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CERVICAL RIBS AND CLEFT ARCHES IN BRAZILIAN *SOTALIA GUIANENSIS*: CONTRIBUTION TO COMPARATIVE STUDIES OF SKELETAL MORPHOLOGY IN NORTHERN AND SOUTHEASTERN SPECIMENS

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ABSTRACT: The Guiana dolphin, *Sotalia guianensis* (Van Bénédén, 1864), is a delphinid species found along the Brazilian coast. Morphological and genetic data point to the existence of different biological populations of the species within this portion of the range. The present paper compares the frequencies of developmental anomalies in two skeletal collections from geographically distant areas. The vertebral column of 43 specimens from Marajó Bay, located on the Pará State coast, and 20 specimens from the central-north coast of the Rio de Janeiro State were analyzed. The only anomalies found were cervical ribs and cleft neural arches, both located on the seventh cervical (C7) vertebra. Cervical ribs were found in 12% of the Pará specimens and in 45% of the Rio de Janeiro specimens; according to the Fisher's Exact Test the difference was significant at a confidence level of $\alpha = 0.01$, thus confirming that the series belong to different populations. The frequencies of cervical ribs observed in the present study were also compared to the frequencies of the same anomaly found in *S. guianensis* from the Ceará and Santa Catarina states, Brazil, and in tucuxi (*S. fluviatilis*) from the Brazilian Amazon. Although the results can be partially explained by demographic and evolutionary models for *Sotalia*, they may be affected by differences in the composition of the collections and in diagnostic methods/criteria used by different authors. Regarding the presence of cervical ribs, Amazon tucuxi were significantly different from the Rio de Janeiro and Pará series, as well as other marine series, as previously stated in the literature. The difference in the frequency of cleft neural arches in specimens from Rio de Janeiro and Pará was also significant, but no comparison could be conducted with other series due to lack of published material. A metric technique to discriminate transverse processes with and without fused cervical ribs is proposed as a methodological contribution for diagnosing the described anomaly.

RESUMO: O 'boto-cinza' *Sotalia guianensis* (Van Bénédén, 1864) é uma espécie de Delphinidae encontrada em diferentes latitudes ao longo da costa do Brasil. Os dados da morfologia e da genética apontam para a existência de diferentes populações da mesma espécie. O presente trabalho contribui para o conhecimento da espécie através da comparação das frequências de anomalias de desenvolvimento em duas séries de esqueletos obtidos em áreas geograficamente distintas. Foram analisadas as colunas vertebrais de 43 espécimes da baía de Marajó, costa do Pará, e de 20 espécimes da costa centro-norte do Rio de Janeiro, e as únicas anomalias observadas foram costela cervical e não fechamento do arco neural, ambos na sétima vértebra cervical (C7). As costelas cervicais ocorreram em 12% dos exemplares do Pará e em 45% dos exemplares do Rio de Janeiro, de acordo com o Teste Exato de Fisher a diferença foi significativa ($\alpha = 0.01$, bilateral), confirmando que as séries examinadas pertencem a diferentes populações. As frequências de costelas cervicais também foram comparadas para *S. guianensis* dos estados do Ceará e Santa Catarina, e para o tucuxi (*S. fluviatilis*) da Amazônia brasileira. Os resultados são parcialmente explicados pelos modelos demográficos e evolutivos para *Sotalia*, mas podem estar sendo influenciados pela composição das séries e pelas diferenças em metodologia e critérios diagnósticos usados pelos diferentes autores. No que se refere à costela cervical, a série amazônica mostrou-se diferente das séries do Rio de Janeiro e do Pará, da mesma forma que das demais séries marinhas, tal como tem sido publicado. As frequências para o não fechamento do arco neural também foram significativamente diferentes entre Rio de Janeiro e Pará, mas não foi possível a comparação com outras séries por falta de material publicado. Uma técnica métrica para comparar os processos transversos com e sem costela cervical fusionada foi proposta como contribuição metodológica ao diagnóstico da anomalia descrita.

KEYWORDS: *Sotalia guianensis*, cervical rib, cleft neural arch, congenital anomalies, Brazil.

Introduction

The Guiana dolphin, *Sotalia guianensis* (Van Bénédén, 1864) is the best-known delphinid along the Brazilian coast. This species is distributed along the western South Atlantic and Caribbean coast, limited to the north at Nicarágua, Central America (Edwards and Schnell, 2001), and to the south at Florianópolis, Santa Catarina State, Brazil, South America (Simões-Lopes, 1988). The first scientific reports regarding the species were published during the 19th century, but their behavior and biological variability are still under investigation. They feed in shallow estuarine waters, in close contact with environments suffering high anthropogenic impacts,

making them one of the most threatened species along the Brazilian coast. *S. guianensis* is best documented from the south and southeastern coast of Brazil; detailed reports at the north coast are relatively recent (Ramos, 2001; Santos *et al.*, 2002; Siciliano *et al.*, 2007; Siciliano *et al.*, 2008; Caballero *et al.*, 2010 this volume).

Smaller dolphins of the same genus are adapted to freshwater habitats and can be found along the Amazon River. These are the tucuxi, who play an important role in the mythology of the people living along the river basins of the Solimões, Amazonas and Orinoco Rivers (da Silva, 1990). The marine and river *Sotalia*, once considered a single species, have differences including the size and shape of skeletal

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parts (Rice, 1977; da Silva and Best, 1994; Monteiro-Filho *et al.*, 2002). When the haplotypes could finally be studied in detail the two species were separated. Comparing the haplotypes for the cytochrome *b*, and others, for different regions of the Brazilian coast, Cunha *et al.* (2005) confirmed stronger similarities between the marine *Sotalia* when compared to the Amazon basin *Sotalia*. Caballero *et al.* (2007) also confirmed the dissimilarities between river and marine animals, as well as the existence of *S. fluviatilis* and *S. guianensis*, presumed to be sympatric Amazon River estuary (Marajó Bay). Additionally, the haplotypes reported from the Amazon estuary suggest that *S. guianensis* is the prevalent species there (Sholl, 2010).

Other genetic researches points to a less marked differentiation between the coastal *Sotalia* (Cunha *et al.*, 2005; Caballero *et al.*, 2007; Caballero *et al.*, 2010 this volume), suggesting three sets of populations, respectively south-southeastern, northeastern and northern, consistent to the present models of holocenic expansion of the species from the north to the south (Castilho and Simões-Lopes, 2008). A unique haplotype along the south-southeastern coast of Brazil strongly suggests a founder effect following demographic expansion (Caballero *et al.*, 2007, Caballero *et al.*, 2010 this volume). *Sotalia* skeletal morphology, pathology and epigenetic studies, especially the *Sotalia* spinal anomalies, have been described (Ramos, 2001; Monteiro-Filho *et al.*, 2002; Van Bresseem *et al.*, 2007; Simões-Lopes *et al.*, 2008; Fettuccia *et al.*, 2009; Laeta *et al.*, 2010).

Developmental anomalies in *Sotalia*, occur during onthogenesis, similar to human's and other mammals (Ortner and Putschar, 1985; Williams *et al.*, 1989; Barnes, 1994; Hall, 2005). Embryos can be affected by chemical, physical and biological factors that may cause change, delay or interruption of normal development; and cases of agenesis, fusion and hypoplasia of bones may also occur for genetic determination (Barnes, 1994). Otherwise, the study of developmental minor defects in cetacean skeletons incipient and little information is available. The present study aims to be a contribution to this field.

