UNIVERSIDADE FEDERAL DO ACRE

LUZIVALDO CASTRO DOS SANTOS JÚNIOR

APARELHO RESPIRATÓRIO POSTERIOR DE *Inia geoffrensis* (BLAINVILLE, 1817) E *Sotalia fluviatilis* (GERVAIS & DEVILLE, 1853) (CETARTIODACTYLA): ESTRUTURA E ULTRAESTRUTURA

RIO BRANCO
ACRE – BRASIL
MAIO – 2017

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Dissertação apresentada à Universidade Federal do Acre, como parte das exigências do Programa de Pós-Graduação em Sanidade e Produção Animal Sustentável na Amazônia Ocidental, para a obtenção do título de Mestre em Ciência Animal.

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APROVADA: 05 de maio de 2017.

Dr. Francisco Glauco A. Santos
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(Coorientadora)

Dr. Yuri Karaccas de Carvalho
UFAC
(Orientador)

Aos meus pais,
os melhores professores que eu tive na vida.

Dedico.

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À Deus, pelo dom da vida, pela graça de estar aprendendo tanta coisa boa nesse mundo e por me conceder a oportunidade de estar vivendo essa vida intensamente.

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E a todos que de alguma maneira contribuíram para a conquista desta etapa tão importante na minha vida! Muito, mas muito obrigado, de coração!

"Toda Noite de baile, no dia do padroeiro, quando a festa vai bem animada, chega um rapaz. Rapaz muito bonito, todo de branco, de chapéu de palhinha na cabeça. Este rapaz escolhe a cabocla mais formosa e dança com ela, e só com ela, sem tirar o chapéu... senão saltam peixinhos por um buraco que ele tem no meio da cabeça. Dança até o sol raiar. Depois o rapaz sai correndo e se lança no rio lá do alto do barranco. Muita moça donzela, quando aparece de barriga, diz com a cara mais limpa desta floresta encantada: foi o boto!"

Thiago de Mello (2002)

CERTIFICADO DO COMITÊ DE ÉTICA NO USO DE ANIMAIS – UFAC

Título do projeto: Aparelho respiratório posterior de *Inia geoffrensis* e *Sotalia fluviatilis*: estrutura e ultraestrutura.

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RESUMO

SANTOS JUNIOR. Luzivaldo Castro dos. Universidade Federal do Acre, maio de 2017. **Aparelho respiratório posterior de *Inia geoffrensis* (Blainville, 1817) e *Sotalia fluviatilis* (Gervais & Deville, 1853): estrutura e ultraestrutura.** Orientador: Yuri Karaccas de Carvalho. Este estudo teve como objetivo caracterizar as estruturas do sistema respiratório posterior de duas espécies de golfinhos-de-rio: *Inia geoffrensis* e *Sotalia fluviatilis*. O trato respiratório de ambas as espécies foi avaliado utilizando técnicas macro e microscópicas. Foram identificadas quatro estruturas anatômicas macroscópicas: traqueia, brônquio principal, brônquio traqueal e pulmão. A presença de brônquio traqueal sugeriu a facilidade de trocas gasosas. A análise histológica revelou a presença de dutos alveolares e esfíncter mioelástico nestes cetáceos amazônicos. A porção respiratória posterior dos golfinhos amazônicos apresenta similaridade com outros odontocetos e o conhecimento desta estrutura pode contribuir também para a compreensão da fisiologia do mergulho e como essas espécies são adaptadas ao seu habitat.

Palavras-chaves: boto, tucuxi, anatomia.

ABSTRACT

SANTOS JUNIOR. Luzivaldo Castro dos. Universidade Federal do Acre, May, 2017. **Posterior respiratory apparatus of *Inia geoffrensis* (Blainville, 1817) and *Sotalia fluviatilis* (Gervais & Deville, 1853): structure and ultrastructure.** Advisor: Yuri Karaccas de Carvalho. This study aimed to characterize the structures of the posterior respiratory system of two species of river dolphins: *Inia geoffrensis* and *Sotalia fluviatilis*. The respiratory tract of both species was evaluated using macro and microscopic techniques. Four macroscopic anatomical structures were identified: trachea, main bronchus, tracheal bronchus and lung. The presence of the exuberant tracheal bronchus suggested ease of gas exchanges. Histological analysis revealed the presence of alveolar ducts and myoelastic sphincter in these Amazonian cetaceans. The posterior respiratory portion of the Amazonian dolphins presents similarity with other odontocetes and the knowledge of this structure can contribute to the understanding of the physiology of diving and how these species are adapted to their habitat.

