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ANIMAL SCIENCE

# Morphological comparison of the larynx and trachea of *Chelonia mydas* (Linnaeus, 1758), *Caiman yacare* (Daudin, 1802) and *Caiman latirostris* (Daudin, 1802)

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**Abstract:** The larynx is in the lower respiratory tract and has the function of protecting the airways, controlling, and modulating breathing, assisting the circulatory system, and vocalizing. This study aims to describe the anatomy and histology of the skeleton of the larynx and trachea of the species *Chelonia mydas*, *Caiman yacare* and *Caiman latirostris*. The study was conducted at the Federal University of Espírito Santo (UFES), using nine specimens of *Ch. mydas*, 20 of *Ca. yacare* and four of *Ca. latirostris*. Samples of the larynx and trachea were collected, fixed, and sent for dissection of the structures and subsequent macroscopic analysis. For histology, samples were processed by the routine paraffin embedding method and stained with hematoxylin-eosin and Verhoeff. For the three species, two arytenoid cartilages, a cricoid cartilage, a hyoid apparatus composed of a base and two horns were found. In *Ch. mydas*, two structures called thyroid wings were observed, not found in crocodilians. The trachea of crocodilians presented incomplete tracheal rings and musculature, while the trachea of *Ch. mydas* presented complete tracheal rings. Histologically, the entire cartilaginous skeleton of the larynx of the three species, as well as the tracheal rings, are constituted by hyaline cartilage.

**Key words:** Morphology, anatomy, laryngeal skeleton, respiratory system, reptiles.

## INTRODUCTION

"Reptiles" is a group with great diversity of species that differ morphologically and physiologically from each other (Kardong 2016, Shine 2005). Within this group, Testudines and Crocodylia have a respiratory system adapted to aquatic environments. The larynx is a part of this system found in the lower respiratory tract. It has four main functions among vertebrate: protecting the airways, controlling and modulating breathing, assisting in the circulatory system, and vocalization. This structure performs its function by reflex, closing and blocking the passage of air, or protecting the respiratory tract against the entry of water (Kirchner 1993). Among the structures found in the larynx, the glottis is in the caudal portion of the tongue and is supported by the hyoid apparatus. The glottis controls the passage of air, opening and closing during the breathing process (Kardong 2016, Wyneken 2001). The larynx connects caudally to the trachea, which is a long structure composed of cartilaginous rings. The trachea branches into two pulmonary bronchi, which are responsible for moving air to the lungs (Kardong 2016).

Comparative studies about the anatomy of reptiles are scarce, and little is known about the morphology of their respiratory system.

However, research on ecology (Filippini & Bullhões 1988, Freitas-Filho, unpublished data), nutritional patterns (Aleixo et al. 2002, Sarkis 2002, Sazima & Sazima 1983), and reproduction of *Chelonia mydas* (Linnaeus, 1758), *Caiman yacare* (Daudin, 1802), and *Caiman latirostris* (Daudin, 1802) are commonly found (Camillo et al. 2009, Silva & Antas 1981), as these animals have high ecological and commercial value.

Describing the morphology of the species *Ch. mydas*, *Ca. yacare,* and *Ca. latirostris* is essential to understand their physiological and behavioral patterns. Also, the delineation of animal morphological characteristics is necessary to understand differences and similarities that group different taxa. Research on the larynx of testudines is scarce, and for crocodilians they are non-existent. Comparative morphological data about animals that live on the borderline of the aquatic and terrestrial environment help us understand how different groups evolved in such conditions (Grigg & Kirshner 2015). Therefore, this study aims to describe the anatomy and histology of the skeleton of the larynx and trachea of the species *Ch. mydas*, *Ca. yacare,* and *Ca. latirostris* and to compare the three species, reporting the similarities and differences in each of them.

## MATERIALS AND METHODS

The study was conducted at the Federal University of Espírito Santo (UFES), Alegre campus, Espírito Santo, Brazil, and approved by the Animal Use Ethics Committee (CEUA) of the University, under protocol no. 13/2020.

### Characterization of specimens

Nine free-living female specimens of the species *Ch. mydas* were used. The turtles were found lifeless on the coast of Anchieta municipality, Espírito Santo, Brazil, collected, and donated to Universidade Federal do Espírito Santo (UFES) for research by the Marine Research and Conservation Institute (IPCMar), also located in Anchieta (Table I).

Four specimens of the species *Ca. latirostris* were used, three females and one male. The females were captive adults, and the adult male was a free-ranging animal. The three females were from the "Criadouro 2C", a private breeding area in Cachoeiro de Itapemirim municipality, Espírito Santo, Brazil. They were euthanized for sanitary reasons due to the closure of the site. The adult male specimen was captured in the wild and taken to the Caiman Project facilities for examination, but later died. All specimens were donated by Projeto Caiman to UFES for research.

