and Rio de Janeiro (RJ), using 21 morphometric measurements. Multivariate analysis showed significant distinction mainly between the units of northern (AM/PA and MA/PI) and south-eastern (RJ) Brazilian coast. The timpanic bone showed variation, reaching larger sizes in the Brazilian south coast unit, corroborating current molecular data on the geographic variation of* S. guianensis.

**Keywords:** *Sotalia guianensis*, periotic–timpanic, traditional morphometry

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## INTRODUCTION

The Atlantic coast of South America is included in the distribution area of several cetaceans, including resident species of dolphins such as *Sotalia guianensis* (Van Bénéden, 1864). This species occurs in tropical and subtropical waters from Honduras, in the Caribbean Sea, to Florianópolis, on the southern coast of Brazil (Flores & Da Silva, 2009), showing high site fidelity and coastal habits usually associated with estuary bays (Santos *et al.*, 2001; Flores & Bazzalo, 2004; Azevedo *et al.*, 2005; Rossi-Santos *et al.*, 2007; Nery *et al.*, 2008; Dias *et al.*, 2009). The other member of this genus, *S. fluviatilis*, also known as the tucuxi, is a riverine dolphin species completely adapted to fresh water and restricted to the Amazon region.

A recent study has shown that the morphological variation of *S. guianensis* follows a phylogeographic pattern (Cunha *et al.*, 2010) along the coast, and as geographic variation in morphology may result from distinct selective pressures in different environmental conditions (Gould & Johnston, 1972), it may be a great tool to define population substructure in this species. However, despite the recent taxonomic re-evaluation of the genus *Sotalia* (see Monteiro-Filho *et al.*, 2002; Cunha *et al.*, 2005; Caballero *et al.*, 2007) there are few studies focused on *S. guianensis* geographic variation, especially the morphometric aspects of the periotic–timpanic bone. Among previous studies analysing morphometric variation in *S. guianensis*, Ramos (2001) used morphometric skull variation, while other studies focused on interspecific comparisons between the two species of the genus (see Monteiro-Filho *et al.*, 2002; Fettuccia, 2006; Fettuccia *et al.*, 2012).

However, acoustic adaptations to the aquatic environment determined several morphological changes in the auditory apparatus of cetaceans. Both auditory capsules, known as the periotic–timpanic bone complex, are probably the most divergent structures of the skull of cetaceans (Mead & Fordyce, 2009) and are relevant in taxonomic studies of both Odontoceti (Kasuya, 1973) and Mysticeti (Geisler & Luo, 1996). In this study, we evaluated the use of traditional morphometrics of the periotic–timpanic bone to identify

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geographic variation in *S. guianensis* populations and improve the taxonomic resolution and biogeographical aspects (see Perrin, 1975).

## MATERIALS AND METHODS

We analysed 142 specimens of *Sotalia guianensis* from collections of the following institutions and research groups: Museu Paraense Emílio Goeldi (MPEG), Belém, Pará (N = 38); Instituto Ilha do Caju Ecodesenvolvimento e Pesquisa (PROCEMA/ICEP), Ilha do Caju, Maranhão (N = 10); Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (AQUASIS), Caucaia, Ceará (N = 28); Universidade Estadual do Norte Fluminense (UENF), Campos dos Goytacazes (N = 43), Rio de Janeiro; and Grupo de Estudos de Mamíferos Marinhos da Região dos Lagos (GEMM-Lagos; N = 19), Rio de Janeiro.

We grouped samples from different localities in four operational taxonomic units (OTUs; Vanzolini, 2002; Heyer, 2005) based on geographic proximity, and assuming ecological homogeneity (Peloso & Avila-Pires, 2010), in three Brazilian regions: north (Unit Amapá/Pará, AP/PA); north-east (Unit Maranhão/Piauí, MA/PI; and Ceará, CE); and south-east (Unit Rio de Janeiro, RJ) (Figure 1).