Keywords: Amazon river dolphin, tucuxi, anatomy.

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1 ARTIGO

1.1 Artigo 1

Posterior respiratory apparatus of *Inia geoffrensis* and *Sotalia fluviatilis*: structure and ultrastructure.

Luzivaldo Castro dos Santos Júnior, Miriam Marmontel, Francisco Glauco de Araújo Santos, Rose Eli Grassi Rici, Maria Angélica Miglino e Yuri Karaccas de Carvalho.

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Posterior respiratory apparatus of *Inia geoffrensis* and *Sotalia fluviatilis*: structure and ultrastructure

Aparato respiratorio posterior de *Inia geoffrensis* y *Sotalia fluviatilis*: estructura y ultraestructura

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Summary

This study aimed to characterize the structures of the posterior respiratory system of two species of river dolphins: *Inia geoffrensis* and *Sotalia fluviatilis*. The respiratory tract of both species was evaluated using macro and microscopic techniques. Four macroscopic anatomical structures were identified: trachea, main bronchus, tracheal bronchus and lung. The presence of the exuberant tracheal bronchus suggested ease of gas exchanges. Histological analysis revealed the presence of alveolar ducts and myoelastic sphincter in these Amazonian cetaceans. The posterior respiratory portion of the Amazonian dolphins presents similarity with other odontocetes and the knowledge of this structure can help also contribute to the understanding of the physiology of diving and how these species are adapted to their habitat.
Keywords: Amazon river dolphin, tucuxi, anatomy.

1. Introduction

Cetartiodactyla is one of the most diversified orders of mammals and includes cetaceans, animals that have acquired anatomical adaptations to adaptation aquatic life, such as a fusiform body and modified limbs (De Muizon, 2009; Uhen, 2010; Reeves et al. 2011; Hassanin, 2012). The Amazonian cetaceans boto *Inia geoffrensis* (Reeves et al., 2011) and tucuxi *Sotalia fluviatilis* (Secchi, 2012) are listed as "Data Deficient" according to the International Union for the Conservation of Nature and Natural Resources.

Both botos and tucuxis are distributed in the Amazon and Orinoco River basins (Best, 1993; Da Silva, 1996; Da Silva, 2007).

Studies on the biological characteristics of freshwater dolphins started when the first taxonomic study of these species was performed by Gray in 1863 (Kida, 1990). These animals possess a set of anatomical specializations that, due to evolutionary needs, improve diving capacity, including an increase in size of body and of cavities oxygen storage (Piscitelli, 2009). The respiratory system which begins at the blowhole, passes through the trachea and ends in the lungs (Berta *et al.* 2005).

The morphological pattern of the tracheobronchial tree is similar among the different species of cetaceans, confirming the phylogenetic relationships between these animals, which can be verified in *Lipotes vexillifer*, *Platanista* sp. and *Pontoporia* blainvillei (Kaiya, 1982; Endo *et al.*, 1999).

Amazonian cetaceans present conflicts with fishing, often in a negative way (Santos Junior & Marmontel, 2016). This inter-specific interaction between fishermen and cetaceans may lead to death (Parsons & Jefferson, 2000).

This work aimed to characterize the posterior respiratory system of the Amazon river dolphins (boto and tucuxi), from macroscopic and microscopic analysis of the trachea, bronchi and lungs.

2. Material and methods

Carcasses of Amazon river dolphins were collected in Lake Tefé and the Mamirauá and Amanã sustainable development reserves (Figure I), located in the region of mid Solimões river, near the town of Tefé, Amazonas, Brazil. These carcasses were collected between 1995 and 2015 and underwent a process of biometry and necropsy.