The larynx, trachea, and lungs of twenty specimens of *Ca. yacare* were collected at Caimasul breeding company, located in Corumba municipality, Mato Grosso do Sul, Brazil. It was not possible to identify the sex of these animals, as the carcasses came from the slaughter line

**Table I. Identification of the specimens of *Chelonia mydas* used in the research. ID: Identification. CCC: Curvilinear carapace length. LCC: Curvilinear carapace width. F: Female.**

| ID | Sex | Weight (kg) | CCC (cm) | LCC (cm) |
|---|---|---|---|---|
| N123/21 | - | 7,18 | 42,50 | 38,00 |
| N125/21 | F | 7,42 | 41,00 | 36,50 |
| N126/21 | F | 4,16 | 34,50 | 29,00 |
| N61/22 | F | 9,04 | 50,00 | 43,00 |
| N62/22 | F | 13,40 | 50,00 | 45,00 |
| N63/22 | F | 4,42 | 34,50 | 31,00 |
| N64/22 | F | 9,32 | 46,00 | 40,50 |
| N65/22 | F | 4,66 | 39,00 | 36,50 |
| N66/22 | F | 3,06 | 32,50 | 29,00 |

production without the reproductive tract. All animals came from the 2018 egg harvest; thus, all specimens were four years old.

The specimens of *Ch. mydas* and *Ca. latirostris* were necropsied, following routine techniques according to the sanitary parameters of the Animal Pathology Laboratory of the Veterinary Hospital at UFES, where larynx and trachea samples were collected from females of both species. For the free-ranging male of *Ca. latirostris*, only a laryngeal sample was collected (Table II). For the species *Ch. mydas*, the animals were previously identified, and their weight, curved carapace length (CCC), and curved carapace width (LCC) were measured (Table I). They were also photographed. For specimens of *Ca. latirostris*, the animals were previously identified (ID), photographed, and their total length (CT), rostrum-cloacal length (CRC), and skull length (CC) were measured (Table II).

The specimens of *Ca. yacare* were not weighed or measured, being only identified from N1 to N20, due Caimasul breeding company protocols. However, the total length of the specimens ranged from 100 to 150 centimeters. Samples were collected from the viscera section, while the larynx, trachea, and lung were separated from the remaining organs, such as the stomach and intestine.

### Preparation of specimens

The following procedures were performed for *Ch. mydas* and *Ca. latirostris*. The collected samples were fixed in a 10% formaldehyde solution, labeled and separated into identified plastic containers. All procedures were done at UFES. For *Ca. yacare*, the samples were collected and taken to Caimasul laboratory, where they were fixed in a 10% formaldehyde solution. After about seven days, the samples were taken to the Laboratory of Animal Anatomy at UFES. Then, all larynges were dissected using tweezers, scissors, and a scalpel to expose the cartilaginous structures. For the macroscopic analysis of the trachea, the number of cartilaginous rings present in each specimen studied was counted. After counting, the arithmetic mean and standard deviation were estimated for each species.

**Table II. Identification of specimens of *Caiman latirostris* used in the research. ID: Identification; CT: Total length; CRC: rostrum-cloacal length.**

| ID | Sex | CT (cm) | CRC (cm) |
|---|---|---|---|
| N164 | Female | 178 | 90 |
| N165 | Female | 172 | 90 |
| N166 | Female | 168 | 94 |
| N01/21 | Male | 217 | 110 |

### Histological analysis

The cartilaginous skeleton of the larynx was cleaved, and the fragments were placed in separately identified cassettes. Then, they were soaked in 70% alcohol and taken to the Laboratory of Animal Pathology of the Veterinary Hospital at UFES for processing according to the laboratory's routine histology techniques, embedded in paraffin and cut in a microtome, with a 5µm thickness, and stained in hematoxylin-eosin and Verhoeff. The slides were read using a Leica DM500 photomicroscope, and the material was photographed.

## RESULTS

### Anatomical characterization of the larynx

In the three species studied, the larynx is the third structure of the respiratory system, being observed in the lower respiratory tract. It is found at the base of the tongue and is in the caudomedial portion of the oral cavity, in the cranial trachea (Figure 1).

The skeleton of the larynx of female *Ch. mydas* shows no intraspecific variation (Figure 1a), being narrow, positioned in the caudomedial

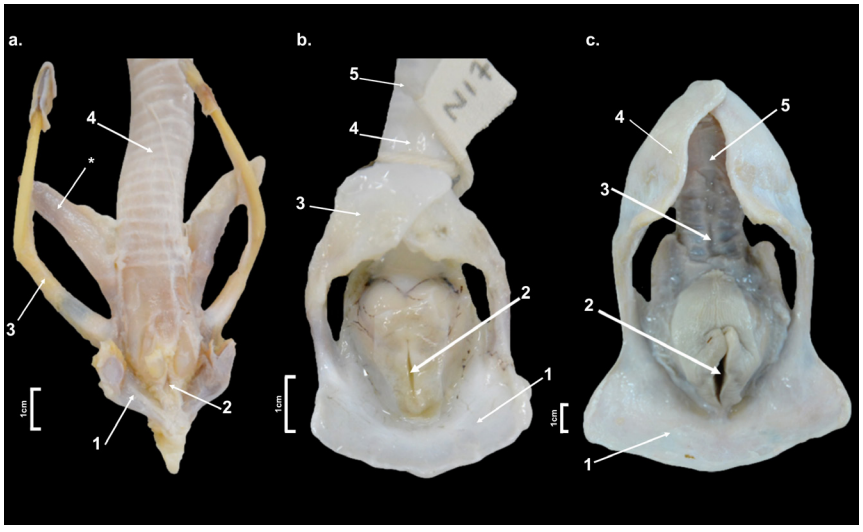


**Figure 1. Dorsal view of the larynx of the species *Chelonia mydas* (a), *Caiman yacare* (b) and *Caiman latirostris* (c) in the study. a) *Chelonia mydas*. 1. Base of the hyoid apparatus. 2. Opening of the glottis. 3. Hyoid bone. 4. Trachea. Asterisk: Thyroid wing. b) *Caiman yacare*. 1. Basihyoid. 2. Opening of the glottis. 3. Horn of the hyoid apparatus/hyoid bone. 4. Tracheal muscle. 5. Trachea. c) *Caiman latirostris*. 1. Basihyoid. 2. Opening of the glottis. 3. Horn of the hyoid apparatus/hyoid bone. 4. Tracheal muscle. 5. Trachea.**

