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Morphological characterization of the laryngeal cartilages of the *Bradypus variegatus* sloth (Schinz, 1825)

Caracterização morfológica das cartilagens laríngeas da preguiça Bradypus variegatus *(Schinz, 1825)*

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ABSTRACT: The common sloth, *Bradypus variegatus* is an arboreal mammal, belonging to the order Pilosa of the Xenarthra superorder. It has a wide geographical distribution in some countries in Central and South America. However, there is a need for anatomical studies on the *B. variegatus* larynx, as this organ is important for the functioning of the respiratory system and for vocalization. Thus, the objective was to describe macro and microscopically the cartilaginous structures of the larynx of the *B. variegatus* sloth. For this purpose, 10 specimens were used, obtained after natural death. Of these, six specimens were fixed with 20% formaldehyde and kept in a 30% saline solution tank, being subsequently submitted to dissection and separation of the laryngeal cartilages. In the remaining specimens, fixation took place in 10% buffered formaldehyde with 0.01M sodium phosphate buffer (PBS) and pH of 7.3, followed by the preparation of slides stained with hematoxylin-eosin. Macro and microscopic analysis were performed. The sloth's larynx is at the level of the first cervical vertebrae, and as for its composition, the presence of unpaired cartilages, cricoid, thyroid and epiglottis and pairs, the arytenoids, was verified. All of them of the hyaline type, being observed in the thyroid cartilage, process of endochondral synostosis. The epiglottis and cricoid cartilages showed different morphology in comparison with other mammals. The arytenoids showed very evident corniculate processes. And the thyroid cartilage stood out in size compared to the others.

KEYWORDS: Xenarthra; three-toed sloth; upper airways; laryngeal cartilages.

RESUMO: A preguiça-comum, *Bradypus variegatus*, é um mamífero arborícola, pertencente à ordem Pilosa da superordem Xenarthra. Possui ampla distribuição geográfica em alguns países da América Central e do Sul. Porém, há necessidade de estudos anatômicos da laringe de *B. variegatus*, pois este órgão é importante para o funcionamento do sistema respiratório e para a vocalização. Assim, objetivou-se descrever macro e microscópicamente as estruturas cartilaginosas da laringe da preguiça *B. variegatus*. Para tanto, foram utilizados 10 espécimes, obtidos após morte natural. Destes, seis espécimes foram fixados com formol 20% e mantidos em tanque de solução salina 30%, sendo posteriormente submetidos à dissecção e separação das cartilagens laríngeas. Nos demais espécimes, a fixação ocorreu em formol a 10% tamponado com tampão fosfato de sódio (PBS) 0,01M e pH 7,3, seguida da preparação de lâminas coradas com hematoxilina-eosina. Foram realizadas análises macro e microscópicas. A laringe da preguiça está ao nível das primeiras vértebras cervicais, e quanto à sua composição foi verificada a presença de cartilagens desemparelhados, cricóide, tireóide a epiglote e pares, as aritenóides. Todas elas do tipo hialina, sendo observadas na cartilagem tireóidea, processo de sinostose endocondral. A epiglote e as cartilagens cricoides apresentaram morfologia diferente em comparação com outros mamíferos. As aritenóides apresentavam processos corniculados muito evidentes. E a cartilagem tireóide se destacou em tamanho em relação às demais.

PALAVRAS-CHAVE: Xenartra; preguiça de três dedos; vias aéreas superiores; cartilagens laríngeas.

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INTRODUCTION

Sloths are representatives of the Pilosa order and Xenarthra superorder. They are divided into two genera: *Bradypus* Linnaeus, 1758 and *Choloepus* Illiger, 1811, and are popularly known as three-toed sloth and two-toed sloth, respectively (Gardner, 2005; Medri *et al.*, 2011). Among the bradypodidae, the species *Bradypus variegatus* SCHINZ, 1825 stands out due to its wide distribution in certain countries of Central and South America. In Brazil, they inhabit the forested areas of the Amazon, Atlantic Forest and Cerrado biomes (Cassano, 2006; Moraes-Barros *et al.*, 2022). In the Pernambuco state, it can be found from the coast to the forest zone (Cabral, 2000).

