







Morphology and Morphometry of the Magellanic Penguin (*Spheniscus magellanicus*) Trachea

Morfologia e morfometria da traqueia do pinguim-de-Magalhães (*Spheniscus magellanicus*)

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Abstract

Objective: This work aimed to describe the trachea of the Magellanic Penguin (*Spheniscus magellanicus*) through descriptive and morphometric approaches. **Methods:** ten adult male *Spheniscus magellanicus* were used, which were collected, fixed, and kept immersed in an aqueous solution of 10% formaldehyde. **Results:** the trachea of the penguin began caudally from the cricoid cartilage of the larynx, represented by 121.3 ± 5.65 cartilaginous rings joined together, which extended to the syrinx (19.75 ± 0.70 cm). Considering its five segments (I-V), the number of tracheal rings decreased in a cranio-caudal direction. The medial tracheal septum, which started caudally from the cricoid cartilage of the larynx, was approximately 2 ± 0.08 cm and was formed by approximately 6.75 ± 0.25 rings. The dimensions of the lumen of the trachea decreased cranio-caudally, with a statistical difference between segment I and the others (II to V). The syrinx of the tracheobronchial type presented 7.52 ± 0.65 rings. **Conclusions:** these data will contribute to the understanding of the anatomy of this species, as well as to the diagnosis and treatment of possible tracheal pathologies found in it.

Keywords: Marine Birds; Anatomy of Birds; Respiratory.

Resumo

Objetivo: o objetivo deste trabalho foi descrever a traqueia do pinguim-de-Magalhães (*Spheniscus magellanicus*), por meio de abordagens descritivas e morfométricas. **Metodologia:** foram utilizados dez *Spheniscus magellanicus*, adultos, machos que foram após coletas, fixados e mantidos imersos em solução aquosa, a 10% de formaldeído. **Resultados:** a traqueia do pinguim iniciou-se, caudalmente, a cartilagem cricoide da laringe, representada por $121,3 \pm 5,65$ anéis cartilagosos unidos entre si, que se estenderam até as sírinxes ($19,75 \pm 0,70$ cm). Considerando seus cinco segmentos (I-V), o número de anéis traqueais diminuiu seu número em sentido craniocaudal. O septo traqueal medial teve início caudalmente a cartilagem cricoide da laringe, aproximadamente $2 \pm 0,08$ cm, e foi formado por, aproximadamente, $6,75 \pm 0,25$ anéis. As dimensões do lúmen da traqueia diminuíram craniocaudalmente, com diferença estatística entre o segmento I e os outros (II a V). As sírinxes, do tipo traqueobronquiais, apresentaram $7,52 \pm 0,65$ anéis. **Conclusões:** esses dados contribuirão para o entendimento da anatomia dessa espécie, bem como para o diagnóstico e tratamento de possíveis patologias traqueais nela encontradas.

Palavras-Chave: Aves Marinhas; Anatomia das Aves; Respiratório.

INTRODUCTION

The Magellanic penguin (*Spheniscus magellanicus*) is the most frequent and only of the penguins found on the Brazilian coast. They live in the waters of the Atlantic and South Pacific oceans, on the coasts of Argentina, Chile, and the Falkland Islands. Under the influence of winter, they migrate to the Brazilian coast from Rio Grande do Sul to Pernambuco in search of warmer waters and richer food¹.

During the winter migration, *Spheniscus magellanicus* can reach the beaches, presenting a combination of severe dehydration, hypoglycemia, malnutrition, and hypothermia¹. This clinical condition requires procedures such as tracheal probing and intubation, but the particularities inherent in the anatomy of the species' trachea impose difficulty².

In this context, the morphology of the bird's respiratory system is of great relevance for the preservation and conservation of this species. To this end, specific knowledge of the trachea of the *Spheniscus*, in particular its topography, arrangement, trajectory, components, and dimensions, is essential.