portion of the animals' oral cavity, connecting to the trachea in its caudal portion (Figure 2b). It presents the hyoid apparatus from which four thin prominences arise: two bony and two cartilaginous, which project craniocaudally in relation to the animal's body. The hyoid apparatus is formed by the hyoid bone (Figure 2a item 2), by the thyroid wings (Figure 2b item 5), and by an internally ossified base covered by cartilage (Figure 2a item 4). The two tapered structures in the rostral region are the hyoid bone, located laterally on both sides of the apparatus, compact, tapered, and with an arch projected laterally in the middle of the structure. They are formed by bone tissue and articulate with the base of the hyoid apparatus (Figure 2a). The two most caudal structures are the wings of the thyroid cartilage, formed by cartilaginous tissue and observed on both sides of the hyoid apparatus, flattened dorsoventrally, and tapered laterocaudally (Figure 2a).

The opening of the glottis is formed by three cartilages, two of which are connected arytenoids, and a cartilaginous ring called the cricoid (Figure 2b and 2c). The arytenoid cartilages are found dorsally, being positioned side by side, supported by the cricoid cartilage. In the craniodorsal portion of the arytenoid cartilages, two prominences can be observed, forming two small and rounded structures (Figure 2c item 6). The cricoid cartilage is concave in relation to the arytenoids; however, it is convex when viewed ventrally, in relation to the hyoid apparatus.

The larynges of *Ca. yacare* (Figure 3) and *Ca. latirostris* (Figure 4) are in the caudomedial portion of the oral cavity, as observed in *Ch. mydas*. Despite showing the same structures, the larynx of the crocodilians is wider when compared with *Ch. mydas*. Four cartilaginous structures are observed; the basihyoid, a cricoid cartilage, which forms a ring and connects to the first cartilaginous ring of the trachea (Figure 3d and 4d), two arytenoid cartilages, which are surrounded by the cricoid cartilage, and together form the opening of the glottis (Figure 3c and 4c). The basihyoid has two other identical structures constituted partly of cartilage and bone, which project craniocaudally in relation to the body of the animal and are articulated in the left and right lateral portions of the basihyoid, like two horns. These structures unite and form a bridge dorsally to the arytenoid and cricoid cartilages and to the beginning of the trachea (Figure 3b and 4b).

The arytenoid cartilages in *Ca. latirostris* form two semicircles when observed

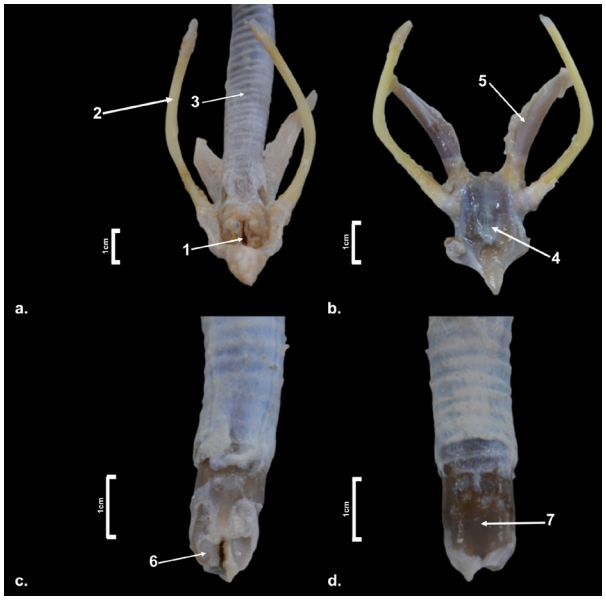


**Figure 2. Photomacroscopy of the larynx of *Chelonia mydas*. a) Dorsal view. 1. Opening of the glottis. 2. Hyoid bone. 3. Trachea. b) Dorsal view. 4. Base of the hyoid apparatus. 5. Thyroid wing. c) Dorsal view. 6. Arytenoid cartilage. d) Ventral view. 7. Cricoid cartilage.**