The common sloth (*B. Variegatus*) has morphophysiological and behavioral adaptations to live in the treetops and feed on leaves and sprouts, with long forelimbs and short hindlimbs, both equipped with three long and curved claws. In addition, it has eight or nine cervical vertebrae that allow this animal to turn its head sharply (Medri *et al.*, 2011; Miranda, 2017; Pinheiro, 2008). Its natural predators are the harpy eagle (*Harpia harpyja*) and big cats such as the jaguar *(Panthera onca*) (Marchini *et al.*, 2011; Silva, 2007).

Despite *B. Variegatus* species being classified as less worrying in the red list of threatened animals of the International Union for Conservation of Nature - IUCN in 2022, due to its wide distribution, this species suffers from several anthropic actions such as deforestation, which bring as consequence the loss and fragmentation of habitats, as well as trafficking and illegal hunting, which may lead to local extinction (Moraes-Barros *et al.*, 2022).

Sloths are solitary animals, carrying out social interactions only during the reproductive period, in which, above all, the female vocalizes to the male before and during copulation, and presents parental care that lasts up to six months (Bezerra *et al.*, 2008; Miranda, 2017). The larynx is an organ of the respiratory system that makes the connection between the pharynx and the trachea, and participates in the mammalian vocalization process (König; Liebich, 2021). Therefore, it is necessary to study the laryngeal cartilages of *B. variegatus* to add information about the respiratory system of this species, and thus contribute to its medical practice and the conservation of this wild mammal.

MATERIAL AND METHODS

To be performed, this research was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SinGen), under the A74BCB6 number, and was carried out with the release of the Authorization and Information System on Biodiversity of the Chico Mendes Institute (SisBio/ICMBio), nº 46665/11. Ten cadavers of *B. variegatus* sloths, which were acquired after natural death through the Wild Animal Screening Center (CETAS-TANGARA), were used. All stages were photo documented, and the naming of anatomical terms was guided by Nomina Anatómica Veterinária, 2017.

Six sloth corpses (one female and one juvenile male, and four juveniles of undetermined sex) were fixed with 20% formaldehyde and kept in a 30% saline solution tank. Subsequently, they were submitted to laryngeal dissection, in which a scalpel, tweezers and blunt and fine-tipped scissors were used. Initially, they received a median sagittal incision in the neck, with the formation of lateral windows, removal of the skin and musculature, in order to gain access to the larynx, once done, its syntopy and skeletony were indicated and then it was removed and investigated *ex situ*. The laryngeal cartilages were characterized according to shape, quantity and measurements, the latter were made with a 150mm/0.02mm steel caliper.

From the cricoid cartilage, the median length of its blades, ventral and dorsal, was obtained; the width of the upper end of the cartilage, measured at the level of the cricothyroid ligament; the width of the cricoid at the level of articulation with the caudal processes of the thyroid cartilage; and the width of the lower extremity, measured at the height of the cricotracheal ligament. In the thyroid cartilage case, the length of its body was measured; the distance between the ends of the processes, rostral and caudal in the two antimeres; and the width of its blades, measured from the body of the cartilage to the most lateral point of each blade. From the epiglottis cartilage, the median length was obtained; the maximum width of the body, measured at the most rostral region of the cartilage; and the width at the level of the petiole. While, for the right and left arytenoid cartilages, the maximum length was measured, starting from the cricoarytenoid joint to the most rostral region of the cartilage; and the maximum width, measured from the aryepiglottic ligament to the median border of the arytenoid.