The works of³⁻⁶, as well as⁷ on different birds, were compiled and mentioned trachea beginning at the level of the cricoid cartilage of the larynx, directing caudally with variable trajectory, until ending within the celomic cavity at the syrinx. The trachea was formed by the union of tracheal rings, cartilaginous and complete, with different shapes⁸ and dimensions along its entire extension⁵⁻⁷. In relation to the morphological aspects, mentioned that in penguins and some marine species^{12,13}, a tracheal septum

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was present in the trachea lumen, responsible for dividing the lumen of the organ ventrodorsally along its entire length, acting against the occurrence of tracheal collapse.

The knowledge of the morphology of the trachea of penguins is relevant when generating anatomical and clinical data for the species. The aim of this study was to describe the trachea of the Magellanic penguin (*Spheniscus magellanicus*) through descriptive and morphometric approaches.

METHODS

Ten Magellanic penguins (*Spheniscus magellanicus*) were used. Five animals were from the Veterinary Anatomy Laboratory of the Federal University of Paraná, and the other five were from the Veterinary Anatomy Laboratory of the University of Brasília. All were males, the length of the body extends from the atlanto-occipital interarcual space to the last coccygeal vertebra 42,4±3,8 cm, fixed, and kept immersed in an aqueous solution of 10% formaldehyde (LABSYNTH - Laboratory Products Ltda). This study did not require official or institutional ethical approval. The animals were handled according to high ethical standards.

The access and visualization of the trachea were obtained through a ventral section of the skin of the cervical region, from the rostral end of the gnathotheca to the sternum. The sternum had its articulations with the right and left coracoid bones sectioned, allowing the visualization of the organs arranged cranially in the celomatic cavity. The vessels of the base of the heart were dissected and displaced laterally by sections, allowing the visualization of the bifurcation of the trachea, the syrinx, and finally, the right and left main bronchi. Then, with the help of a binocular stereoscope (Global Optics® NO106), the underlying tissues were divulged until the identification and isolation of the trachea were achieved. Its anatomical description, components, trajectories, and arrangements were determined, as well as its beginning - caudally to the cricoid cartilage of the larynx; and its end - cranially to the syrinx when emitting the right and left main bronchi.

Using a digital caliper (Starret EC799-A), the total length of the trachea (cm) was measured, from its beginning caudally to the cricoid cartilage of the larynx, to its end caudally to the syrinx. Using the binocular stereoscope (Global Optics® NO106), the trachea was divided into five segments, I to V, in the cranio-caudal direction. We have adopted a criterion for determining the segments (I-V) based on regional skeletopy, specifically involving cervical vertebrae and the first thoracic vertebra. This ensures safety for clinical/surgical/anesthetic procedures in *Spheniscus*. Their lengths (cm) were also evaluated in the same way as the total length of the trachea.

With the binocular stereoscope (Global Optics® NO106), the total number and each of the segments of tracheal rings was determined. Ventrally, the tracheal rings presented a median groove that projected internally as a complete septum, the medial tracheal septum. This septum was responsible for the

bilateral division of the trachea into two fully individualized lumens, from segment I to the right and left syrinx. The dimensions of the right and left lumen were determined using the digital caliper (Starret EC799-A) along the segments.

All variables were presented as mean ± standard error of the mean. The numbers of tracheal rings, lengths, and lumen, total and of each segment, were evaluated for normality using the Kolmogorov-Smirnov test. Then, submitted to the application of the paired Student's t-test, $p \leq 0.05$. With the help of the GraphPad Prism program (Prism version 6.0c for Mac, GraphPad Software, La Jolla, CA).

RESULTS

Anatomical Description, Components, Pathways, and Arrangements

The trachea of the Magellanic penguin (*Spheniscus magellanicus*) displayed a tracheobronchial arrangement. Starting immediately caudal to the cricoid cartilage of the larynx, represented by interconnected cartilaginous rings, extending to the right and left syrinx.

It was possible to observe a standardized route between the evaluated specimens. The trachea was located in its initial third along the cervical region, ventral to the esophagus, and in a median position with the cervical vertebrae. In its middle third, the trachea shifted laterodorsally to the right of the esophagus, and finally, in the final third, it was located lateroventrally to the right of the esophagus due to the presence of a sharp bend. From then on, it continued its ventromedial route to the esophagus, extending caudally until ending in the right and left syrinx. Along the route, syntopic relationships were established with cervical structures such as cervical vertebrae, esophagus, thyroid glands, and right and left brachiocephalic arteries.