We measured 21 metric characteristics of each periotic–timpanic bone complex. Linear measures were taken, according to Kasuya (1973), with a 200 mm caliper (0.1 mm precision), except the measures 12, 14 and 21 that were first reported in this study (Figure 2; Tables 1 and 2). All

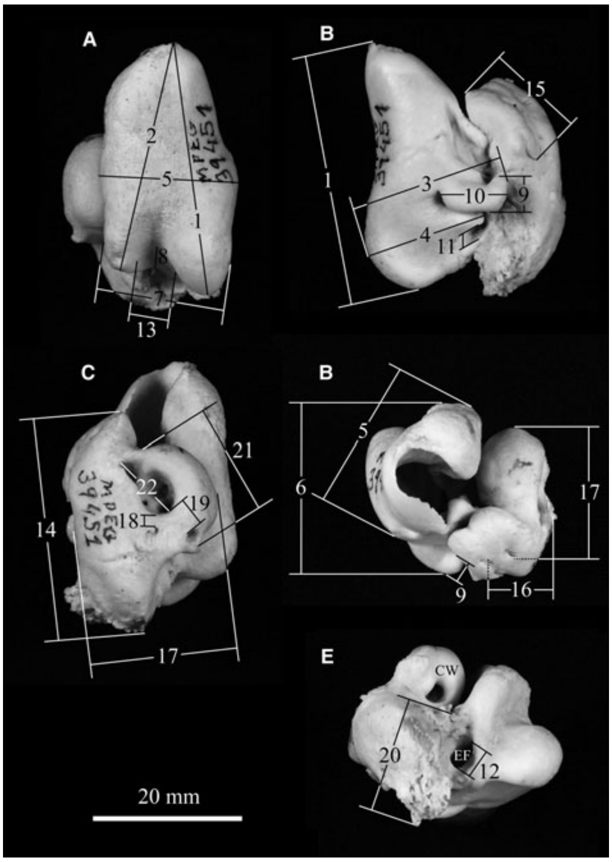


**Fig. 2.** Five views of left side periotic–timpanic of *Sotalia guianensis* (MPEG 39451) showing the measures used: A, ventral; B, lateral; C, dorsomedial; D, anterior; E, posterior. CW, cochlear window; EF, elliptical foramen). Photographs: D.L. Arcoverde.


**Fig. 1.** Location of the five operational taxonomic units (OTUs) on the Brazilian coast by region. North: unit Amapá/Para (AP/PA); north-east: units Maranhão/Piauí (MA/PI) and Ceará (CE); south-east: unit Rio de Janeiro (RJ). Map: D.L. Arcoverde.

measurements were taken by DLA to minimize the variation attributed to different collectors (Perrin *et al.*, 1994).

Assuming the absence of sexual dimorphism in *Sotalia* (Borobia, 1989; Monteiro-Filho *et al.*, 2002) as well as the absence of ontogenetic variation in the periotic–timpanic bone complex of Odontoceti (Kasuya, 1973), both sexes and different stages of development were grouped together in the analysis. According to Kasuya (1973) the periotic–timpanic bone complex had no significant asymmetry, which was later confirmed for *S. guianensis* (Parente *et al.*, 1999). Therefore, for this study we used the data from the right periotic–timpanic complex, and from the left complex when the right was not available. The definition of the periotic–timpanic bone complex followed Simões-Lopes (2006), while the osteological terminology and anatomic orientation followed Mead & Fordyce (2009).

We evaluated the morphometrics using a discriminant analyses function (DAF) for all the OTUs in order to identify the more powerful measurements in discriminating groups (Tabachnick & Fidell, 2001). The DAF analyses were conducted in STATISTICA® v.7.1 (StatSoft Inc., USA) (StatSoft, 2005) considering a level of significance of $P \leq 0.05$.

## RESULTS

Discriminant analysis of the periotic–timpanic bone complex showed significant geographic variation among OTUs (Wilks'

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**Table 1.** List of measurements of periotic–timpanic complex bone taken of *Sotalia guianensis* specimens from Brazil according to Kasuya (1973). The measures 12, 14 and 21 were first presented in this study.