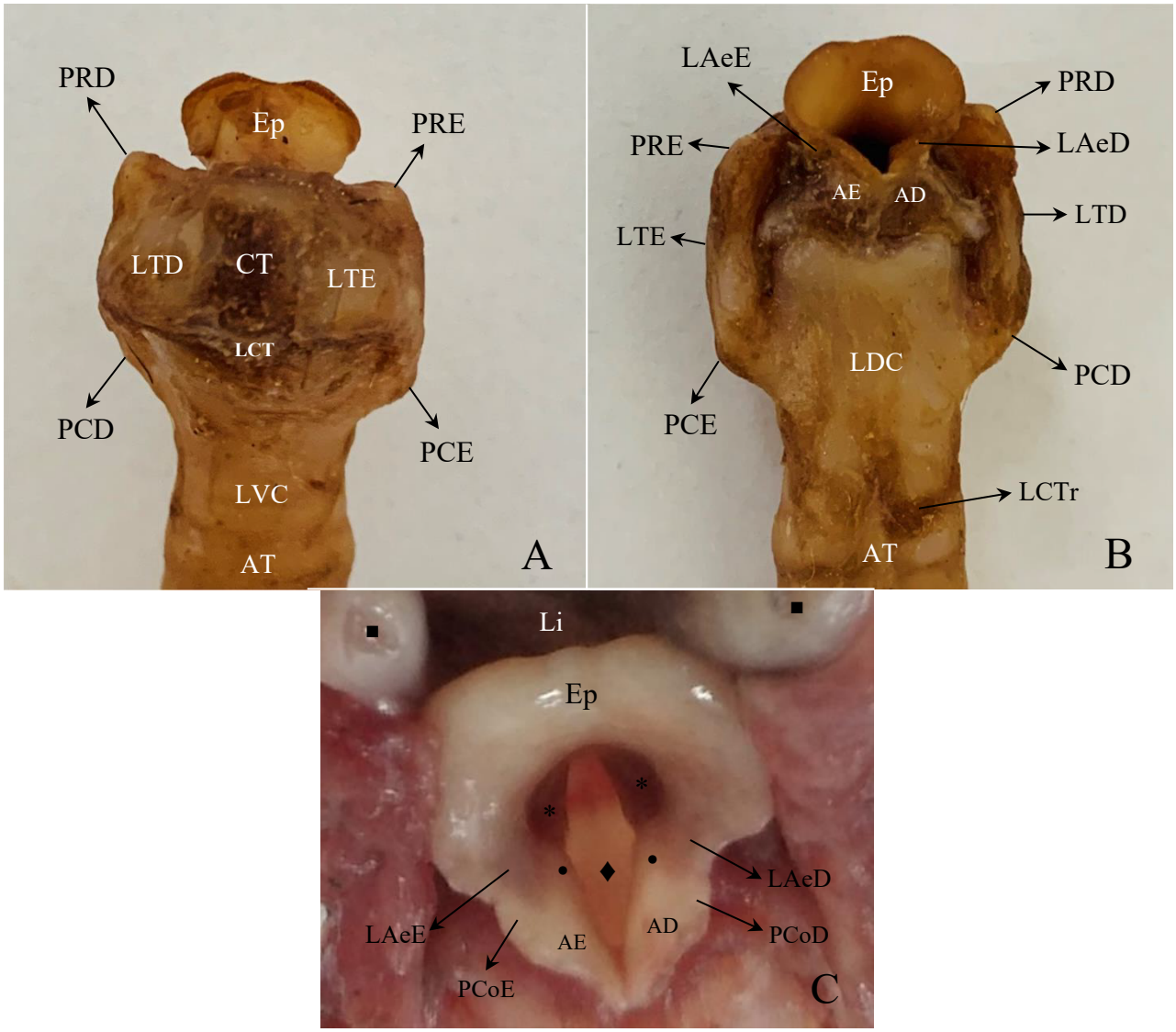
The larynx was collected for histological processing immediately after death in four *B. variegatus* specimens (two females and two adult males), in order to identify the types of present cartilage. The organ was fixed in formaldehyde, buffered at 10% with sodium phosphate buffer (PBS) 0.01M and pH 7.3. Further on, the cartilages were separated and transported to the DMFA/UFRPE Histology Laboratory, where they were submitted to paraffin inclusion processing, in which the blocks were cut in a microtome adjusted to 4 micrometers (µm). The prepared slides were stained with hematoxylin-eosin and observed under an optical microscope. Exclusively, the thyroid cartilage was decalcified in 10% nitric acid, when necessary.