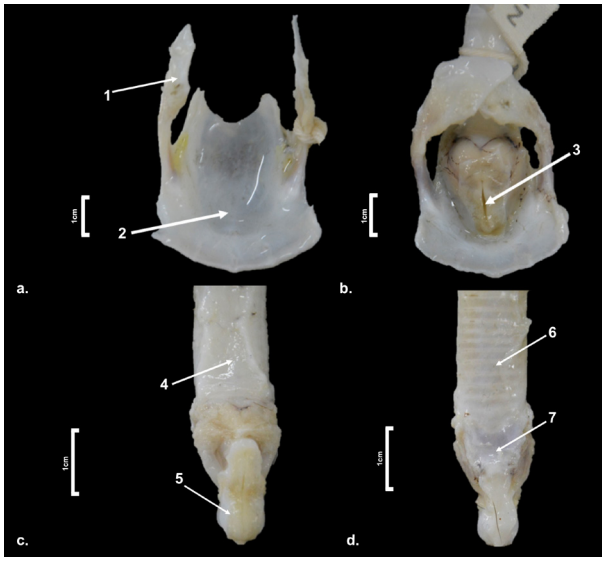


**Figure 3. Photomacroscopy of the larynx of *Caiman yacare*. a) Dorsal view. b) Dorsal view. c) Dorsal view. d) Ventral view. 1: Hyoid bone; 2: Basihyoid. 3: Opening of the glottis. 4: Tracheal muscle. 5: Arytenoid cartilage. 6: Trachea. 7. Cricoid cartilage.**

dorsoventrally (Figure 4c item 5), whereas the same cartilages in *Ca. yacare* have a more laterally angled prominence in their caudal portion (Figure 3c item 5). The cricoid cartilage is connected caudally to the trachea, being the most caudal structure in the laryngeal region. It is observed in the ventral view of the larynx. Its wide and circular base is ring-shaped, and its rostral portion forms an inverted V shape, where the arytenoid cartilages are connected by the cricoarytenoid joint (Figures 3d and 4d). The main difference observed in the larynx of *Ch. mydas* compared to the larynx of *Ca. yacare* and *Ca. latirostris* was the presence of the thyroid cartilage wing (Figure 2b), which is not observed in the larynx of crocodilians. All three species do not have epiglottis cartilage. Also, the hyoid apparatus in the three species supports the tongue and floor of the mouth (Figure 2b, Figure 3a, and Figure 4a). Although it was not possible to determine the sexes of *Ca. yacare* specimens, no anatomical differences were observed in the skeleton of the larynx. As in *Ca. latirostris*, no differences were observed between females and males.

## Histological characterization of the larynx

The cartilaginous skeleton of the larynx of the three species is constituted of hyaline cartilage (Figures 5, 6, 7, and 8), the most common cartilaginous tissue, which contains mostly fibrils of type II collagen, with the absence of elastic fibers observed by Verhoeff staining. The chondrocytes had an elongated shape and are located within gaps formed by cavities in the extracellular matrix and form isogenous groups at the base of the hyoid apparatus of *Ch. mydas* and *Ca. yacare* (Figure 6). The perichondrium, a connective sheath bathed by nerves and blood and lymphatic vessels, lining the cartilaginous tissue, was observed.

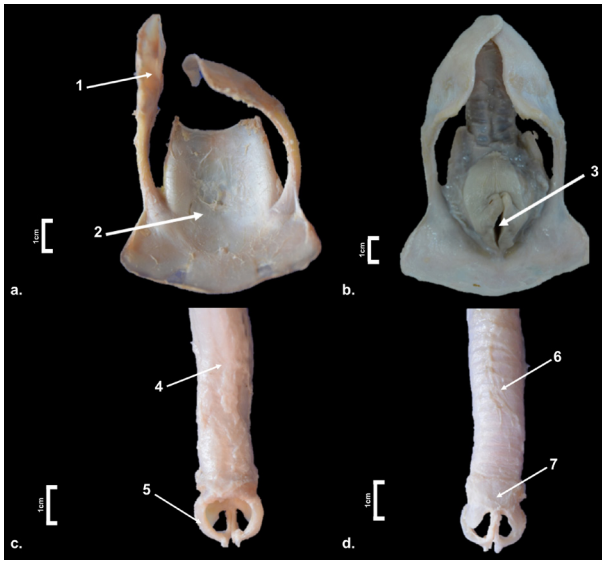


**Figure 4. Photomacroscopy of the larynx of *Caiman latirostris*. a) Dorsal view. b) Dorsal view. c) Dorsal view. d) Ventral view. 1: Hyoid bone. 2: Basihyoid. 3: Opening of the glottis. 4: Tracheal muscle. 5: Arytenoid cartilage. 6: Trachea. 7. Cricoid cartilage.**

### Morphological characterization of the trachea

The trachea of the three species is similar (Figure 2a item 3, Figure 3d item 6, and Figure 4d item 6). In all animals, it is positioned in the body mediastinum, from the neck to the thorax, reaching the lungs. It is a tube-shaped structure, slightly flattened dorsoventrally, and throughout its length is formed by a sequence of cartilaginous rings, united by the annular ligament (Figure 2a item 3, Figure 3d item 6, and Figure 4d item 6). In the caudal portion, it branches into two pulmonary bronchi through the carina of the trachea, which reach the lungs. The tracheal rings of the *Ch. mydas* species are complete (Figure 2a item 3), with the absence of the tracheal muscle. For *Ca. yacare* and *Ca. latirostris*, the tracheal rings are incomplete, with tracheal muscle in the dorsal portion of the trachea (Figure 3c item 4 and Figure 4c item 4). The number of tracheal rings varied according to the species (Table III). *Ch. mydas* had a mean of 40 tracheal rings, and *Ca. yacare* 69 and *Ca. latirostris* had a mean of 64 tracheal