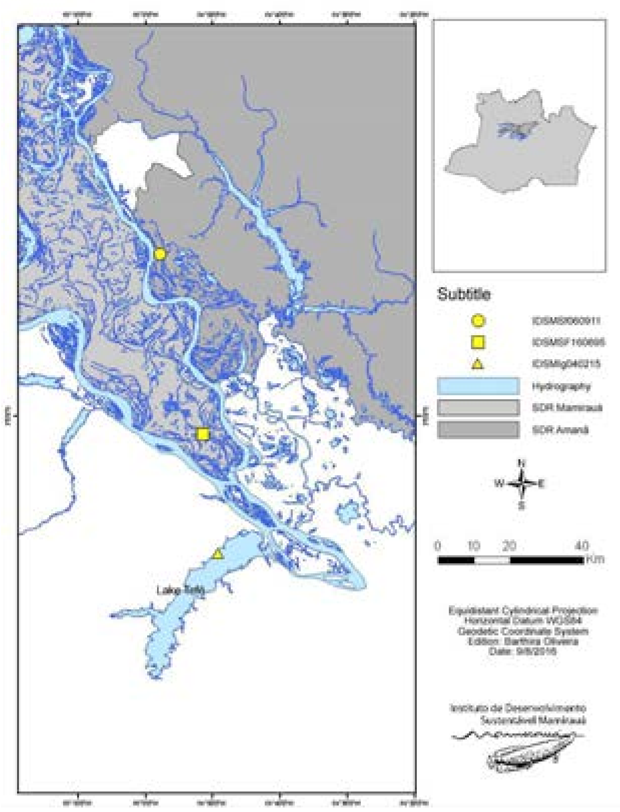

Figure 1- Study area and collection points of carcasses.

The research was approved by the Federal University of Acre (UFAC) Committee on Ethics in the Use of Animals (protocol number 05/2017) and registered in the Biodiversity Information and Authorization System (IBAMA) under the numbers 44557-1/2014 and 44577-2/2015.

Samples of the posterior respiratory system (Table I) were preserved in 10% formaldehyde solution in the Aquatic Mammals Laboratory of the Mamirauá Institute for Sustainable Development (MISD).

Table I – Data of the river dolphins used in this study.

Species	Code	Length (cm)	Age class	Collected materials
S. fluviatilis	IDSMSf160695	82	Calf	Trachea, bronchi, right and left lungs
S. fluviatilis	IDSMSf060911	148	Adult	Right and left lungs
I. geoffrensis	IDSMIg040215	213	Adult	Trachea, bronchi, right and left lungs

The structures that make up the posterior respiratory system of boto and tucuxi were evaluated macroscopically at the UFAC's Laboratory of Animal Anatomy (Center for Biological and Nature Sciences) and the following biometric parameters were taken: length, width and thickness of the components of the trachea, tracheal bronchus, main bronchi and lungs. For the nomenclature of the structures and their components, the Nomina Anatomica Veterinaria was used (ICVGAN, 2012).

After the macroscopic analysis, fragments of the trachea, tracheal bronchus, main and secondary bronchi, and lungs were removed from the parietal and mediastinal surfaces in their cranial, middle and caudal regions. Optical microscopy was performed at the MISD's Histology Laboratory. Samples were submitted to conventional histological processing with five-micrometer sections on a Slee Cut 5062® microtome. For each fragment, slides were prepared with the selection of five non-serial sections each, which were stained in Harris Hematoxylin and Eosin (HE). The slides thus processed were observed and photodocumented in distinct fields on the Motic BA 410® microscope with coupled camera.

Scanning Electron Microscopy was performed at the Advanced Center for Diagnostic Imaging (CADI) of the School of Veterinary Medicine and Animal Science of the University of São Paulo (CF-FMVZ/USP). We collected 1.0 cm-long fragments of the cranial and caudal portions of the trachea, tracheal bronchus, main bronchi and lungs, washed in distilled water for 20 min and post-fixed in 1% osmium tetroxide solution (Polyscience®, Inc. USA) for 2 hours. The fragments were dehydrated in ascending series of ethanol (50% to 100%) for 30 min each. Dehydration to the critical point of CO_2 occurred in Balzers® CPD 020 (FMVZ/USP). The samples were mounted on metallic bases (stub) using carbon glue and metallized with gold in the EMITECH® K550 (FMVZ-USP) apparatus, analyzed and photographed using a scanning electron microscope (Morgagni 268D - Phillips®).

3. Results and Discussion

3.1 Macroscopic Analysis

The conductive portion of the boto and tucuxi respiratory tract consists of the trachea, tracheal bronchus, right and left main bronchi and secondary bronchi, all of which have incomplete cartilaginous rings with a rounded shape (Figure 2). In other species (e.g. *Tursiops truncatus*, *Grampus griseus* and *Stenella coeruleoalba*), this structure of the tracheal apparatus indicated an increase in its resistance and rigidity, mainly to withstand water pressure (Bagnoli *et al.*, 2011).