RESULTS

Based on the analyzed sloths, the larynx of the *B. variegatus* species is a median organ with a skeleton associated with the first cervical vertebra. Formed by the odd cartilages, cricoid, thyroid and epiglottis and pairs, the arytenoids, its syntopy is marked by the muscles of the neck, ventral and laterally; faces the hyoid bone, the oropharynx and the root of the tongue, rostrally; relates to the esophagus dorsally; and its caudal portion is limited by the trachea. According to the study of the sloth's laryngeal skeleton, the cricoid cartilage is formed by two

layers, one ventral and the other dorsal, which join laterally. In the latter, the arytenoid cartilages are articulated, which have a triangular shape, with vocal processes, and very evident corniculates. The thyroid cartilage has a median and ventral body, from which two laminae depart, one for each antimere. At the dorsal end of these blades, the rostral and caudal processes are seen. Thus, the rostral process joins the hyoid, and the caudal one joins the dorsal lamina of the cricoid cartilage. The petiole of the epiglottis cartilage articulates in the rostral dorsal portion of the thyroid, whose body has broad, rounded edges (Figure 1).

In terms of laryngeal morphometry, generally speaking, there were little discrepant variables between the used specimens. As for the cricoid cartilage, higher measurements were obtained for the adult male, to the detriment of the adult female and young animals (Table 1). The thyroid cartilage showed higher values for the adult male regarding body length and the distance between the processes, in both antimeres. However, concerning the width of the blades, higher measurements were observed for the adult female and the juveniles, which presented values corresponding to each other (Table 2). From the measurement of the epiglottis cartilage, it was noticed the greater median length in the adult female, the petiole width on average higher in the young sloths and the higher maximum width in the adult male (Table 3). The arytenoid cartilages, in turn, were slightly larger in the adult female than in the others (Table 4).



Source: Helen Lylyan da Silva, 2023

Figure 1. Photo macrographs of the dissected larynx of the *Bradypus variegatus* sloth. A- Ventral view. B- Dorsal view. C- Rostral view. Epiglottis (Ep), thyroid body (CT), right thyroid lamina (LTD), left thyroid lamina (LTE), right rostral process (PRD), left rostral process (PRE), right caudal process (PCD), left caudal process (PCE), cricothyroid ligament (LCT), right arytenoid (RA), left arytenoid (AE), right corniculate process (PCoD), left corniculate process (PCoE), vocal processes (●), right aryepiglottic ligament (LAeD), ligament left aryepiglotticus (LAeE), ventral cricoid lamina (LVC), dorsal cricoid lamina (LDC), cricotracheal ligament (ICTr), tracheal ring (AT), tongue (Li), laryngeal inlet (♦), vocal folds (*), ducts of salivary glands (■).

Through the microscopic investigation of the laryngeal cartilages, it was verified that all of them are of the hyaline type, with the observation of endochondral synostosis in the thyroid cartilage, which made it able to identify the existence of bone tissue surrounding the bone marrow and hypertrophic chondrocytes with pyknotic nuclei, filled with bone matrix, in the transition zone, with the presence of a sedimentation line indicating the limits between the hyaline cartilage and the bone tissue. The ossification process is more advanced in males (Figure 2).

DISCUSSION

The cartilaginous composition of the larynx of the *B. variegatus* sloth was shown to be similar to that generally described for mammals, with the cricoid, thyroid, epiglottis and arytenoid cartilages being observed (König; Liebich, 2021). However, it differs from birds, such as the African ostrich (*Struthio camelus*), the quail (*Nothura maculosa*) and the hawk (*Buteo rufinus*), in which the absence of thyroid and epiglottis cartilages was observed, and the existence of pro cricoid cartilage. (Abidu-Figueiredo *et al.*, 2012; Çevik-Demirkan *et al.*; 2007; Kabak *et al.*, 2007).

In some species, such as the crab-eating fox (*Cerdocyont hous*), sesamoid cartilages were identified located between the corniculate process of the arytenoids and the rostral margin of the cricoid lamina, which were not seen in sloths. These animals also differ regarding the laryngeal skeleton, which is located at the level of the atlas in the xenarthra, and extending

Table 1. Dimensions of the cricoid cartilage of dissected *Bradypus variegatus* sloths.