The trachea of the Magellanic penguin was represented by thin and complete cartilaginous rings, joined together by tracheal annular ligaments (Figures 1A and 1B). The tracheal rings showed an oval shape, flattened dorsoventrally, and their greatest dimension occurred in a transverse axis, arranged laterolaterally. The rings presented a deep sulcus on their ventromedial surface (Figures 1A and 1B). The sulcus was reflected in the lumen of the trachea, continuing as a complete septum, the medial tracheal septum (Figure 1C). Together, the union of the septa of each of the tracheal rings was arranged, ventrodorsally, and caudally from the cricoid cartilage of the larynx to the syrinx. Making the lumen of the trachea divided along the entire route (Figures 1C and 1D).

The syrinx right and left were characterized as tracheobronchial and were arranged inside the celomatic cavity, caudally to the trachea bifurcation (Figures 1B and 1E). They presented externally as a very pronounced narrowing, laterolaterally, and they continued caudally into the corresponding main bronchi, right and left (Figures 1B and 1E).

Morphometry

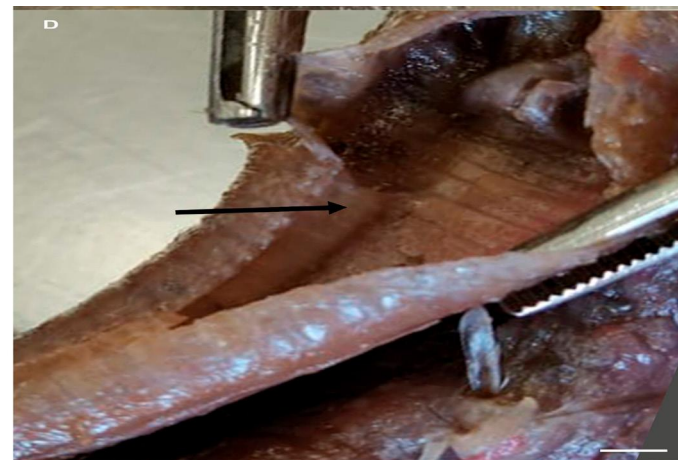
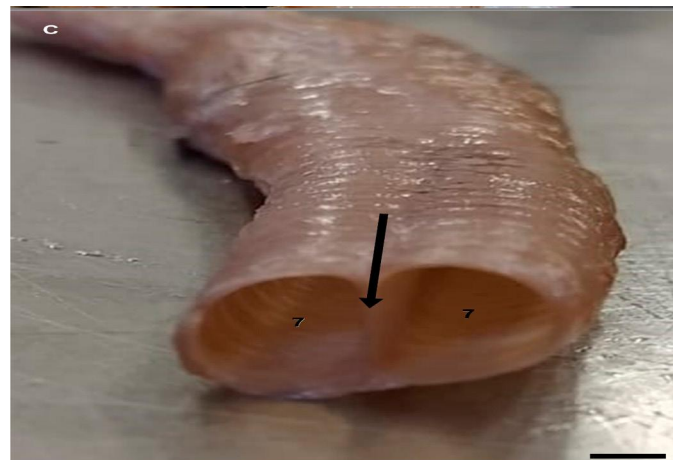
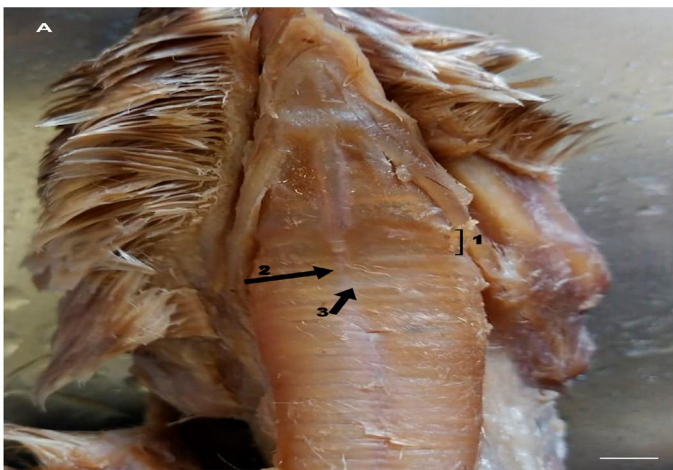
The trachea of the Magellanic penguin presented a total length, from its beginning caudally to the cricoid cartilage of the larynx to the syrinx 19.75±0.70 cm. Formed by the union of the tracheal rings between them, which varied 121.3±5.65 rings along its entire extension. Considering its five segments arranged cranially to caudally until its end, it was possible to observe that the number of tracheal rings decreased compared to the first segment (I) 21.5±4.22. In comparison to the second segment (II), this decrease was approximately 2.33%, third (III) 12.56%, fourth (IV) 7.90%, and fifth (V) 17.21% (Figure 1E). The absolute data, mean, and standard error of the mean, were submitted to the application of the Student's t-test and did not present a statistical difference $p \leq 0.05$.