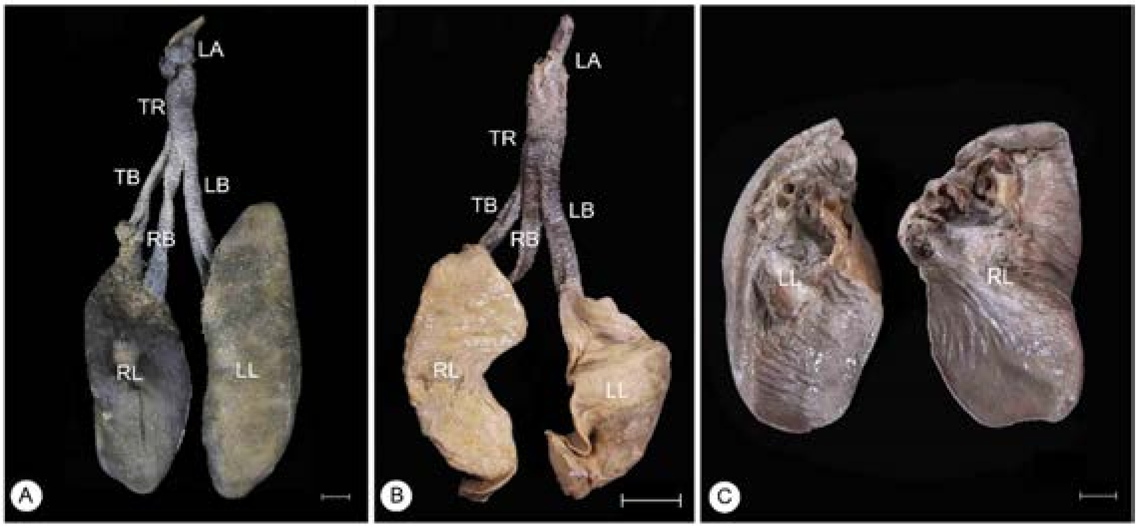

Figure 2 - A: Photomacrograph of the parietal face of the adult boto respiratory complex; B: Photomacrograph of the mediastinal face of the tucuxi calf respiratory complex; C: Photomacrograph of the mediastinal face of the adult tucuxi lung. LL-Left Lung Lobe, RL-Right Lung Lobe, LA-Larynx, LB-Left Main bronchus, RB-Right Main Bronchus, TB-Tracheal Bronchus, TR-Trachea. Scale = 3cm.

The trachea is a tube that begins in the larynx and ends in the tracheal bifurcation of the main bronchi. The trachea of the boto and tucuxi is conical and wide, similar to those found in other odontocetes, as *Platanista gangetica*, and *Pontoporia blainvillei* (Kida, 1990) and *Lagenorhynchus acutus* (Moore *et al.* 2014).

These results are similar to those found in the study by Henry *et al.* (1983), which shows that tracheal rings of many cetaceans vary in their cartilages in different regions of the organ, these being generally dorsally open (Smodlaka *et al.* 2009). In our findings, both in the cranial, middle and caudal regions, the cartilages were dorsally open. These cartilages have the function of coating the flat C-shaped rings present on the tracheal wall of some aquatic mammals (Gray *et al.*, 2006).

The trachea of the analyzed boto and tucuxis also demonstrated trabecular surface pattern, a result compatible with Reidenberg and Laitman (2014), who concluded that such a tracheal pattern in these animals facilitates the contraction of volume, since this portion of the tracheal wall is flexible. Unlike the mysticete cetaceans, the boto and the tucuxi did not present a pattern of parallel tracheal folds, because these two species are considered shallow-diving animals and thus do not need to adapt to larger volume changes necessary to make deep dives (Reidenberg & Laitman, 2014).

Both species presented spiral cartilaginous rings in both the trachea and the tracheal bronchus and main bronchi, similar to those found in *Delphinus delphis* and *Lagenorhynchus acutus* by Moore *et al.* (2014). These authors suggest that the spiral format of these rings is related to evolutionary issues in the different species of odontocete cetaceans, such as an adaptation to withstand pressure while diving.