The vertebral column and associated structures are formed from three embryonic developmental fields: the notochord, neural tube and paraxial mesoderm (Almeida, 1999). Most of the vertebral anomalies occur during or after the neurulation phase in those fields (Williams *et al.*, 1989; Barnes, 1994). The notochord is the primordial tissue that induces backbone development; problems on this structure will basically affect the vertebral bodies (Hall, 2005). The neural tube forms the central nervous system; its anomalies are generally severe, affecting neural structures and adjacent bones, causing spina bifida among other problems. The paraxial mesoderm is the precursor of the skeletal organs; the mesoderm along the notochord axis will form the vertebrae, ribs and skull base; a long list of relatively severe anomalies can be associated to this field (Barnes, 1994). Cervical ribs and cleft neural arches are

two different anomalies associated to the paraxial mesoderm and have been described in mammals of different species, including humans (Hall, 2005).

The embryonic somites form the vertebrae. Along the thoracic somites are bilateral mesoderm projections that form the transverse processes, with the potential to also form separate ossification centers for ribs (Hall, 2005). Cervical ribs occur when the segmentation of the somites is dislocated to the cervical border, increasing the potential for rib formation at a cervical vertebra (Williams *et al.*, 1989; Barnes, 1994). The cervical ribs may be unilateral or bilateral, being rudimentary extensions of the anterior part of the transverse processes. Occasionally there may be a slight decrease in the middle of the process diameter, pointing to the transition between the process and the fused rib. Literature reports cervical ribs in different vertebrae (Schmorl and Junghanns, 1971), but C7 shows a stronger tendency in the presentation of this anomaly, due to the separate ossification center for its transverse process. As a genetically defined characteristic, cervical ribs occur in different frequencies in different populations.

Cervical ribs and other vertebral anomalies have been described in Cetacea (Cowan, 1966; De Smet, 1977; Rommel, 1990), and *S. fluviatilis* from the Amazon River (Fettuccia, 2006). In a more recent study, Fettuccia *et al.* (2009) compared *S. fluviatilis* and *S. guianensis* from Brazil and observed a high frequency of cervical ribs (87%, N = 31), most of them bilateral, in skeletons of *S. fluviatilis*, in contrast to low frequencies of the same anomaly in *S. guianensis* from Santa Catarina, in southern Brazil (19%, N = 32) and from Ceará, in northeastern Brazil (9%, N = 23). Most of the cervical ribs were present at C7, except for one observed at C3. Other important reports about *Sotalia* bone anomalies are present in the Brazilian literature (Fragoso, 2001; 2006).

At the mammal embryo, the fusion of the neural arches may be delayed resulting in cleft arches (Almeida, 1999; Hall, 2005). Different from spina bifida occulta, the cleft arch - to what this paper is concerning - causes no widening of the neural channel (Barnes, 1994). Among humans, the cleft arch is the most frequent vertebral anomaly (Saluja, 1988; Barnes, 1994). Deficiency of the vitamin B complex, especially folic acid (B9), is its main cause (Minns, 1996; Fleming and Copp, 1998; Jablonsky and Chaplin, 2000). Captive animals (Reiderson, 2003) can present this deficiency; on the other side, in the natural environment, ultraviolet radiation causing photodecomposition of folic acid and derivates (Jablonsky and Chaplin, 2000; Licht and Grant, 1997) may change the availability of these nutrients in river and marine environments.

Contrary to the cervical rib, the cleft neural arch has not been usually described for wild species, especially in cetaceans (Cowan, 1966; Reiderson, 2003). In Brazil, Fragoso (2001) describes only one case at the first thoracic vertebra in a skeleton of *S. guianensis* from Rio de Janeiro (Laeta, 2007; Van Bresseem, *et al.*, 2007; Laeta *et al.*, 2010).

The goal of the present paper is to increase the knowledge regarding *Sotalia guianensis* skeletal morphology, describing vertebral anomalies in two geographically distinct populations from Brazil. Comparisons of the frequencies of these anomalies between a Rio de Janeiro State collection and Pará State collection will also contribute to the comprehension of differences and similarities between the northern and eastern populations of dolphins living along the Brazilian coast.

Material and Methods

Specimens were collected along the north-central coast of the Rio de Janeiro State, between Saquarema (22°55'S 42°30'W) and Quissamã (22°06'S 41°28'W); and at the Marajó Bay, between the eastern coast of the Marajó Island (Soure, Salvaterra) (00°40'S 48°30'W) and the northeastern coast of mainland Pará (Curuçá, Marapanim, Salinópolis, Algodual, Bragança, Maracanã) (00°34'S 47°30'W; Figure 1). Considering 50 vertebrae as the average number characteristic for the species (Carvalho, 1963; da Silva and Best, 1996; Fettuccia and Simões-Lopes, 2004), only

skeletons with 60% or more of the vertebrae and intact cervical segments were selected for this study.

Rio de Janeiro specimens included 20 skeletons collected, treated and stored by the group that studies marine mammals from the Região dos Lagos area (Grupo de Estudo de Mamíferos Marinheiros da Região dos Lagos, GEMM-Lagos). All specimens originated from beach monitoring of stranded animals and drifted carcasses, collected from August 2001 to February 2006 (Table 1), when ocean currents flow in a southwest direction (Bassani *et al.*, 1999; Yoneda, 1999²). The collections of 85% of these specimens inside the coastal range between Barra de São João and Barra do Furado is consistent with the direction of the ocean currents favoring the drift to those shores. These carcasses were supposed to belong to the *Sotalia* population living along the São João River estuary (Siciliano *et al.*, 2006). The skeletal maturity of the animals (*sensu* Perrin, 1975) confirmed that 15 (75%) of the specimens were immature, and the other five (25%), mature.

Pará specimens were composed of 43 skeletons belonging to the Museu Paraense Emílio Goeldi (MPEG) collection.