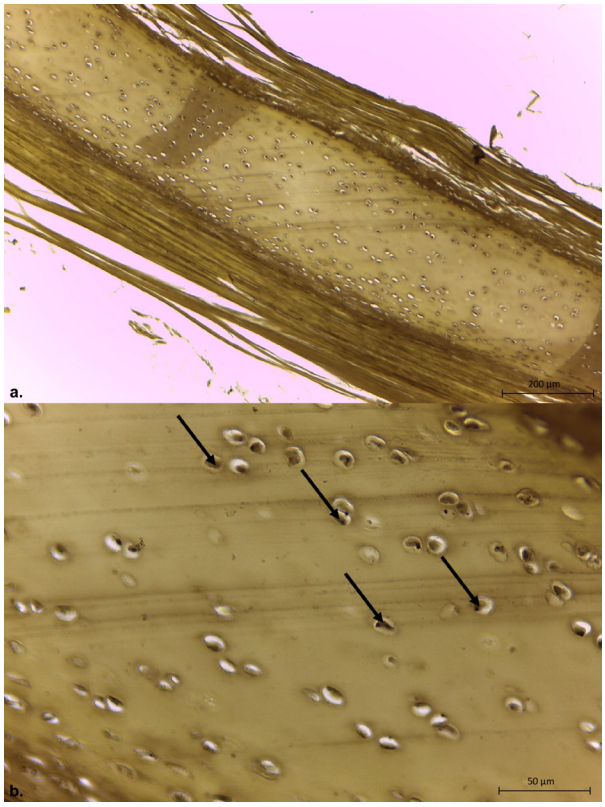


**Figure 5. Photomicroscopy of the thyroid wing of *Chelonia mydas*. Verhoeff staining. a) 10× objective. b) Arrows: Chondrocytes within the gap. 40× objective.**

rings. Histologically, the trachea of the three species presents a pseudostratified cylindrical ciliated epithelium, and the tracheal rings are constituted of hyaline cartilage, formed by elongated chondrocytes within gaps in the extracellular matrix, forming isogenous groups (Figure 9).

## DISCUSSION

In tetrapods in general, the larynx functions as a vocal organ, however, exclusively in birds the specific structure responsible for phonation is the syrinx. However, because crocodilians and birds are phylogenetically close, birds will be used in comparing the anatomical structure of the larynx and trachea. As observed in other tetrapods, both *Ch. mydas*, and *Ca. yacare* and

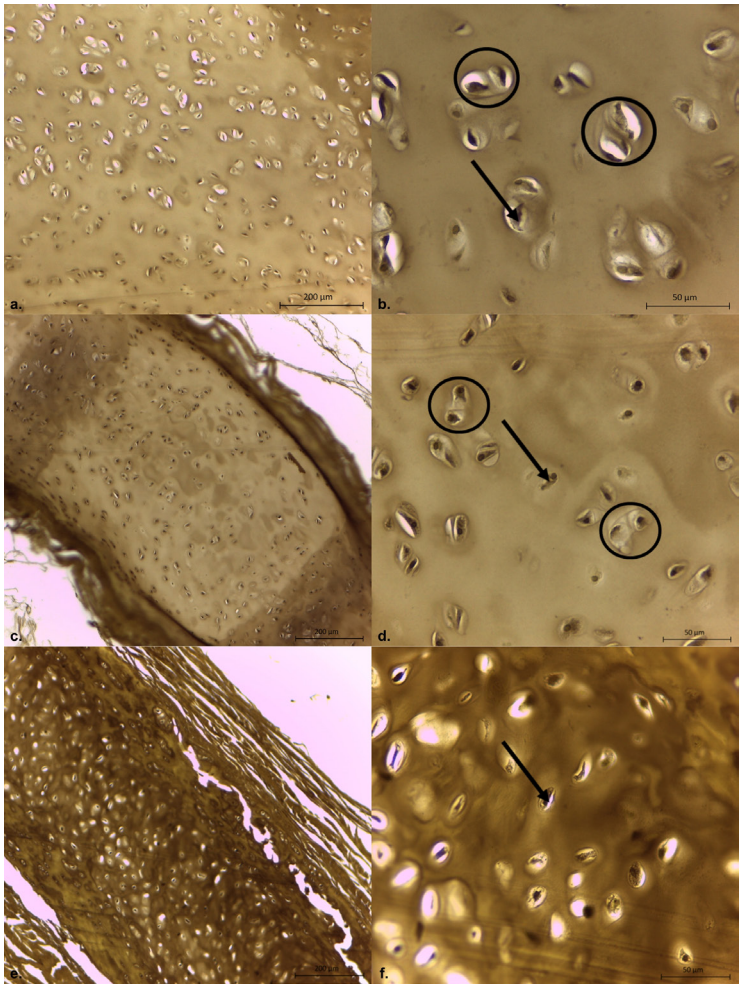


**Figure 6. Photomicroscopy of the base of the hyoid apparatus of *Chelonia mydas*, *Caiman yacare*, and *Caiman latirostris*. Verhoeff staining. a) *Chelonia mydas*. 10× objective. b) *Chelonia mydas*. Arrows: Chondrocytes within the gap forming isogenous groups (circles). 40× objective. c) *Caiman yacare*. 10× objective. d) *Caiman yacare*. Arrows: Chondrocytes within the gap forming isogenous groups (circles). 40× objective. e) *Caiman latirostris*. 10× objective. f) *Caiman latirostris*. Arrow: Chondrocytes inside the gap. 40× objective.**

*Ca. latirostris* have two arytenoids and one cricoid cartilage.