The number of rings in the trachea of the boto was 16, in the tracheal bronchus there were 24 rings and on the right and left main bronchi there were 30 and 55 rings, respectively. In the tucuxi calf the number of rings was not counted, due to impossibility of visualization.

The length of the trachea of the adult boto was 6.93 cm, whereas in the tucuxi calf 4.41 cm (Table II); such lengths are compatible with the size range of these species. Moore *et al.* (2014) when describing an adult individual of *Delphinus delphis*, a marine dolphin species, claim that its trachea had length of 10cm. The size attributed to the boto, whether of its body and one of its anatomical structures, can be attributed to its displacement in narrow environments of the Amazonian rivers (Martin & Silva, 2004).

Specimen	**Organ**	**Antimer**	**Measure**		
			L	**W**	**H**
Calf Tucuxi IDSMSf160695	Traquea	-	4.41	2.17	0.86
	Tracheal bronchus	-	5.91	1.34	0.24
	Main bronchus	R	6.09	0.94	0.35
		L	8.67	1.17	0.26
	Lung	R	12.35	2.39	3.10
		L	12.72	2.76	5.93
Adult boto IDSMIg040215	Traquea	-	6.93	3.74	2.77
	Tracheal bronchus	-	9.75	1.75	1.97
	Secondary tracheal bronchus	R	4.45	0.11	1.74
		L	4.07	0.83	2.05
	Main bronchus	R	10.2	0.53	1.99
		L	12.09	1.40	1.70
	Lung	R	31.60	10.85	30.3
		L	30.55	11.14	41.1
Adult tucuxi IDSMSf060911	Lung	R	23.89	13.80	87.0
		L	27.79	13.92	77.8

Table II - Morphometric measures (in centimeters) of length (L), width (W) and height (H) of the portions of the posterior respiratory apparatus of boto and tucuxi.

The tracheal bronchus of the boto and tucuxi is inserted into the apex of the right pulmonary lobe near the insertion of the main right bronchus. This tracheal bronchus is a notable feature for cetacean species, facilitating gas exchange and increasing diving time, as previously described in other river dolphins, such as *Platanista gangetica* (ENDO *et al.*, 1999). The tracheal bronchus, as well as the left main bronchus, also presented two bronchial branches, inserted in the pulmonary apex.

This tracheal bronchus in boto and tucuxi corresponds to type II of the Nakakuki model, because in these Amazonian cetaceans, the bifurcation in the tracheal bronchus starts in the middle portion of the trachea when compared to other river dolphins

(Nakakuki, 1980 apud Kida, 1990). The right and left lungs of the boto and tucuxi exhibit a single cylindrical shaped lobe whose length is greater than its width. These findings are in line with those described by Piscitelli *et al*. (2013) in marine cetaceans.

3.2 Microscopic Analysis

The tracheas of both species presented incomplete rings (Figure 3A) and this characteristic supports the idea that the rings become less rigid. However, union with other rings forms an apparently firmer structure, aiding in compression under pressure (Moore, 2014).

Microscopically the trachea presented hyaline cartilage composed of chondrocytes (Figure 3B) and covered by a fibrous adventitial tunica composed of collagen fibers, blood vessels and adipose tissue.