| Number | Measure | Figure |
|---|---|---|
| 1 | Standard length of tympanic bone, distance from anterior tip to the posterior end of the outer posterior prominence | 1A/1B |
| 2 | Distance from the anterior tip to the posterior end of the inner posterior prominence | 1A |
| 3 | Distance from the postero-ventral tip of the outer posterior prominence to the tip of the sigmoid process | 1B |
| 4 | Distance from postero-ventral tip of outer posterior prominence to tip of conical process | 1B |
| 5 | Width of the tympanic bone at the level of the sigmoid process | 1A/1D |
| 6 | Height of tympanic bone, from tip of the sigmoid process to ventral keel | 1D |
| 7 | Width across the inner and outer posterior prominences | 1A |
| 8 | Greatest depth of interprominential notch | 1A |
| 9 | Width of upper border of sigmoid process | 1B/1D |
| 10 | Width of posterior branch of lower tympanic aperture | 1B |
| 11 | Presence of elliptical foramen. If present, its greatest diameter | 1E |
| 12 | Internal width between internal and external posterior prominence | 1A |
| 13 | Standard length of the periodic, from the tip of anterior process to the posterior end of posterior process, measured on a straight line parallel with cerebral border | 1C |
| 14 | Length of the parabullary ridge | 1B |
| 15 | Thickness of superior process at level of upper tympanic aperture | 1D |
| 16 | Width of periodic across cochlear portion and superior process, at level of upper tympanic aperture | 1C/1D |
| 17 | Least distance between the margins of fundus of internal auditory meatus and of ductus endolymphaticus (vestibular aqueduct) | 1C |
| 18 | Least distance between the margins of fundus of internal auditory meatus and of aquaeductus cochleae | 1C |
| 19 | Length of articular facet of the posterior process of the periodic to the posterior process of the tympanic bone | 1E |
| 20 | Antero-posterior diameter of cochlear portion | 1C |
| 21 | Diameter of the internal acoustic meatus, from the margin of endocranial opening of facial canal to margin of the area cribrosa media | 1C |

$\lambda$: 0.04292, F (48.295) = 11.578; $P < 0.01$). Root 1 showed 75% of the variation while root 2 showed 20% of the variation. The classification matrix showed 96.6% of correct classification, and the RJ unit had the best correct classification (98.3%) followed by the AP/PA and the CE units (both with 95.8%), while the MA/PI unit showed 87.5% of correct classification.

Root 1 completely discriminated the AP/PA unit from the RJ unit. The MA/PI unit greatly overlapped with the AP/PA and partially overlapped with the CE unit, but showed almost no overlap with the RJ unit. The CE unit overlapped with all other units in our analysis (Figure 3).

According to the DFA, eight measurements showed significant differences among the OTUs: 1, 2, 3, 5, 7, 13, 14 and 19 (see Tables 1 & 3). The tympanic bone concentrated the variation identified and showed a pattern of growth from north to south along the geographical distribution on the Brazilian coast. The smallest specimens belonged to the AP/PA unit, on the north coast, and the largest specimens were found in the RJ unit, on the south-east coast.

## DISCUSSION

Previous morphology studies focused on the comparison between the two currently known species of the genus *Sotalia* (Casinos *et al.*, 1981; Borobia, 1989; Silva & Best, 1996; Ramos, 2001; Monteiro-Filho *et al.*, 2002, Fettuccia, 2006; Fettuccia *et al.*, 2012). Few have analysed variation within *S. guianensis* or its riverine ecotype *S. fluviatilis*. According to Borobia (1989) and Monteiro-Filho *et al.* (2002), the skull of *S. guianensis* showed no morphometric variation, although the former author expected to find a growth pattern from north to south along the Brazilian coast. Casinos *et al.* (1981) suggested that marine populations would show the variability found by Ramos (2001), who demonstrated a decrease in skull length with the increase of latitude, comparing specimens only from the south-eastern coast of Brazil.

The growth of the tympanic bone in this study follows the growth of the skull in higher latitudes, as previously shown by molecular genetic markers (Cunha *et al.*, 2005; Cunha *et al.*, 2010). This variation may be attributed to Bergmann's rule. Data from more comprehensive studies corroborate the expectations of Borobia (1989); that is, on average the skull of *Sotalia guianensis* populations increases with increasing latitudes. Casinos *et al.* (1981), in the Macaibo Lake, Venezuela, recorded an average length of cranium of 335.5 mm, while Fettuccia (2006), along the Brazilian coast, observed three different areas on the coast, and found cranium lengths of 351 mm in Amapá (northern region), 392 mm in Ceará (north-eastern region) and 387.5 mm in Santa Catarina (southern region). This is in disagreement with Ramos (2001), probably because of the low sampling of some geographic areas, (see Parente *et al.*, 1999) or differences in classification criteria of adults used by each author (Borobia, 1989; Fettuccia, 2006).