Morphometric Variables of Cricoid Cartilage (cm)	Adult Female	Adult Male	Average±Standard Deviation Juveniles of Undetermined sex
Average Length of the Ventral Cricoid Plate	0,95	1,15	1,06±0,09
Average Length of Dorsal Cricoid Plate	1,01	1,31	1,09±0,08
Width of the Superior End of the Cricoid Cartilage	0,94	0,95	0,91±0,09
Width of the Cricoid Cartilage at the Level of Articulation with the Caudal Processes of the Thyroid Cartilage	1,05	1,16	1,03±0,05
Width of the Inferior End of the Cricoid Cartilage	0,68	0,77	0,73±0,04

Table 2. Dimensions of the thyroid cartilage of dissected *Bradypus variegatus* sloths.

Morphometric Variables of thyroid cartilage (cm)	Adult Female	Adult Male	Average±Standard Deviation Juveniles of Undetermined sex
Body Length	0,73	0,78	0,68±0,06
Distance between the ends of the Rostral Process and the Right Caudal Process	0,95	1,11	1,04±0,08
Distance between the ends of the Left Rostral Process and Left Caudal Process	0,93	1,19	1,01±0,08
Right Blade Width	1,03	0,94	1,02±0,05
Left Blade Width	1,05	0,94	1,01±0,06

Table 3. Dimensions of the epiglottis cartilage of dissected *Bradypus variegatus* sloths.

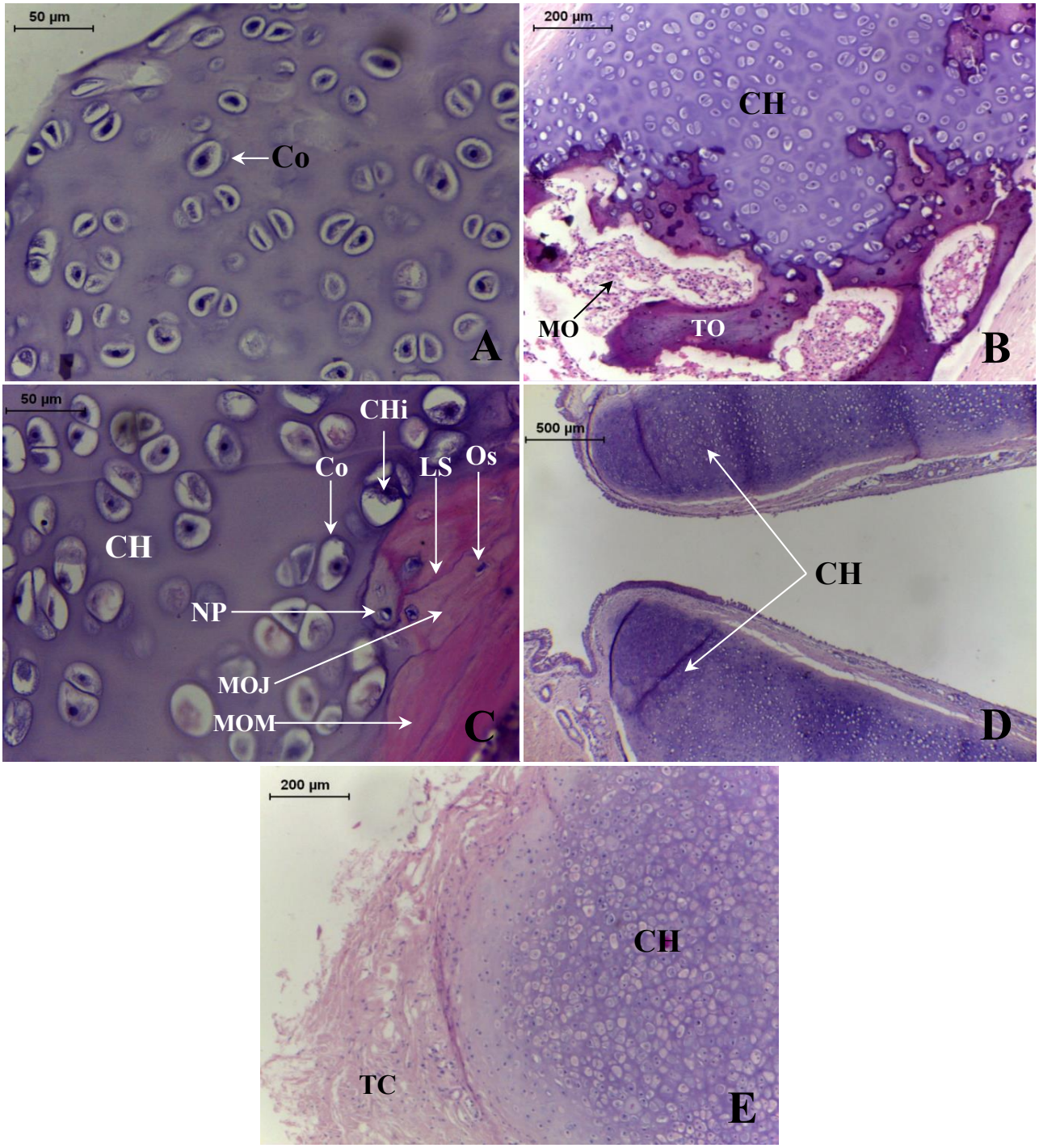
Morphometric Variables of epiglottis cartilage (cm)	Adult Female	Adult Male	Average±Standard Deviation Juveniles of Undetermined sex
Average length	0,46	0,36	0,35±0,06
Maximum Body Width	0,66	0,67	0,65±0,04
Width at Petiole Level	0,43	0,41	0,45±0,04