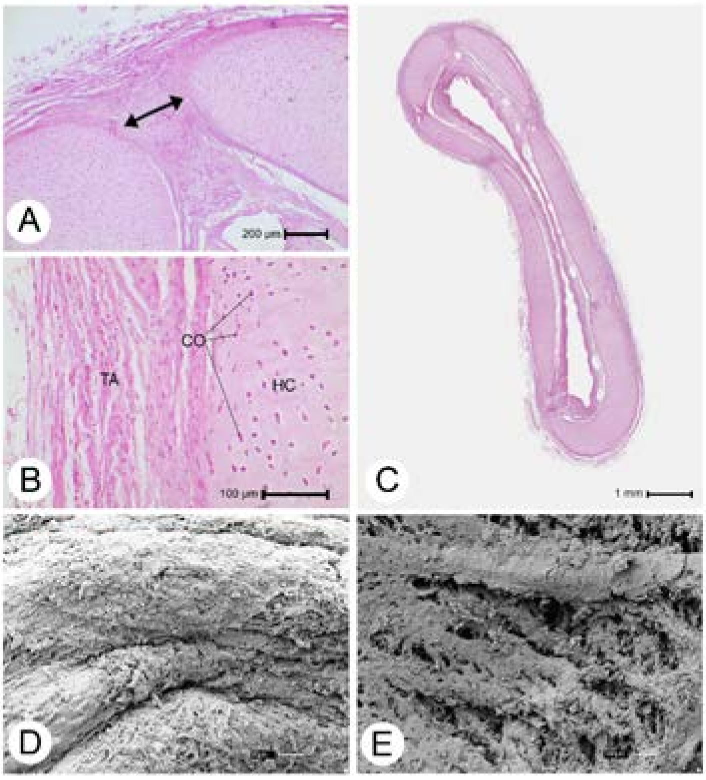

Figure 3- A: Photomicrograph part of the tracheal bronchus of the tucuxi calf, with spacing between the rings (arrow) (HE); B: Photomicrograph of trachea with elastic fibers of tucuxi calf (HE); C: Tracheal bronchus photomicrograph of tucuxi calf (HE); D: Photomicrograph of fibrous tunic of the tucuxi calf (MEV); E: Visceral pulmonary pleura photomicrograph of adult boto (MEV). HC-hyaline cartilage, TA- Tunica adventitia fibrosa, CO-chondrocytes.

The trachea, as well as the tracheal bronchi and main bronchi of boto and tucuxi, presented elastic fibers in its fibrous adventitial tunica (Figure 3B). These are related to the depth of the dive, which was reported by Moore et al. (2011) on deep divers. However, for the boto and tucuxi the presence of elastic fibers likely guarantees higher ventilatory rates, since they live in limnetic regions (shallow regions).

Cetacean lung size has been described in several ways, including total lung mass, total lung volume and a variety of air storage capacity measurements (Piscitelli *et al*., 2010). The lungs have no lobes and the main bronchus is gradually reduced in its caudal and branched region in successive segmental bronchi (Piscitelli, 2009).

The pulmonary pleura (Figure 4B) of the boto and tucuxi has a lining function, and is formed by a broad serous layer of pseudo-stratified epithelium containing smooth muscle, elastic fibers, and blood vessels, which present in greater quantity in deep divers (Piscitelli et al., 2013). This thick pleura in aquatic mammals is suggested to confer rigidity to the lung, giving it greater resistance to compression during diving (Gray et al., 2006). There is also a layer of collagen fibers and fibroblasts, besides the smooth muscle that appears in the alveolar opening, forming the alveolar wall.

The lungs of both species have alveolar sacs composed of simple pulmonary alveoli, formed by connective tissue and small blood vessels (Figure 4A). The alveolar walls have dense alveolar septa composed of connective tissue at their ends (Figure 4B). It is believed that these septa serve for oxygen storage, as described by Shimokawa et al. (2011). Although Yamasaki (1977) did not mention the existence of alveolar ducts in *Platanista* sp. (Piscitelli, 2013), the alveolar ducts appear clearly in the lungs of river dolphins the present study.