The growth pattern shown by the tympanic bone in this study follows the growth of the skull with increasing latitudes, which may be related to water temperature, a distribution pattern known as Bergmann's rule; that is, a homoeothermic animal that lives in cold waters is larger than one of the same species that lives in warmer waters (see Rensch, 1938). In fact, Schnell *et al.* (1986), studying patterns of geographic variations of *Stenella attenuata,* found strong correlations between skull measurements and environmental variables.

Cunha *et al.* (2010) suggested the existence of at least six distinct populations of *Sotalia guianensis* along the Brazilian coast using mtDNA analyses: Pará, Ceará, Rio Grande do

**Table 2.** Descriptive statistic of periotic–timpanic bone complex of *Sotalia guianensis* from north: Ampá/Pará (AP/PA), north-east: Maranhão/Piauí (MA/PI) and Ceará (CE), south-east regions of Brazil: Rio de Janeiro (RJ). N, total number; X, average; Min, minimum; Max, maximum; SD, standard deviation; CV, coefficient of variation in %. Measurements are in millimetres.

| Measurements | AP/PA (N = 38) | | | | | MA/PI (N = 10) | | | | | CE (N = 28) | | | | | RJ (N = 66) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X | Min | Max | SD | CV | X | Min | Max | SD | CV | X | Min | Max | SD | CV | X | Min | Max | SD | CV |
| 1 | 32.64 | 30.63 | 34.13 | 0.91 | 2.78 | 33.36 | 31.86 | 34.76 | 0.93 | 2.78 | 34.03 | 32.66 | 36.14 | 1.04 | 3.06 | 34.90 | 33.84 | 36.04 | 0.54 | 1.54 |
| 2 | 30.18 | 28.71 | 31.75 | 0.95 | 3.14 | 31.15 | 30.12 | 32.54 | 0.77 | 2.47 | 32.01 | 30.19 | 33.79 | 1.07 | 3.33 | 32.40 | 30.88 | 33.99 | 0.71 | 2.20 |
| 3 | 21.72 | 20.92 | 22.29 | 0.40 | 1.82 | 22.33 | 21.86 | 22.67 | 0.26 | 1.17 | 22.79 | 21.47 | 24.20 | 0.58 | 2.56 | 23.64 | 22.53 | 24.95 | 0.47 | 2.01 |
| 4 | 16.20 | 15.68 | 16.86 | 0.34 | 2.12 | 17.02 | 16.12 | 17.55 | 0.46 | 2.70 | 17.02 | 15.99 | 18.17 | 0.56 | 3.30 | 17.51 | 16.58 | 18.34 | 0.39 | 2.24 |
| 5 | 18.39 | 17.14 | 19.45 | 0.65 | 3.52 | 18.76 | 18.16 | 19.16 | 0.35 | 1.86 | 17.63 | 16.57 | 19.47 | 0.62 | 3.53 | 18.63 | 17.03 | 20.24 | 0.75 | 4.01 |
| 6 | 22.35 | 21.27 | 23.22 | 0.52 | 2.35 | 22.70 | 21.77 | 23.85 | 0.66 | 2.93 | 23.20 | 22.22 | 25.44 | 0.73 | 3.14 | 23.82 | 22.57 | 25.67 | 0.54 | 2.27 |