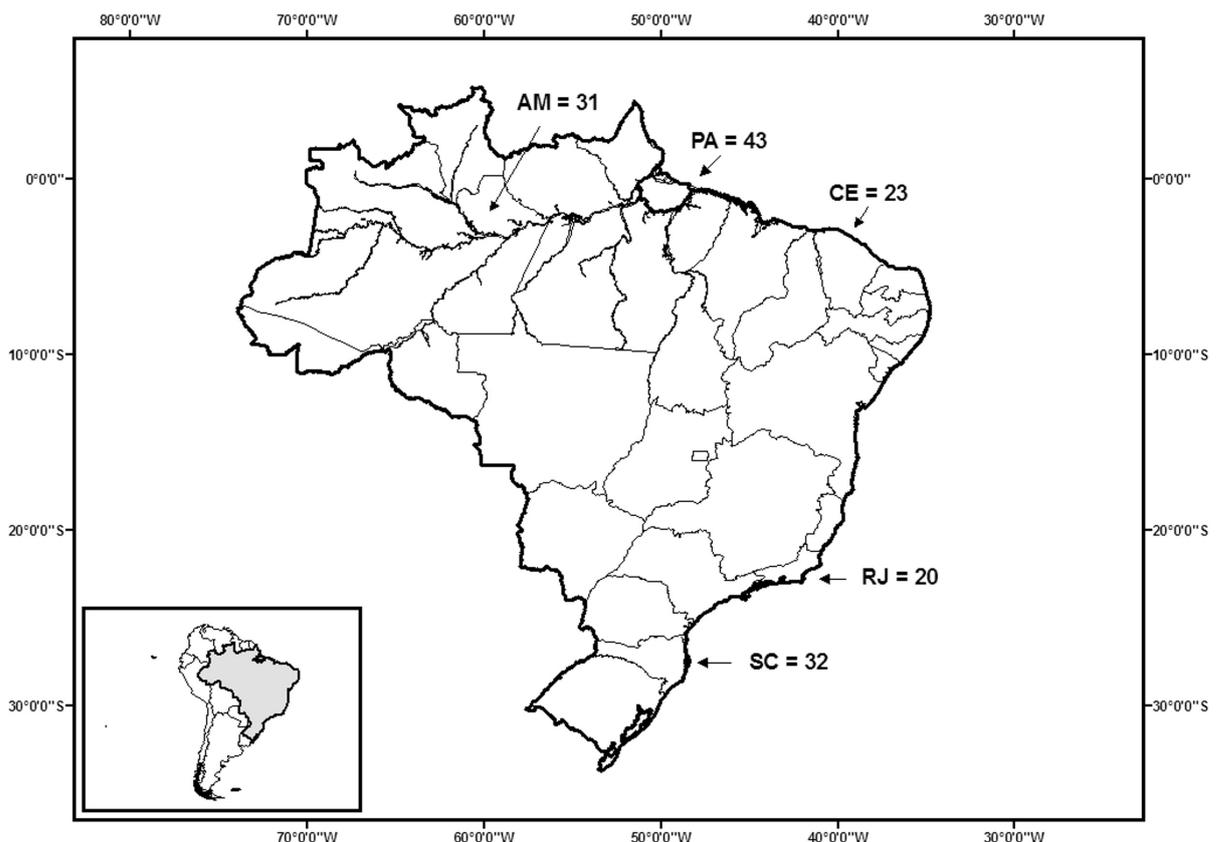


Figure 1. Map of Brazil indicating the approximate geographical origin of the *Sotalia* specimens from Pará (PA) and Rio de Janeiro (RJ) analyzed in the present paper, and from Amazonas (AM), Ceará (CE) and Santa Catarina (SC) analyzed by Fettuccia *et al.* (2009).

² Yoneda, N.T. (1999) Área temática: plâncton. 53 pp. [Available from <http://www.anp.gov.br/brnd/round6/guias/PERFURACAO/PERFURACAO_R6/refere/pl%E2ncton.pdf>].

Most of the skeletons (84%) were collected at different beaches of the Marajó Bay by the team that studies aquatic mammals of the Amazon (Grupo de Estudos de Mamíferos Aquáticos da Amazônia, GEMAM), during beach monitoring between December 2005 and July 2008 (Table 2). The timing of carcass findings was consistent with Costa *et al.* (2007)³, who found they predominantly occur in last months of the year. The remaining (16%) skeletons belonged to a former collection of the same museum. The skeletons were all labeled as *S. guianensis*: 27 (63%) of them originated from Soure and Salvaterra, at Marajó Island and the other 13 (30%) from Curuçá, Bragança, Marapanim, Algodual, Maracanã and Salinópolis, at Pará mainland. Only three skeletons (7%) had no precise information of origin. The skeletal maturity was estimated as follows: 21 (49%) immature individuals, 19 (44%) mature individuals, and three (7%) that could not be aged.

Neither collection could not be sexed because most of the carcasses were badly decomposed or skeletonized. Weathering, tunnels and erosions caused by bioactivity, marks of human manipulation and others changes were described before the pathological

analysis was performed (Mello, 1999). Also, neither collection can be considered random samples since they are biased by differential mortality risks, preservation chances, stranding, and drifting, among others. The authors assume that they do not represent the age structure of the living populations. On the other hand, most of the anomalies are not related to differential mortality, and are expected to be represented in these groups with no special bias. The skeletal collections are equally comparable to others obtained in similar conditions (Souza *et al.*, 2003), and, in some cases, may be a unique source of data regarding the species.

The methods chosen for this investigation were the visual examination and description of the bones, followed by metric analysis. The comparative method based on the species morphology (Carvalho, 1963; da Silva and Best 1994; Fettuccia and Simões-Lopes 2004) was used to identify bone anomalies. The diagnosis was based on similar human developmental anomalies (Barnes, 1994), supported by the assumption of ontogenetic similarities among mammals (Black and Scheuer, 1997; Almeida, 1999; Pough *et al.*, 2008).

Table 1. Specimens of *Sotalia guianensis* from GEMM-Lagos, Rio de Janeiro State, Brazil used in the present study.

SPECIMEN	COLLECTION DATE	LOCALITY	MATURITY
GEMM 023	August 30, 2001	Fazenda São Lázaro, Macaé	Immature
GEMM 031	July 11, 2002	Praia Seca, Araruama	Immature
GEMM 033	August 10 2002	Praia de João Francisco, Quissamã	Immature
GEMM 039	November 09, 2002	Praião, Barra de São João	Mature
GEMM 041	November 23, 2002	Praia do Abricó, Rio das Ostras	Immature
GEMM 047	October 18, 2003	Macaé	Immature
GEMM 048	November 05, 2003	Barra do Furado, Quissamã	Immature
GEMM 049	November 05, 2003	Barra do Furado, Quissamã	Immature
GEMM 065	September 02, 2004	Praia da Enseada, Rio das Ostras	Mature
GEMM 068	October 23, 2004	Barra de São João	Mature
GEMM 070	October 31, 2004	Quissamã	Immature
GEMM 071	October 31, 2004	Quissamã	Mature
GEMM 073	November 18, 2004	Praia Rasa, Búzios	Mature
GEMM 076	December 11, 2004	Praia de Flexeiras, Quissamã	Immature
GEMM 082	August 02, 2005	Praia de Flexeiras, Quissamã	Immature
GEMM 083	August 02, 2005	Praia de Flexeiras, Quissamã	Immature
GEMM 084	August 02, 2005	Praia de Flexeiras, Quissamã	Immature
GEMM 087	September 03, 2005	Figueira, Arraial do Cabo	Immature
GEMM 089	September 24, 2005	Praião, Barra de São João	Immature
GEMM 096	February 13, 2006	Praia de João Francisco, Quissamã	Immature

³ Costa, A.F., Emin-Lima, N.R., Rodrigues, A.L.F., Sousa, M.E.M. and Siciliano, S. (2007) Encalhes do boto-cinza *Sotalia guianensis* em praias da costa leste da ilha de Marajó, Pará. Pages 212-216 in Abstracts, VIII Workshop ECOLAB: Ecossistemas Costeiros Amazônicos, 06-12 August, Macapá, Brasil.

Table 2. Specimens of *Sotalia guianensis* from GEMAM and MPEG, Pará State, Brazil used in the present study.