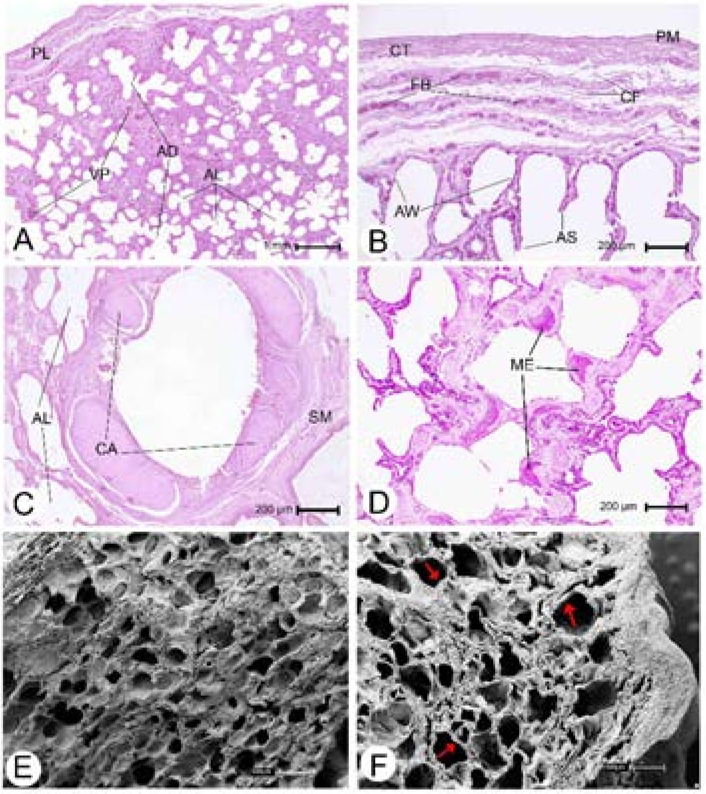

Figure 4- A: Adult tucuxi left lung photomicrograph; B: Photomicrograph of the detail of adult tucuxi (HE) left lung; C: Adult bronchodilator (HE) bronchodilator photomicrograph; D: Photomicrograph of detail of the presence of myoelastic sphincters in the adult tucuxi lung; E: Photomicrograph of adult tucuxi alveolar sacs; F: Photomicrograph of the alveolar walls of adult boto alveolar sacs (arrow). AS-Alveolar septum, PL-Pulmonary pleura, PM- Pleural mesothelium, CT- Pleural connective tissue, ME- Myoelastic sphincter, FB- Fibroblasts, CF- Collagen fibers, AW- Alveolar wall, AL- Alveolar sacs, PV- pulmonary vessels, AD-alveolar ducts, CA-cartilage, SM-submucosa.

The respiratory bronchioles (Figure 4C) of the boto and tucuxi are shaped like irregular rings and isolated parts, surrounded by hyaline cartilage. The bronchiole has its layer formed by serous tunic, with small amount of smooth muscle fibers. Yamasaki (2007) identified smooth muscles well developed in bronchioles in the lungs of *Pontoporia blainvillei* and Gray et al. (2006) describe a large number of smooth muscle fibers in the wall of the respiratory bronchioles in the leopard seal *Leptonychotes weddellii*, attributed by the authors to the species' need to reach great depths of diving. However, these characteristics are not present in boto and tucuxi, which are species of shallow dives and do not require large fibers of elastic fibers to perform their functions.

Although Yamasaki *et al.* (1977) did not describe the presence of myeloelastic sphincters in the river dolphins lungs, our findings demonstrated that myoelastic sphincters (Figure 4D) can be found in the proximities of the alveolar sacs of the two species of Amazonian cetaceans, a fact that resembles the study of Crespo & Cidre (2005), who showed the presence of myoelastic sphincter distributed near the alveolar sacs of the lungs of *Pontoporia blainvillei*. It is clear that these sphincters are more developed in species of deep diving, having as the function of regulating the air flow during the dive. However, in shallow divers, these sphincters are less developed and may be associated with the absence of alveolar collapses, that is, retraction of some or all of the alveoli (Crespo & Cidre, 2005).

4. Conclusion

Our findings indicate that the respiratory apparatus of Amazonian river dolphins is similar to other groups of aquatic mammals, including other cetaceans. Boto and tucuxis present tracheal bronchi, which is the main characteristic that differs from the lower respiratory system of these river dolphins with several species of terrestrial mammals, such as man or dog, that do not have this structure. In addition, the knowledge of this apparatus helps in understanding the physiology of diving and suggests the adaptation of the species to their habitat.

Resumen

Este estudio tuvo como objetivo caracterizar las estructuras del flujo respiratorio de dos especies de delfines de agua dulce: *Inia geoffrensis* y *Sotalia fluviatilis*. Los tractos respiratorios fueron estudiados con las técnicas de evaluación macroscópica y microscópica. En ambas especies se identificaron cuatro estructuras anatómicas macroscópicas: tráquea, bronquios principales, bronquio traqueal y los pulmones. La presencia de un bronquio traqueal exuberante sugiere aumento en el intercambio de gases y el aumento de tiempo de inmersión de las especies. El análisis histológico reveló la presencia de los conductos alveolares, y del esfínter mioelástico en los cetáceos amazónicos. La porción posterior respiratoria de los delfines del Amazonas tiene similitud con otras ballenas dentadas y su conocimiento puede contribuir a la comprensión de la fisiología del buceo y cómo estas especies están adaptadas a su hábitat.

Palabras clave: delfín rosado, delfín gris, anatomía.

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