The larynx of *Ch. mydas* is in the caudomedial portion of the oral cavity and connects caudally to the trachea, a tubular structure of complete cartilaginous rings, as described by Stuart (unpublished data) for the species *Chelonoidis carbonaria* (Spix, 1824), commonly known as the red-footed tortoise. According to this author, the larynx and its muscles are supported by the hyoid apparatus, which is formed by three cartilages: two arytenoids and one cricoid, lacking the epiglottis cartilage, which was also absent in the *Ch. mydas* specimens observed in this study. Despite anatomical differences, all observed and absent cartilaginous structures are the same in *Ca. yacare* and *Ca. latirostris*, except for the thyroid wings, which are only absent in crocodilians.

In a study conducted by Habova et al. (2022), three common structures were described in the larynx of 19 species from the Testudines group – *Centrochelys sulcata* (Miller, 1779), *Chelonoidis carbonarius* (Spix, 1824), *Stigmachelys pardalis* (Bell, 1828), *Testudo graeca* Linnaeus, 1758, *Testudo hermanni* Gmelin, 1789, *Testudo horsfieldii* Gray, 1844, *Testudo kleinmanni* Lortet, 1883, *Testudo marginata* Schoepff, 1792, *Cuora galbinifrons* Bourret, 1939, *Cuora mouhotii* (Gray, 1862), *Emys orbicularis* (Linnaeus, 1758), *Geoemyda spengleri* (Gmelin, 1789), *Chelodina rugosa* Ogilby, 1889, *Chelus fimbriatus* (Schneider, 1783), *Lissemys scutata* (Peters, 1868), *Malaclemys terrapin* (Schoepf, 1793),

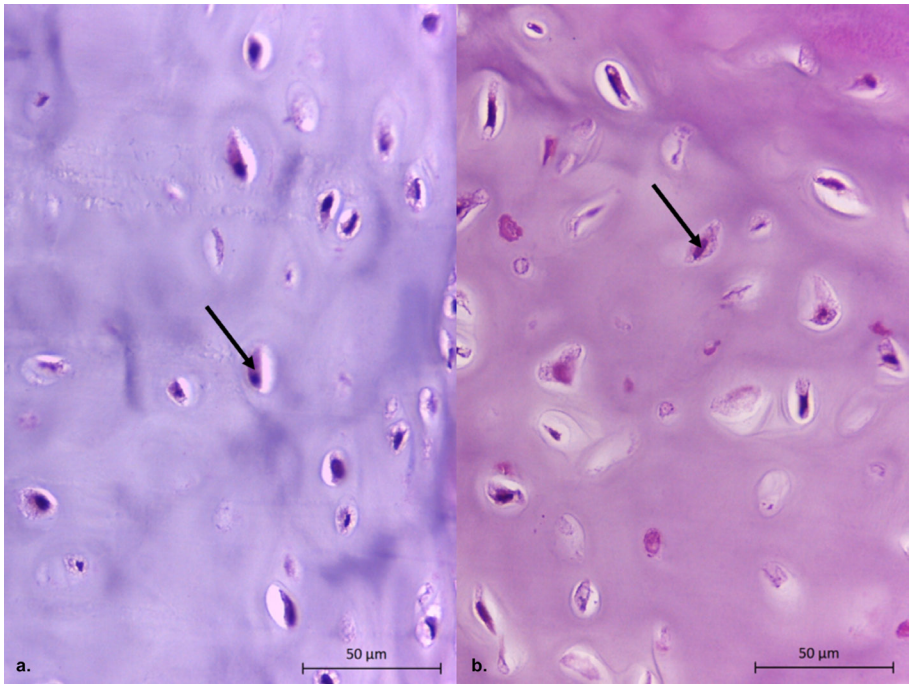


**Figure 7. Photomicroscopy of the basihoid horns of *Caiman yacare*, and *Caiman latirostris*. Hematoxylin-Eosin staining. a) *Caiman yacare*. b) *Caiman latirostris*. Arrows: Chondrocytes within the gap. 40× objective.**

*Mauremys caspica* (Gmelin, 1774), *Platysternon megacephalum* Gray, 1831, and *Trachemys scripta elegans* (Thunberg, 1792) – all of them showed two arytenoid cartilages and one cricoid cartilage, as observed for *Ch. mydas* in this study. Furthermore, all species described by Habova et al. (2022) had a glottis formed by a narrow slit located at the base of the tongue, and the same condition is present in *Ch. mydas*. For aquatic and semi-aquatic species, this narrowing of the laryngeal entrance is an adaptation mechanism that protects the airways and lower respiratory tract from the intake of water during diving.

The laryngeal structures of *Ca. yacare* and *Ca. latirostris* are anatomically like those of *Alligator mississippiensis* (Daudin, 1802), commonly known as the American alligator. The skeleton of the larynx of *A. mississippiensis* was described by Riede et al. (2011) as showing a cricoid cartilage with annular shape, found at the cranial end of the trachea and caudal to the arytenoid cartilages; and two arytenoid cartilages, which are long structures that form two semicircles with connected-ends in the dorsal and ventral portion, being ventrally connected by the cricoarytenoid joint. The hyoid apparatus was described as cup shaped.