The median tracheal septum (Figures 1C and 1D), which

longitudinally separated the trachea, began caudally to the cricoid cartilage of the larynx, approximately 2±0.08 cm. The 6.75±0.25 rings were observed along this space.

Regarding the variations of the lumen of the trachea, it was possible to observe that in comparison to the lumen of the first segment (I) 1.49±0.03 cm, there was a relative decrease of 20.25% for the second (II), 23.59% for the third (III), 31.50% for the fourth (IV) and 27.81% for the fifth segment (V) (Figures 1C, 1D and 1E). A statistically significant difference was evidenced when applying the Student's t-test, $p \leq 0.05$ between segment I and each of the others (Table 1). Caudally, the diameters between the segments showed small variations among themselves, revealing a more uniform pattern along the trajectory (Table 1).

Figure 1. (A) Ventral view of the cervical region of Magellan penguin - delimitation of the first tracheal rings (1), tracheal ring (2), and ventral median sulcus (3), magnification x5. (B) The final third of the trachea, segment V of the trachea (6), circle and arrow (4) highlighting the end of the trachea, immediately cranial the presence of the syrinx (5), magnification x10. (C) Rostral view of the trachea, sectioned transversely and highlighting the medial tracheal septum (black arrow) and the different lumen (7) of the trachea, magnification x10. (D) Ventral view of a longitudinal section of the trachea, medial tracheal septum (black arrow), magnification x10. (E) A right lateral-lateral radiograph of a penguin, illustrating each segment (I to V) of the trachea, yellow circle delimiting and pointing to the left syrinx (white arrow-asterisk), magnification x2. (F) Ventral view of the cervical region of penguin, representing through the dashed white line the flexure of the trachea, esophagus (white arrow), thyroid glands (yellow arrows), brachiocephalic arteries (red arrows), magnification x10.



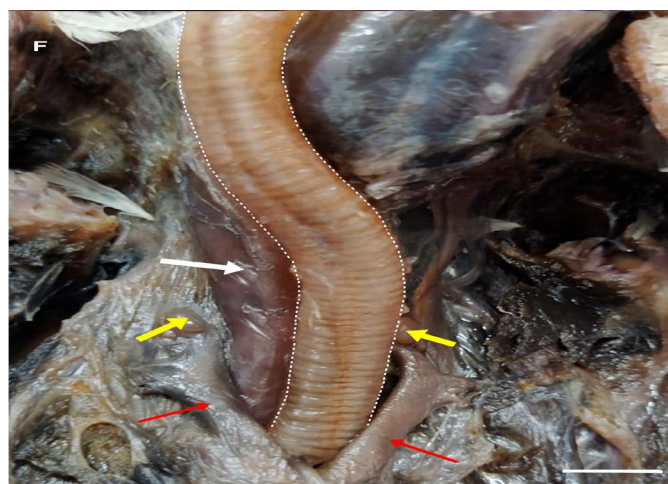
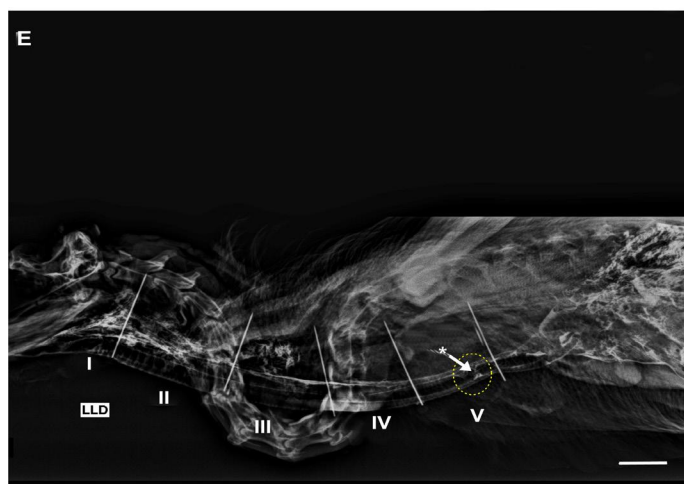