SPECIMEN	COLLECTION DATE	LOCALITY	MATURITY
MPEG 10945	December 9, 1982	Barrado da Tiririca, Coroa Seca, Marajó island	Immature
MPEG 24548	December 29, 1995	Praia Grande, Salvaterra, Marajó island	Immature
MPEG 37816	2005	Praia do Pesqueiro, Soure, Marajó island	Immature
MPEG 37825	-	Bacuriteua, Bragança	Mature
MPEG 37826	February 18, 2004	Bacuriteua, Bragança	Immature
MPEG 37827	February 18, 2004	Bacuriteua, Bragança	Mature
MPEG 38430	April 2005	Baía de Marapanim	Immature
MPEG 38433	September 23, 2006	Praia de Joanes, Salvaterra, Marajó island	Mature
MPEG 38436	September 25, 2006	Praia Grande, Salvaterra, Marajó island	Mature
MPEG 38440	November 08, 2006	Praia do Pesqueiro, Soure, Marajó island	-
MPEG 38441	November 24, 2006	Praia do Araruna, Soure, Marajó island	Mature
MPEG 38447	November 25, 2006	Vila do Pesqueiro, Soure, Marajó island	Mature
MPEG 38448	November 25, 2006	Igarapé do Tatu, Praia do Pesqueiro, Soure, Marajó island	Immature
MPEG 38449	November 25, 2006	Vila do Pesqueiro, Soure, Marajó island	Mature
MPEG 38455	November 26, 2005	Caju-una, Soure, Marajó island	Mature
MPEG 38456	November 26, 2005	Caju-una, Soure, Marajó island	Immature
MPEG 38460	November 27, 2006	Praia Grande, Salvaterra, Marajó island	Mature
MPEG 38461	November 27, 2006	Praia Grande, Salvaterra, Marajó island	Mature
MPEG 38469	February 9, 2007	Vila do Pesqueiro, Soure, Marajó island	Immature
MPEG 38478	February 11, 2007	Praia do Turé, Soure, Marajó island	Mature
MPEG 38479	February 12, 2007	Praia de Joanes, Salvaterra, Marajó island	Immature
MPEG 38735	May 20, 2007	Praia do Mupéua, Fortalezinha, Maracanã	Mature
MPEG 38739	May 20, 2007	Praia do Mupéua, Fortalezinha, Maracanã	Immature
MPEG 38740	May 20, 2007	Praia do Mupéua, Fortalezinha, Maracanã	-
MPEG 38744	May 30, 2007	Fazenda Redenção, Monsarás, Marajó island	Immature
MPEG 38754	August 25, 2007	Caju-una, Soure, Marajó island	Immature
MPEG 38762	August 28, 2007	Vila do Pesqueiro, Soure, Marajó island	Immature
MPEG 38766	September 14, 2007	Praia do Marco, Maracanã, PA	Mature
MPEG 38768	September 18, 2007	-	Mature
MPEG 38771E	May 11, 2007	Praia do Crispim, Marapanim, PA	Immature
MPEG 39443	December 16, 2007	Praia do Pesqueiro, Soure, Marajó island, PA	Mature
MPEG 39448	December 16, 2007	Praia do Pesqueiro, Soure, Marajó island, PA	Mature
MPEG 39449	December 16, 2007	Praia do Pesqueiro, Soure, Marajó island, PA	Immature
MPEG 39450	December 16, 2007	Praia do Pesqueiro, Soure, Marajó island, PA	Mature
MPEG 39457	December 17, 2007	Praia do Céu, Soure, Marajó island, PA	Mature
MPEG 39458	December 17, 2007	Praia do Céu, Soure, Marajó island, PA	-
MPEG 39545	February 24, 2008	Praia do Porto, Joanes, Salvaterra, Marajó island, PA	Immature
MPEG 39548	March 8, 2008	Praia do Atalaia, Salinópolis, PA	Immature
MPEG 39555	April 26, 2008	Praia do Mupéua, Fortalezinha, Maracanã, PA	Immature
MPEG 39556	May 2, 2008	Praia da Romana, I. Romana, Curuçá, PA	Immature
MPEG 39682	-	-	Mature
GEMAM 183	May 31, 2008	Praia da Princesa, Algodoal, Maracanã, PA	Immature
GEMAM 192	-	-	Immature

The diagnosis of cervical ribs was based on the existence of an abnormal elongated transverse process, on one or both sides of the vertebrae. A strangulated area along the anomalous process could be present or absent, and none of the processes showed a true articulation to a cervical rib. Since the species presents a large variation in the size and shape of vertebral body/processes, this was an additional problem regarding the diagnosis of cervical ribs. Thus, metric analysis was checked against the bone's visual identification (Laeta, 2007).

The midline complete aperture separating the two pointed extremities of the semi-arches identified the possible cleft neural arch. This anomaly was carefully examined with a magnifying glass, in order to exclude possible taphonomic causes for bone aperture. The presence of normal cortical bone covering the pointed extremities confirmed the cleft arch. The differential diagnosis of spondilolysis and other arch fractures were based on Ortnier and Putschar (1985).

Metric analysis provided the measurements used to estimate the proportion between the transverse process and the corresponding half of the vertebral body (Laeta, 2007; Laeta *et al.*, 2010). The pictures of the vertebrae (cranial view) were obtained with a digital 5.1 megapixels camera, at a constant distance of 17cm from the vertebral body on a black surface, with a graphic scale positioned for reference. A first guideline crossing the vertebral body was used to mark the sagittal plane; two parallel lines, tangent to the right and left borders of the vertebral body, were drawn. Three dimensions were obtained for each side of the vertebra: (A) the perpendicular dimension between the sagittal plane and the tip of the transverse process; (B) the perpendicular dimension between the sagittal plane and the tangent

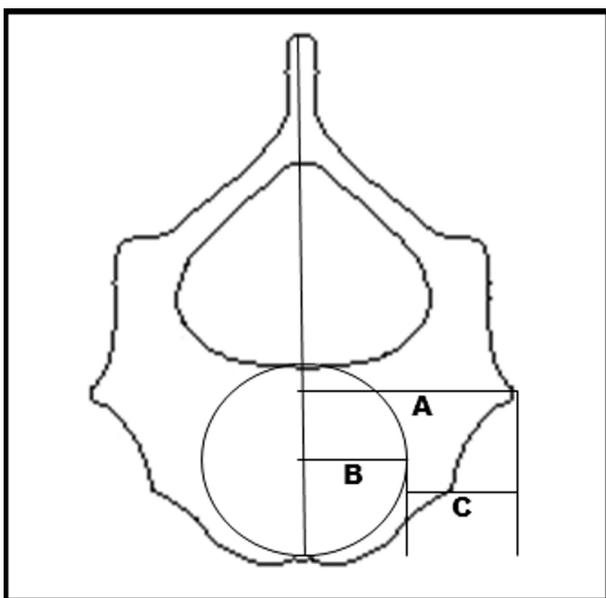


Figure 2. Schematic of a seventh cervical vertebra of *Sotalia*, with guidelines and measurements A, B and C.

to the vertebral disc, at the same side; (C) the length of the transverse process (A-B; Figure 2). A length index was calculated based on the equation $(B \times 100) / A$, in order to separate the normal and abnormal processes.

Samples were tested for homogeneity (absence of cervical rib x presence of cervical rib) with Fisher's Exact Test, for two-tailed confidence levels of $\alpha = 0.01$ and $\alpha = 0.05$. Data was treated with BioEstat 5.0 (Ayres *et al.*, 2007).