Ferguson (1981) described the hyoid cartilage of *A. mississippiensis* as the hyoid apparatus, a dorsal flap that closes the oral cavity and meets and seals the secondary palate, preventing water inlet in the glottis. For *Ca. yacare* and *Ca. latirostris*, these flaps were found in the caudodorsal portion of the horns of the hyoid apparatus, formed by cartilaginous tissue. Thus, the hyoid apparatus found in crocodilians ensures that in these animals, even when submerged, air enters the nostrils and flows along the nasal cavity, which is formed cranially by the primary palate and caudally by the secondary palate, passing through the opening of the glottis, and allowing breathing and vocalization even with the oral cavity submerged. Besides the respiratory function, crocodilians use the larynx as the main structure in vocalization. The sound emitted by the passage of air through this structure is a fundamental instrument for signaling the

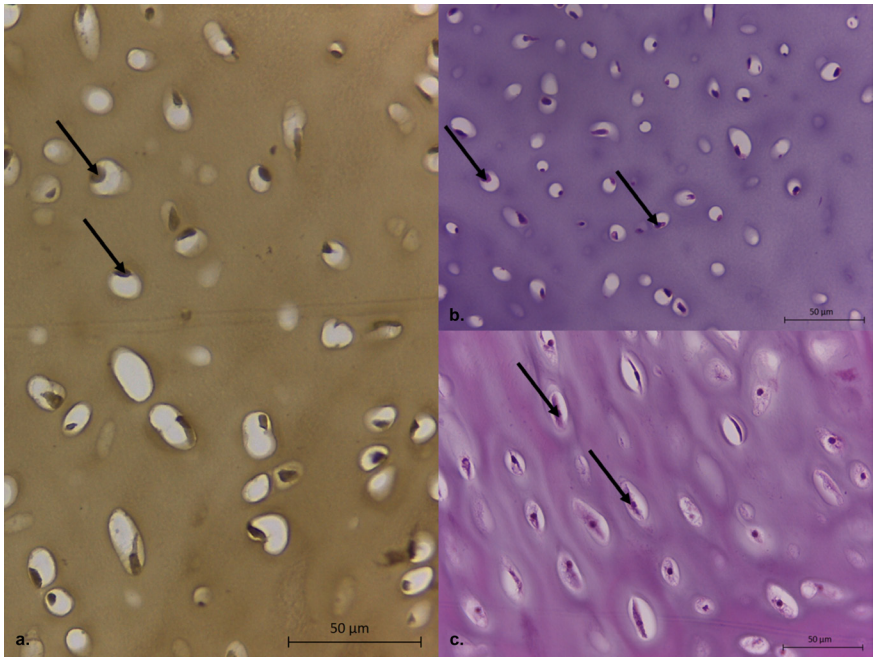


**Figure 8. Photomicroscopy of the arytenoid and cricoid cartilages of *Chelonia mydas*, *Caiman yacare*, and *Caiman latirostris*. a) *Chelonia mydas*. Verhoeff staining. b) Caiman yacare. Hematoxylin-Eosin staining. c) *Caiman latirostris*. Hematoxylin-Eosin staining. Arrows: Chondrocytes within the gap. 40× objective.**

reproductive season and the hatching of eggs by the hatchlings (Grigg & Kirshner 2015, Kirchner 1993).

Vocalization is made from the air that passes through the nostrils and reaches the larynx, emitting the sound by the vibration of the arytenoid cartilages. However, we found no anatomical structure responsible only for the modulation of the air flow between the nostrils and the glottis for this process to occur. During vocalization, the nostrils are widely opened to allow air flow, causing the arytenoid cartilages to open the glottis and the emission of sounds to occur (Riede et al. 2011). The structures observed in the laryngeal skeleton of the studied species likely participate in an active way in vocalization, as well as playing a fundamental role in the breathing of such animals.

The anatomical structures found in the larynx of testudines and crocodilians in our study are similar, as they perform the same functions to meet the physiological needs of feeding and breathing in an aquatic environment. When testudines, crocodilians, and birds are compared, similarities and differences between the three taxonomic groups can be observed. Testudines and crocodilians do not have the procricoid cartilage, whereas birds have this cartilage; thyroid wings are found in testudines and absent in crocodilians and birds; testudines, crocodilians, and birds lack the epiglottis cartilage; and testudines, crocodilians, and birds have a hyoid apparatus.

Regarding the trachea, *Ch. mydas* have complete cartilaginous rings, like that observed in birds and other crocodiles (Kingsley et al. 2018). However, *Ca. latirostris* and *Ca. yacare* have incomplete tracheal rings joined by the tracheal muscle, differing them from other species, such as *A. mississippiensis* (Kingsley et al. 2018). The animals of the Cryptodira group, which includes *Ch. mydas*, have a short trachea that allows the head to be easily retracted (O'Malley 2017). For the 19 species of Testudines studied by Habova et al. (2022), complete cartilaginous rings were observed in the formation of the trachea and pulmonary bronchi, which connect through connective tissue, as found in the *Ch. mydas* specimens from this study. Additionally, a dorsoventral flattening of the trachea was