| 7 | 16.22 | 15.19 | 17.53 | 0.60 | 3.72 | 16.69 | 15.33 | 17.76 | 0.84 | 5.05 | 17.47 | 16.61 | 19.17 | 0.60 | 3.46 | 17.71 | 16.59 | 19.18 | 0.49 | 2.79 |
| 8 | 3.35 | 2.81 | 3.77 | 0.27 | 7.95 | 3.45 | 3.10 | 3.76 | 0.24 | 7.04 | 3.70 | 2.97 | 4.32 | 0.29 | 7.79 | 3.48 | 2.80 | 4.30 | 0.30 | 8.65 |
| 9 | 4.87 | 4.31 | 5.53 | 0.26 | 5.32 | 4.98 | 4.50 | 5.66 | 0.41 | 8.17 | 5.09 | 4.50 | 5.50 | 0.27 | 5.27 | 5.31 | 4.76 | 5.98 | 0.30 | 5.69 |
| 10 | 1.35 | 0.80 | 1.93 | 0.21 | 15.49 | 1.52 | 1.15 | 1.74 | 0.21 | 13.86 | 1.66 | 1.19 | 2.06 | 0.23 | 13.70 | 1.71 | 1.17 | 2.51 | 0.25 | 14.71 |
| 11 | 3.81 | 2.22 | 4.67 | 0.55 | 14.37 | 3.88 | 3.16 | 4.29 | 0.35 | 8.92 | 3.68 | 1.59 | 4.92 | 0.88 | 23.95 | 4.44 | 2.89 | 5.61 | 0.63 | 14.10 |
| 12 | 4.85 | 4.24 | 5.52 | 0.33 | 6.77 | 5.29 | 4.66 | 5.94 | 0.44 | 8.26 | 5.16 | 4.08 | 5.74 | 0.39 | 7.63 | 5.74 | 4.59 | 6.68 | 0.43 | 7.44 |
| 13 | 29.08 | 26.90 | 30.29 | 0.85 | 2.92 | 28.48 | 27.41 | 30.31 | 0.92 | 3.25 | 30.55 | 27.95 | 33.99 | 1.52 | 4.96 | 31.04 | 29.05 | 34.56 | 1.03 | 3.31 |
| 14 | 13.54 | 12.39 | 14.39 | 0.52 | 3.84 | 13.95 | 13.38 | 14.76 | 0.45 | 3.26 | 14.15 | 12.28 | 15.27 | 0.83 | 5.89 | 14.16 | 12.17 | 16.05 | 0.75 | 5.30 |
| 15 | 10.73 | 9.43 | 12.50 | 0.75 | 7.03 | 10.57 | 9.55 | 11.11 | 0.48 | 4.50 | 11.28 | 10.11 | 12.26 | 0.67 | 5.97 | 11.88 | 10.18 | 13.89 | 0.71 | 5.97 |
| 16 | 19.14 | 18.15 | 20.43 | 0.65 | 3.41 | 19.28 | 18.43 | 20.12 | 0.57 | 2.95 | 19.70 | 18.08 | 20.94 | 0.71 | 3.62 | 20.20 | 19.32 | 21.26 | 0.50 | 2.48 |
| 17 | 1.57 | 1.07 | 2.20 | 0.27 | 17.14 | 1.46 | 1.25 | 2.01 | 0.24 | 16.31 | 1.75 | 1.31 | 2.33 | 0.31 | 17.89 | 2.02 | 1.31 | 2.89 | 0.29 | 14.46 |
| 18 | 1.60 | 1.07 | 2.06 | 0.28 | 17.24 | 1.59 | 1.05 | 2.32 | 0.42 | 26.32 | 1.80 | 1.19 | 2.34 | 0.27 | 14.88 | 2.12 | 1.46 | 2.89 | 0.31 | 14.65 |
| 19 | 14.65 | 12.82 | 16.35 | 0.78 | 5.32 | 14.44 | 13.40 | 15.00 | 0.51 | 3.56 | 15.47 | 13.87 | 17.63 | 0.90 | 5.79 | 16.02 | 13.13 | 18.42 | 1.00 | 6.26 |
| 20 | 13.86 | 12.99 | 14.90 | 0.51 | 3.69 | 14.15 | 12.78 | 14.62 | 0.61 | 4.34 | 14.07 | 13.43 | 14.93 | 0.44 | 3.10 | 14.81 | 12.57 | 16.47 | 0.54 | 3.62 |
| 21 | 10.92 | 9.40 | 12.70 | 0.60 | 5.47 | 11.45 | 9.80 | 12.11 | 0.71 | 6.24 | 11.16 | 10.01 | 12.57 | 0.65 | 5.85 | 11.22 | 10.06 | 13.12 | 0.60 | 5.36 |