Results

Two congenital vertebral anomalies, the cervical rib and the cleft neural arch, both affecting only the seventh cervical vertebra (C7), were found in the collections. From a total of 63 skeletons, 26 (41%) presented anomalies. The frequencies of cervical rib and cleft arch in Pará and Rio de Janeiro were different, but in both cases the anomalies were minor, and probably asymptomatic for the animals.

Cervical ribs were present in nine (45%) of the 20 skeletons from Rio de Janeiro and in five (12%) of the 43 skeletons from Pará. In both series this anomaly was observed at the right, left or both sides of the vertebrae. Despite the small number of skeletons in both collections, the difference between the frequency of cervical ribs in specimens from Rio de Janeiro and Pará was significant ($p = 0.071$, two-tailed) for a confidence level of $\alpha = 0.01$ (Fisher's Exact Test), suggesting that the Pará and Rio de Janeiro collections may be from different populations.

When plotting $A \times C$ measurements to the left and right sides of the C7 in the Rio de Janeiro specimens, it was possible to cluster the normal individuals, separating them from those presenting cervical ribs (Figure 3). From nine (45%) individuals presenting visually identified cervical ribs, three (33%) were physically mature, and six (67%) were physically immature. Four (44.5%) skeletons had only left cervical ribs; one (11%) had a right cervical rib; four (44.5%) had ribs on both sides (Figure 4). In the Rio de Janeiro specimens 85% of the processes identified as cervical ribs presented a length index under 42; 89% of the normal processes presented an index over 45 (Table 3).

Five (12%) of the 43 skeletons from Pará presented cervical ribs. As in the Rio de Janeiro specimens, visual analysis was confirmed by metric analysis (Figure 5). Pará specimens showed more variation in the size and shape of C7 than those from Rio de Janeiro. Of the five individuals with cervical ribs, four (80%) were physically mature, and one (20%) immature. Concerning symmetry, two (40%) individuals had only left cervical ribs; one (10%) had a right cervical rib; two (40%) had bilateral cervical ribs. The fifth individual (20%) had a cervical rib on the left side, but a *postmortem* fracture at the right side of the vertebra removed the process and the analysis was not conclusive for that side (Figure 6). The length index pointed to the same sectioning interval

between normal processes and processes with cervical ribs. Six of the seven processes with cervical ribs (86%) presented an index under 42, and 100% of the 78 normal ones presented an index above 45 (Table 4).

Two of the cervical rib cases in the Pará collection were found at Maracanã and Salinópolis (mainland), one was found at Soure (Marajó Island), and two were of no defined origin. Regarding this anomaly, the difference between the skeletal series found in areas close to mainland and the ones found in areas close to the Marajó Island was not significant ($p = 0.2421$, two-tailed) at a confidence level of $\alpha = 0.01$ (Fisher's Exact Test), confirming that the two Pará groups of skeletons in the present study are similar.

The cleft neural arch occurred as a small aperture at the midline of the C7 neural arch (0.5 to 2.0mm) in both the Rio de Janeiro and Pará specimens. The contiguous vertebrae (C6 and T1) were always normal. Cleft arches were present in nine (45%) of the twenty Rio de Janeiro

skeletons, and in six (14%) of the Pará skeletons (Figure 7). This difference is significant ($p = 0.0113$, two-tailed) for a confidence level slightly higher than $\alpha = 0.01$. In relation to the Rio de Janeiro series, two (22%) of the skeletons with cleft neural arches belonged to mature individuals and seven (78%) to immature ones.

Four (67%) of the skeletons from Pará presenting cleft neural arches belonged to immature animals, and two (33%) to mature ones. Five of the skeletons with this anomaly came from Marajó Island (four from Soure, one from Salvaterra), and they represent 19% of the island specimens. Only one skeleton was from mainland vicinities, representing 8% of those animals. The difference between the island and mainland specimens was not significant ($p = 0.6434$) for a confidence level of $\alpha = 0.01$. Regarding the Rio de Janeiro skeletons, three specimens presented both anomalies, in contrast to the Pará series, in which they always occurred in different individuals (Table 5).

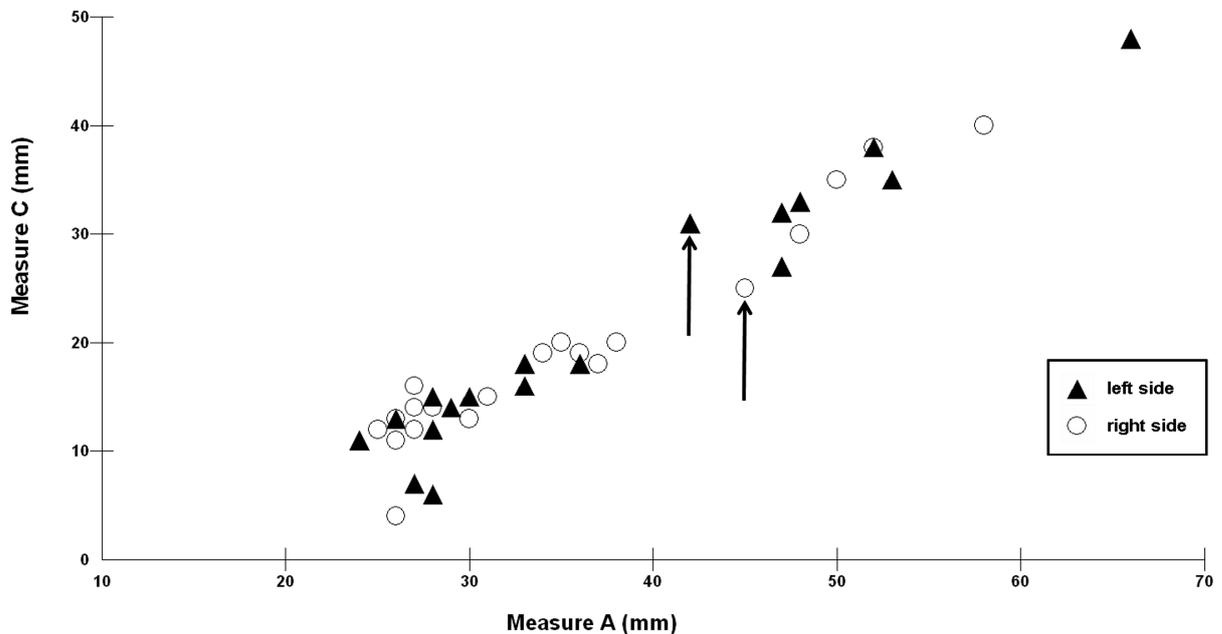


Figure 3. Scatterplots of A and C measurements for the left and the right side of the seventh cervical vertebrae, Rio de Janeiro *Sotalia guianensis* series. Notice the separate clustering of the individuals presenting no cervical ribs and presenting cervical ribs. The arrows point the first case with cervical rib (left and right).



Figure 4. Cranial view of the seventh cervical vertebra from three individuals from the Rio de Janeiro series: GEMM 048 – without cervical rib (A); GEMM 073 – presenting a left cervical rib (B); GEMM 084– presenting bilateral cervical rib (C).

Table 3. Measurements A, B and C (mm) and index estimated for the seventh cervical vertebra (C7) of *Sotalia guianensis* from the Rio de Janeiro series in the present study.