Table 1. Diameter of the lumen of the segments (I to V) of the trachea (cm). The data were submitted to the application of the Student's t-test, comparing the length of segment I with the others (II to V), $p \leq 0.05$.

Segments	Mean±standard error of the mean	p
I	1,49±0,032	-
II	1,19±0,028	0,0022
III	1,14±0,033	0,0017
IV	1,02±0,064	0,0030
V	1,07±0,013	0,0003

The presence of the medial tracheal septum, arranged along the trachea, enabled the verification of different dimensions for the right and left lumen (Figures 1C and 1D) in each of the segments (Table 2).

Table 2. Diameters of the lumen of the segments (I to V) of the trachea (cm), right and left. The data were submitted to the application of the Student t-test, comparing the length of segment I with the others (II to V), $p \leq 0.05$.

Segments	Righth		Left	
	Mean±standard error of the mean	p	Mean±standard error of the mean	p
I	1,49±0,032	-	1,49±0,032	-
II	0,62±0,038	$\leq 0,001$	0,53±0,034	$\leq 0,001$
III	0,54±0,014	$\leq 0,001$	0,56±0,034	$\leq 0,001$
IV	0,49±0,031	$\leq 0,001$	0,49±0,043	$\leq 0,001$
V	0,52±0,028	$\leq 0,001$	0,50±0,043	$\leq 0,001$

The tracheobronchial-type syrinx were arranged within the coelomic cavity, caudally to the trachea bifurcation. They presented 7.52 ± 0.65 rings, marking the end of the trachea and the beginning of the right and left main bronchi. The main bronchi of the penguin presented around 8.5 ± 0.87 rings up to its division into

lobar bronchi within the pulmonary parenchyma (Figures 1B and 1E).

DISCUSSION

Evolutionarily in reptiles, mammals, and birds, considered that tracheal displacement was asymmetrical, along with the esophagus, along the ventral midline of the neck. Being able to move laterally allows the trachea to remain short and straight and accommodate the demands of a mobile and flexible neck in the cervical region. Thus, characterizing a typical arrangement of vertebrates, the trachea itself underwent a slight or accentuated lateral rotation concerning the other organs of the cervical region, especially the esophagus, mainly in birds with short necks. In the Magellanic penguin, the pathway of the trachea in the cervical region can be seen as a common pattern among birds and vertebrates. Due to its behavior along the course and syntopic disposition with the other organs located in the cervical region, even highlighting the flexural arrangement and projections evidenced in the specimens. Similarly to what was proposed by, in different classes of vertebrates, in penguins, the trachea extends to reach a length almost equal to that of the neck.

In the Magellanic Penguin, the start and end of the trachea revealed similarities with in quails and in the Avialae class occurring at the level of the cricoid cartilage of the larynx, heading caudally with a variable path, ending in the celomic cavity in the syrinx. Minor variations were evidenced in *Buteo rufinus* when the trachea started caudally of the cricoid cartilage of the larynx, passed ventrally to the esophagus, then shifted to the right side of the neck, ventromedially supporting the ingluvium, heading caudally and ventrally and ending in the syrinx.

In geese that the trachea at the initial third had a ventral disposition to the midline of the vertebral column and the esophagus, assuming a lateral right position along the neck, and at the entrance of the celomic cavity it became ventral to the esophagus again. The flexion of the neck did not modify the syntopical relation established between the vertebral column

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and the trachea, remaining ventral to the vertebral column⁵. This differed from the reports in birds when they considered that the trachea was cited as a cylindrical.