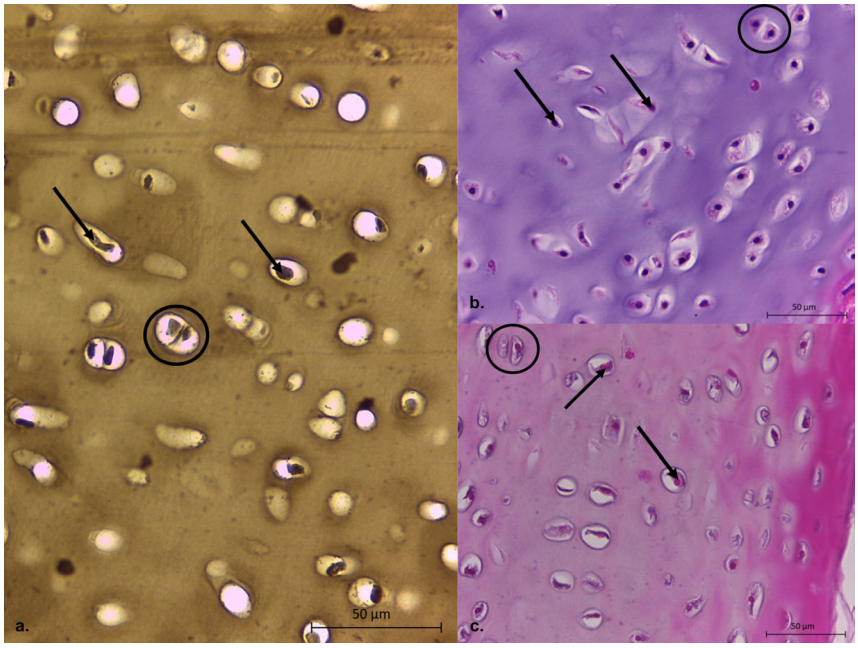


**Figure 9. Photomicroscopy of the tracheal rings of *Chelonia mydas*, *Caiman yacare*, and *Caiman latirostris*. a) *Chelonia mydas*. Verhoeff staining. b) *Caiman yacare*. Hematoxylin-Eosin staining. c) *Caiman latirostris*. Hematoxylin-Eosin staining. Arrows: Chondrocytes within the lacunae. Circles: Isogenous groups. 40× objective.**

described, which aligns with the findings for *Ch. mydas*.

Associations regarding the number of tracheal rings and size of the animals can also be made, since *Ch. mydas* and *Ca. latirostris*, whose body length could be measured, have a considerable size difference. Although specimens of *Ch. mydas* had a curvilinear carapace length (CCL) that varied from 32.50 cm to 50.00 cm, those of *Ca. latirostris* reached a total body length (TL) that varied from 168 cm to 217 cm. The amplitude in these values allows us to state that when comparing testudines and crocodilians, animals with smaller body length have smaller tracheas, such as those of the species *Ch. mydas*, which presented a mean of 40 tracheal rings. However, animals with larger body length have longer tracheas, like the crocodilian species in this study, which had an average of 69 and 64 tracheal rings for *Ca. yacare* and *Ca. latirostris*, respectively.

When comparing the two crocodilian species, it is believed that as they become older, the tracheal rings fuse. A higher number of tracheal rings was found in young individuals of *Ca. yacare* compared to adult individuals of *Ca. latirostris*, with an average amplitude of five rings for both species. However, it is essential to conduct studies between young and adult animals of the same species to confirm this hypothesis.

The histological analysis showed that all species studied here have cartilages of the larynx and tracheal rings of the hyaline type, resembling the histological description of *S. camelus* by Figueiredo et al. (2012). Al-Medhtiy (2013) found the same histological pattern for the cricoid, arytenoid, and procricoid cartilages of the helmeted guineafowl, *Numida meleagris galeata* (Pallas, 1767), all of them consisting of hyaline cartilage. Thus, although the anatomical structures of the larynx and trachea are different

**Table III. Mean number of tracheal rings found in animals of the three species.**

| Species | Mean | Standard deviation |
|---|---|---|
| *Chelonia mydas* | 40 | **± 3,5** |
| *Caiman yacare* | 69 | **± 3,1** |
| *Caiman latirostris* | 64 | **± 2,5** |

in testudines, crocodilians, and birds, the cartilages of these structures present the same tissue constitution.

The larynges of *Ch. mydas*, *Ca. yacare*, and *Ca. latirostris* have three cartilages, which are macroscopically different. Thyroid wings are observed only in *Ch. mydas*, being absent in both crocodilian species. The number of tracheal rings was different for each species, but anatomically the same for *Ca. latirostris* and *Ca. yacare*. Histologically, the three species presented the laryngeal skeleton and the tracheal rings formed by hyaline cartilage.

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## Author contributions

MARIANA DE OLIVEIRA LIMA: wrote the manuscript, collected specimens and data, processed and analyzed the data, and interpreted the results. LEONARDO LIMA GORZA: collected specimens of Caiman yacare. EDUARDO JOSÉ DA SILVA BORGES: donated copies of Caiman yacare. VIVIANE TAVARES DE PAULA: stained the histological samples. LOUSIANE DE CARVALHO NUNES: collected specimens of Chelonia mydas. YHURI CARDOSO NÓBREGA: donated specimens of Caiman latirostris. RODRIGO GIESTA FIGUEIREDO and MARIA APARECIDA DA SILVA: carried out the design, conception, and writing and revision of the manuscript.