SPECIMEN	MEASUREMENTS (mm) LEFT SIDE			INDEX	MEASUREMENTS (mm) RIGHT SIDE			INDEX
	A	B	C		A	B	C	
GEMM 023	33	15	18	45	26	15	11	58
GEMM 031	33	17	16	52	36	17	19	47
GEMM 033	28	13	15	46	27	13	14	48
GEMM 039	29	15	14	52	27	15	12	56
GEMM 041	33	15	18	45	28	14	14	50
GEMM 047	42	11	31	26	27	11	16	41
GEMM 048	30	15	15	50	34	15	19	44
GEMM 049	24	13	11	54	25	13	12	52
GEMM 065	53	18	35	34	48	18	30	38
GEMM 068	36	18	18	50	30	17	13	57
GEMM 070	47	15	32	32	50	15	35	30
GEMM 071	27	20	7	74	45	20	25	44
GEMM 073	48	15	33	31	35	15	20	43
GEMM 076	28	22	6	79	26	22	4	85
GEMM 082	28	16	12	57	31	16	15	52
GEMM 083	26	13	13	50	26	13	13	50
GEMM 084	52	14	38	27	52	14	38	27
GEMM 087	53	18	35	34	58	18	40	31
GEMM 089	47	20	27	43	37	19	18	51
GEMM 096	66	18	48	27	38	18	20	47

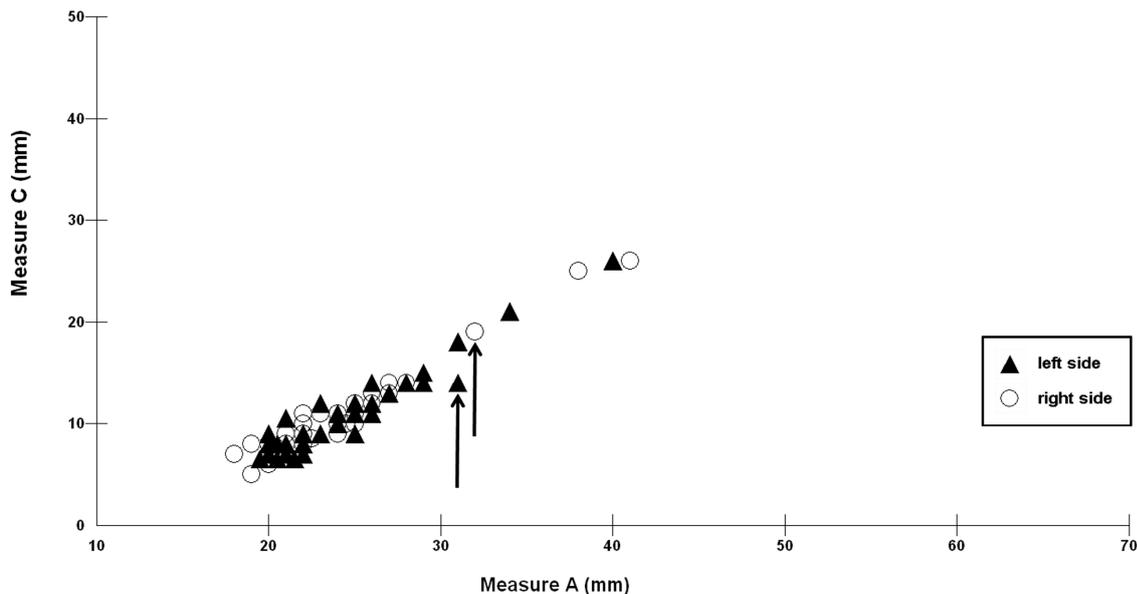


Figure 5. Scatterplots of A and C measurements for the left and the right side of the seventh cervical vertebrae, Pará *Sotalia guianensis* series. Notice that the separation of the cluster of the individuals presenting no cervical ribs is not so clear. This is possibly because of the smaller size of the vertebrae. The arrows point the first case with cervical rib (left and right)

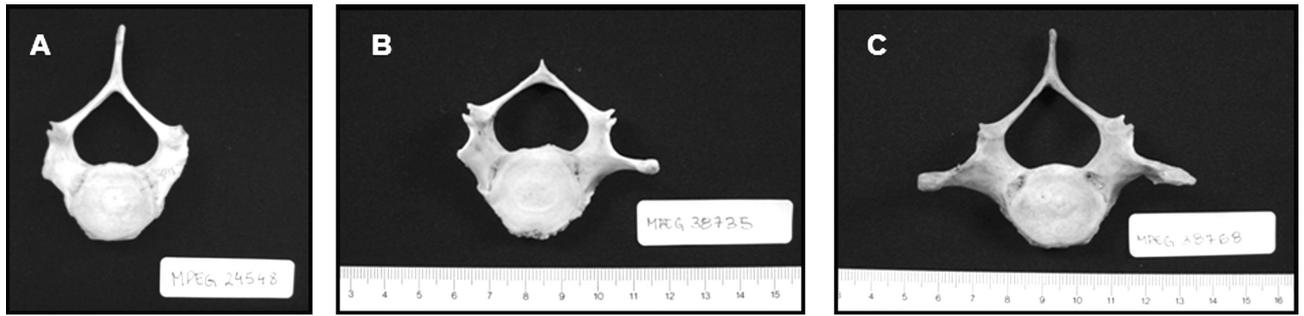


Figure 6. Cranial view of the seventh cervical vertebrae from three individuals from Pará series: MPEG 24548 - without cervical rib (A); MPEG 38735 - presenting a left cervical rib (B); MPEG 38768 - presenting bilateral cervical ribs (C).

Table 4. Measurements A, B and C (mm) and index estimated for the seventh cervical vertebra (C7) of *Sotalia guianensis* from the Pará series used in the present study.

SPECIMEN	MEASUREMENTS (mm)			INDEX	MEASUREMENTS (mm)			INDEX
	LEFT SIDE				RIGHT SIDE			
	A	B	C		A	B	C	
MPEG 10945	23	11	12	48	22	11	11	50
MPEG 24548	20.5	14	6.5	68	24	14	10	58
MPEG 37816	21	10.5	10.5	50	23	12	11	52
MPEG 37825	21.5	15	6.5	70	21	13.5	7.5	64
MPEG 37826	22	13	9	59	25	13	12	52
MPEG 37827	21	13	8	62	22	14	8	64
MPEG 38430	24	13	11	54	26	13	13	50
MPEG 38433	29	15	14	52	28	14	14	50
MPEG 38436	24	13	11	54	25	13	12	52
MPEG 38440	23	14	9	61	27	14	13	52
MPEG 38441	21	14	7	67	21	13	8	62
MPEG 38447	22	14	8	64	22.5	14	8.5	62
MPEG 38448	26	14	12	54	26	14	12	54
MPEG 38449	24	13	11	54	24	14	10	58
MPEG 38455	29	14	15	48	27	13	14	48
MPEG 38456	19.5	13	6.5	67	22	14	8	64
MPEG 38460	24	14	10	58	22	13	9	59
MPEG 38461	24	14	10	58	23	12	11	52
MPEG 38469	26	12	14	46	21	12	9	57
MPEG 38478	25	13	12	52	25	13	12	52
MPEG 38479	25	14	11	56	25	15	10	60
MPEG 38735	34	13	21	38	22	13	9	59
MPEG 38739	20.5	12.5	8	61	24.5	14.5	10	59
MPEG 38740	28	14	14	50	25	14	11	56
MPEG 38744	27	14	13	52	25	13	12	52
MPEG 38754	20	13	7	65	20	14	6	70
MPEG 38762	23	14	9	61	21	14	7	67
MPEG 38766	24	13	11	54	22	13	9	59
MPEG 38768	40	14	26	35	41	15	26	37
MPEG 38771E	22	10	12	45	22	12	10	55
MPEG 39443	31	13	18	42	32	13	19	41
MPEG 39448	24	14	10	58	21	13	8	62
MPEG 39449	22	15	7	68	20	13	7	65
MPEG 39450	25	16	9	64	24	15	9	63
MPEG 39457	23	14	9	61	24	13	11	54
MPEG 38458	20	13	7	65	19	14	5	74
MPEG 39545	20	11	9	55	18	11	7	61
MPEG 39548	31	14	17	45	-	-	-	-
MPEG 39555	20	12	8	60	20	12	8	60
MPEG 39556	26	15	11	58	24	15	9	63
MPEG 39682	28	14	14	50	38	13	25	34
GEMAM 183	22	13	9	59	20	14	6	70
GEMAM 192	20	11	9	55	19	11	8	58