In *Buteo rufinus*⁴, the trachea had a tubular shape with an empty interior, made of complete tracheal rings, narrowed dorsally and ventrally. The narrowing in the dorsal and ventral shape of the tracheal rings was similar to the literature⁸. It is believed that due to the narrowing, the tracheal rings overlapped each other, causing variation in trachea length⁸.

In the domestic goose, verified along the trachea that the shape of the lumen and the diameter varied in their proportions, from rounded to oval⁵. For the same species, the rings in the cranial half of the trachea could interlock dorsally and ventrally, presenting in this way the shape of an "H" with grooves that project internally¹¹. The trachea was described to be composed of complete cartilaginous rings⁷, as observed in the Magellanic penguin. The oval shape of the trachea of the Magellanic penguin revealed, as presented by⁶, a certain rotation so that the lateral points of the trachea are always positioned dorsally and ventrally. We understand this as a preventive mechanism to avoid obliteration and alterations in the shape of the organ.

The number of tracheal rings was found to be quite variable among birds, ranging from 120 in ducks⁹ and about 85 in quails³. In *Buteo rufinus*⁴, found the trachea formed by 89 to 96 tracheal rings. Domestic geese⁵ had 144 complete cartilaginous tracheal rings. Even for the same species¹¹, evidenced from 137 to 140 tracheal rings. The trachea of the king penguin measured from 27-29 cm in length and had around 125 cartilaginous rings¹³. In the birds investigated, the trachea measured about 19 cm and had 121.3±5.65 tracheal rings. Thus, revealing that the values counted were generally within the expected range for birds when compared to the consulted literature. It was not possible, specifically, to establish relationships between the number of tracheal rings and the trachea length or the dimensions of the animals, that is, body length or weight, or even factors specifically related to sexual dimorphism.

Regarding morphological aspects, in quails, there was a variation in the craniocaudal dimensions of the tracheal rings, measuring 4.3±0.3 mm in length and 6.3±0.3 mm in width at

the beginning, 4.1±0.2 mm in length and 4.8±0.2 mm in width in the middle and 3.9±0.2 mm in length and 4.0±0.2 mm in width at the end of the trachea³. In *Buteo rufinus*⁴ there was a narrowing in the shape of the dorsal and ventral sections of the tracheal rings, promoting their overlap. The lumen of the trachea of the domestic goose underwent variation in its shape along its length⁵. In the 90-95th tracheal ring, the diameters were smaller than those evidenced after the 117-118th tracheal rings in the goose¹¹. The king penguin¹³ had its width increased caudocranially, on an average of 16%. The Magellanic penguin showed evident changes in the lumen in the different segments evaluated (I-V) - craniocaudally in the species. Above all, it was not possible to characterize an increasing or decreasing pattern, given the oscillation of the dimensions evidenced by the lumen of the segments. A fact that allowed us to extrapolate specifically for the species in question due to the scarcity of data in the available literature.

The Magellanic penguin presented a tracheal septum medial, dividing the lumen of the trachea ventrodorsally along its entire length, up to the beginning of the syrinx, with varying dimensions between segments (I-V). The trachea of the king penguin was separated by a septum, occupying approximately 78% of its length, acting to prevent tracheal collapse while penguins ingest prey in deep depths¹³. Medial tracheal septum were also present in other penguin species¹².

CONCLUSIONS

The trachea of the Magellanic penguin (*Spheniscus magellanicus*) was formed by the union of tracheal rings, having its beginning caudal to the cricoid cartilage and its end immediately cranial to the syrinx. It was flattened dorsoventrally, with a deep groove on its ventromedial face, internally reflecting the median tracheal septum, which divided the trachea along its path, with different dimensions for the right and left lumen, in each segment.

Therefore, the findings of this study showed that the trachea of *Spheniscus magellanicus* follows a generalized pattern of architecture and function, adapting to the requirements of its species. These data will contribute to the understanding of the anatomy of this species, as well as to the diagnosis and treatment of possible tracheal pathologies found in it.

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