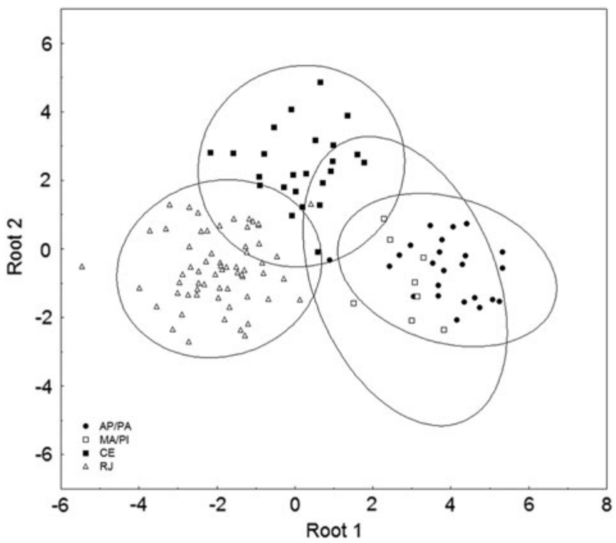


**Fig. 3.** Projection of root 1 and root 2 of canonical analyses based on periotic–timpanic complex bone of *Sotalia guianensis* in four OTUs analysed: (Ampá/Pará (AP/PA), Maranhão/Piauí (MA/PI), Ceará (CE) and Rio de Janeiro (RJ)). Ellipses confidence: 95%.

Norte, Bahia, Espírito Santo and a southern/south-eastern area, from Rio de Janeiro to Santa Catarina. Their classification partially matches the three units suggested here: AP/PA = Pará, CE = Ceará and RJ = Rio de Janeiro. The differentiation between these OTUs suggests possible restrictions to gene flow, corroborating the hypothesis that *S. guianensis* had a series of allopatric expansions southwards along the Brazilian coast, where the distance acted as a geographic barrier restricting the gene flow between subsequent populations and favouring the emergence of distinct mtDNA haplotypes (Cunha *et al.*, 2005; Cunha *et al.*, 2010). According to Möller *et al.* (2007), some physical characteristics of coastal areas (bays and estuaries), as well as site fidelity patterns and behaviour specializations, may cause genetic differences among dolphin populations.

**Table 3.** Coefficients of canonical analyses of 21 morphometric measurements of periotic–timpanic bone complex of *Sotalia guianensis*. Values in bold indicates the measures which best demonstrated the differences between OTUs (Measurements 1, 2, 3, 5, 7, 13, 14, 19).

| Measure | Root 1 | Root 2 |
|---|---|---|
| 3 | −0.783 | 0.063 |
| 5 | 0.437 | −0.939 |
| 7 | −0.229 | 0.526 |
| 19 | −0.462 | −0.184 |
| 13 | −0.192 | −0.431 |
| 2 | −0.151 | 0.697 |
| 4 | 0.203 | −0.118 |
| 14 | 0.064 | 0.350 |
| 1 | −0.347 | −0.414 |
| 12 | 0.160 | −0.241 |
| 8 | 0.293 | 0.135 |
| 15 | 0.175 | 0.224 |
| 16 | −0.106 | −0.239 |
| 18 | −0.261 | −0.070 |
| 11 | −0.241 | 0.054 |
| 21 | 0.120 | −0.258 |

The variation in the characters (see coefficients of variation, Table 2) increased from the AP/PA unit to the CE unit, after which it decreased substantially up to the RJ unit. Such phenotypic variation was also observed in molecular data (Cunha *et al.*, 2005; Cunha *et al.*, 2010), which indicated the populations in northern and north-eastern regions to be more variable, both genetically and morphologically, unlike the more homogenous south-eastern and southern populations. Ramos (2001) explained that pattern as the result of gene flow between those populations, although Cunha *et al.* (2010) had a different explanation, assuming it was caused by a 'founder effect'. In our study it was not possible to access specimens of *S. guianensis* from the southern region to evaluate their variability in the periotic–timpanic bone complex to reveal whether there was a similar pattern.

The traditional morphometrics of the periotic–timpanic bone complex was revealed to be an efficient tool to identify geographic variations of *S. guianensis*. The variation found between the OTUs corroborated previous studies in the literature, involving skull morphometrics and molecular data, confirming the existence of distinct population stocks in the species distribution along the Brazilian coast. Future research might analyse samples from the Brazilian southern region, and from other localities of the Atlantic coast of Central and South America to broaden the understanding of the species stocks in those areas.

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