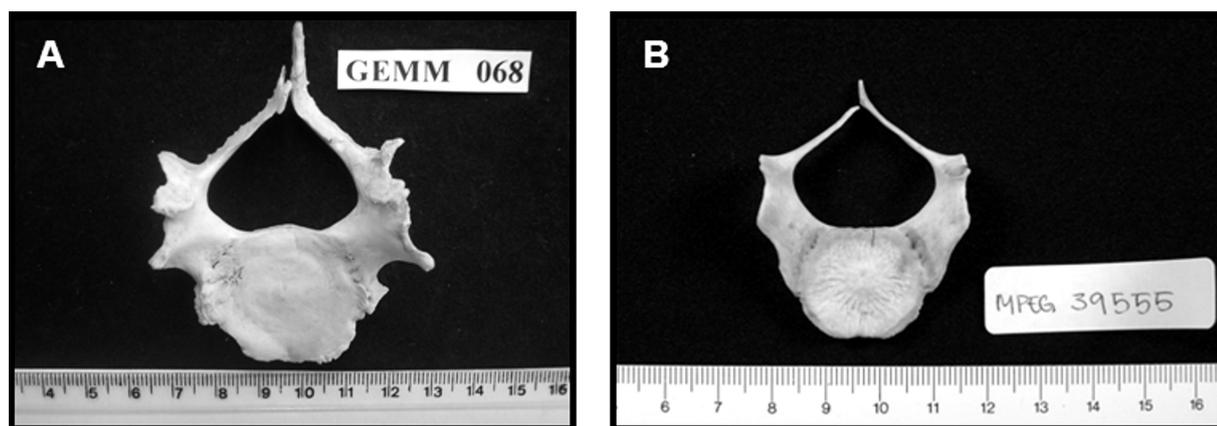


Figure 7. Cranial view of the seventh cervical vertebrae of individuals with cleft neural arch in Rio de Janeiro series GEMM 068 (A), and in the Pará series MPEG 39555 (B).

Table 5. Specimens with anomalies (cervical ribs and cleft neural arch) in the Rio de Janeiro and Pará *Sotalia guianensis* described in the present study.

SPECIMEN	CLEFT NEURAL ARCH	LEFT CERVICAL RIB	RIGTH CERVICAL RIB
GEMM 023	X	-	-
GEMM 033	X	-	-
GEMM 041	X	-	-
GEMM 047	-	X	-
GEMM 065	-	X	X
GEMM 068	X	-	-
GEMM 070	-	X	X
GEMM 071	-	-	X
GEMM 073	X	X	-
GEMM 076	X	-	-
GEMM 082	X	-	-
GEMM 084	X	X	X
GEMM 087	X	X	X
GEMM 089	-	X	-
GEMM 096	-	X	-
MPEG 38447	X	-	-
MPEG 38448	X	-	-
MPEG 38478	X	-	-
MPEG 38754	X	-	-
MPEG 39545	X	-	-
MPEG 39555	X	-	-
MPEG 38735	-	X	-
MPEG 38768	-	X	X
MPEG 39443	-	X	X
MPEG 39548	-	X	-
MPEG 39682	-	-	X

The frequency of cervical ribs in *S. guianensis* from Rio de Janeiro was compared to the data from Fettuccia *et al.* (2009) for *S. guianensis* from Ceará and Santa Catarina, and *S. fluviatilis* from the Amazon. The Rio de Janeiro collection was distinct from the Ceará ($p = 0.0124$, two-tailed) and Amazon specimens ($p = 0.0036$, two-tailed), and this difference was significant at a confidence level of $\alpha = 0.01$, suggesting that these populations are more distant. The difference between the Rio de Janeiro and Santa Catarina series was not significant ($p = 0.0607$, two-tailed) for a confidence level of $\alpha = 0.01$, suggesting they are not separate populations.

When the results for the Pará series are compared to the same literature, the differences are not significant for the same confidence level (Ceará $p = 1.000$; Santa Catarina $p = 0.5128$), and the difference in relation to the Amazon series was the only significant difference, as expected ($p = 0.000$).

Discussion

S. guianensis is among the threatened species possibly affected by various economic and recreational activities along the Brazilian coast (Siciliano *et al.*, 2006). More information concerning the behavior and biological characteristics of these dolphins is necessary before we can estimate the consequences of anthropogenic impacts, in order to mitigate them. Morphologic and genetic data point to the existence of three *S. guianensis* subtypes along the Brazilian coast, and their differentiation may be the consequence of a demographic expansion to the south (Cunha *et al.*, 2005; Caballero *et al.*, 2007; Caballero *et al.*, 2010 this volume). Genetic and environmental factors help explain the distribution of different haplotypes, and probably part of the variation in morphology.

As phenotype markers of biological similarities, skeletal congenital anomalies have been used in the comparison of different human populations, as well as in the study of their possible biological relationships (Barnes, 1994). Their use in the study of dolphin populations can be of some help, tracking their differences and similarities and adding information to the study of genetic and epigenetic traits. They can also help in monitoring of environmental changes that may cause morphological anomalies. There are few references to vertebral anomalies in cetaceans in the literature, probably because most of them are compatible with normal life, causing no disability or expressive mortality impact (Berghan and Visser, 2000; Simões-Lopes, 2005). The investigation of skeletal anomalies in wild living species is very difficult; therefore, skeletal collections, especially those obtained in monitoring projects, provide a unique source of information regarding biological variability of a species. Cervical ribs are accepted as a genetic-dependent character, and the present results can be compared to genetic studies for the species. The genetic data regarding some haplotypes (*i.e.* cytochrome-*b*) suggest

speciation separating *S. fluviatilis* and *S. guianensis*. These same studies propose a different sub-population of marine species along the Brazilian coast, based on haplotype variability from the north to the south (Cunha *et al.*, 2005; Caballero *et al.*, 2007; Caballero *et al.*, 2010 this volume). The previous use of the frequency of cervical ribs for comparisons between river and coastal groups (Fettuccia *et al.*, 2009), was confirmed with new data in this study, consistent with the demographic and micro-evolutionary hypothesis delineated for this species over the last years.

Contrary to Fragoso (2001; 2006), the present results for the Rio de Janeiro central-north collection do not include major vertebral anomalies, and the only vertebral anomalies found were cervical ribs and cleft arches. According to this author, approximately 29.3% of 22 skeletons possessed congenital anomalies such as aplasia, hypoplasia, fused vertebrae, anomalies in the size and shape of the ribs, among others of the axial skeleton. These anomalies were associated with the pollution of the Guanabara Bay area, (Central coast of Rio de Janeiro), from which the animals were obtained. No cervical rib or cleft arch was described. It is possible that the Guanabara Bay collection represents a reduced population affected by an extremely polluted environment, as suggested by Fragoso (2006), in which the anomalies are either expressing teratogenic processes caused by environmental threats, or simply a bias of skeletons stored in old collections. On the other hand, most of the skeletons in the present paper result from recent systematic monitoring activities along the coast of Rio de Janeiro (100% collected after 2001) and along the coast of Pará (84% collected after 2005), and were not selected for rare anomalies.

Pollution is not the only change in the natural environment, and many different impacts affecting wild species must be considered. The cleft arch is a defect hypothesized to be associated with nutritional deficiencies, and it is important to verify if changes in the environment could have an impact on the availability of the vitamin B complex (Barnes, 1994; Jablonsky and Chaplin, 2000; Reiderson, 2003) and if derived products could result from human impact on cetacean habitats, where such defects are frequent. It is also necessary to observe if there are other possible explanations for this defect, and if a possible genetic determination is operating in the case of *Sotalia's* cleft arch located at C7. Until we have more information regarding the processes causing/triggering the anomaly, it should be useful to compare their frequency in different series of well-defined populations and their contexts.

As the Pará collection was divided into two groups of skeletons representing animals from the Marajó Island vicinities and from mainland vicinities, the frequency of cervical ribs in both series was compared, resulting in no significant difference for $\alpha = 0.01$. Thus, both Pará groups were considered as belonging to a single population. The low frequency of cervical ribs and cleft arches was

consistent with a high genetic variability expressed in the population of the northern coast of Brazil (Cunha *et al.*, 2005; Caballero *et al.*, 2007; Caballero *et al.*, 2010 this volume), where no special trace is selected or concentrated by any genetic mechanism. Genetic variability is consistent with more recent surveys (Sholl, 2010) but is also consistent with the anatomical variability of the animals monitored and recovered in the same area (R. Emin-Lima, personal communication). Even though the apparent higher frequency of cleft neural arches in the Marajó island series was not statistically significant, it is still noteworthy. The future improvement of the Pará collection with new specimens could confirm or deny a concentration of cases in the island's vicinity.

Statistical proof of heterogeneity was obtained when comparing the results of cervical ribs and cleft arches from the Rio de Janeiro and Pará specimens; that is they represent different populations. The same result was obtained when the Rio de Janeiro series was compared to the Fettuccia *et al.* (2009) data for Ceará *S. guianensis*. The frequency of cervical ribs and cleft arches is higher in the Rio de Janeiro specimens when compared to the Pará or Ceará populations. As for the presence of cervical ribs, genetic models explain these results. According to Cunha *et al.* (2005), Caballero *et al.* (2007) and Caballero *et al.* (2010 this volume), the reduction of haplotypes in the southeastern and southern groups of *Sotalia* express a founder effect, considering that we could expect animals from those regions to have peculiar frequencies (either higher or lower) for some genetic characteristics. A higher frequency of cervical ribs for a southern collection of skeletons of the same species has already been reported by Fettuccia *et al.* (2009).

When comparing the frequencies of cervical ribs in the Rio de Janeiro specimens with the results published by Fettuccia *et al.* (2009) for the Santa Catarina specimens, we obtained a non-significant statistical difference. This confirms the hypothesis that the Santa Catarina and Rio de Janeiro animals belong to the same stock, since the similarity between the southeastern and the south groups of specimen is consistent with low population variability (Cunha *et al.*, 2005; Caballero *et al.*, 2007; Caballero *et al.*, 2010 this volume). On the other hand, as pointed out earlier, the Rio de Janeiro animals represent samples from a very narrow coastal region, the São João estuary (Siciliano *et al.*, 2006), perhaps due to the fact that the frequency of cervical ribs is especially high (45%). This possibly expresses a closer biological proximity of the animals living in that estuary. Continual monitoring of the coast and an extension of the present study to other groups of skeletons will help in verifying any other possible fluctuations of the frequencies for this genetic characteristic, and perhaps approach a value that can express better the frequency of cervical ribs in southeastern *S. guianensis*.

The comparison of the cervical rib frequency in *S. guianensis* collection with those of freshwater *S. fluviatilis* confirmed morphological (Fettuccia *et al.*, 2009) and genetic (Caballero *et al.*, 2007) studies. According to Ferigolo (1987) the cervical rib is a characteristic of reptiles and birds, and, because of that, it can be considered an atavism. However, the cervical ribs at C6 and C7 can be characteristic of a few mammal species, such as sloths. Hall (2005) reminds us that the identification of an atavism should be based on its low frequency. According to Barnes (1994), cervical ribs in humans, as well as in most mammals, are associated to a genetic or epigenetic condition, and are expected to show a different prevalence in different population series. That is probably not the case of *Sotalia*, a genus that in the Brazilian series is described as presenting cervical rib frequencies between 12% and 87.1%.

As several other anomalies, the cleft arch may also have a genetic component regarding its determination, probably modulated by environmental factors. The literature does not provide other population studies of this anomaly for the Brazilian *Sotalia*, making it harder to interpret the results. The non-closure of the arch can be endemic in disadvantaged and poor human populations (Minns, 1996; Fleming and Copp, 1998; Jablonski and Chaplin 2000; Moalem and Prince, 2007), and nutritional supplements in laboratory rats reduce 70% of their defects (Medical Research Council Vitamin Research Group, 1991; Fleming and Copp, 1998). But the present results are from Cetaceans in natural habitats, not expected to present such a deficiency. To accept the statistically significant different frequencies of this anomaly in *S. guianensis* specimens from Rio de Janeiro and Pará, unique conditions affecting antropic environments must be considered. Teratogenic substances or others types of compounds that could affect development, therefore, must be investigated (Laílson *et al.* 2003; Moura *et al.*, 2008). As bio-accumulators, cetaceans are at the top of the nutritional chain, suffering from an increased effect of any chemical accumulation that could have teratogenic effects, and this fact must also be considered (Watson and Bonde, 1986; Weinstein *et al.*, 1995; Berghan and Visser, 2000).

The continuous variation of vertebral sizes and shapes in dolphins makes the identification of cervical ribs difficult, especially in the absence of a clear limit between the vestigial rib and the process. We assumed here that a change in the anatomical proportions could be measured when a cervical rib was present. The use of special measurements and the length index proved to be easy to implement, expressing the proportion of the process and helping to diagnose borderline specimens. In spite of the variability in size and shape, consistent results were obtained for Rio de Janeiro and Pará skeletons, confirming the visual diagnosis of cervical ribs in *S. guianensis* C7 vertebra in both series.

Indeed, as the Pará skeletons were smaller, their measurements were also more close and separation less clear (Figure 5). Application of the metric analysis proposed here to other series will help to improve the method and to confirm the cutting point interval established and adopted in the present paper.

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