Table 4. Dimensions of the arytenoid cartilages of dissected *Bradypus variegatus* sloths.

Morphometric Variables of arytenoids cartilage (cm)	Adult Female	Adult Male	Average±Standard Deviation Juveniles of Undetermined sex
Maximum Length of the Right Arytenoid Cartilage	0,43	0,36	0,41±0,02
Maximum Length of the Left Arytenoid Cartilage	0,42	0,39	0,40±0,02
Maximum Width of the Right Arytenoid Cartilage	0,44	0,33	0,36±0,13
Maximum Width of the Left Arytenoid Cartilage	0,44	0,35	0,39±0,16

from the tooth to the caudal articular surface of the axis in the canid. However, rostral syntopy with the root of the tongue and oropharynx and dorsal syntopy with the esophagus were mutually recognized (Souza Júnior *et al.*, 2016).

Based on the morphological and morphometric findings of the sloth's larynx, no expressive differences were registered that could indicate factors of sexual dimorphism, which also occurs in domestic animals, such as the dog (*Canis lupus familiaris*) (Tayama *et al.*, 2001), horses (*Equus ferus caballus*) (Charuta *et al.*, 2009) and pigs (*Sus scrofa domesticus*) (Wysocki *et al.*, 2010). The thyroid of bradypodidae can be identified as the largest laryngeal cartilage, coinciding with the carried out analysis in crab-eating foxes (Souza Júnior *et al.*, 2016) and nine-banded armadillos (*Dasypus novemcinctus*) (Fonseca *et al.*, 2017).



Source: Helen Lylyan da Silva, 2023

Figure 2. Photomicrographs of the laryngeal cartilages of the *Bradypus variegatus* sloth. A- Cricoid cartilage. B and C- Thyroid cartilage in the process of endochondral synostosis. D- Arytenoid cartilages. E- Epiglottis cartilage. Chondrocyte (Co), hyaline cartilage (CH), bone tissue (TO), bone marrow (MO), hypertrophic chondrocyte (CHi), pyknotic nucleus in chondrocyte filled with bone matrix (NP), osteocyte (Os), sedimentation line (LS), young bone matrix (MOJ), mature bone matrix (MOM), connective tissue (TC).

In a very particular way, the cricoid cartilage does not have a ventral ring in sloths, but a lamina. This condition promotes a more vertical articulation with the thyroid, differing from the already published data for other species (König and Liebich, 2021). This arrangement seems to promote greater stability for the sloth's airways, especially during movement of its neck, whose rotation can reach 270° (Almeida *et al.*, 2011).

Concerning the thyroid cartilage, it did not demonstrate evident differences between males and females in *B. variegatus*. The positioning of the plates of this cartilage in relation to its body, associated with the dimensions of the vocal folds generate a varied vibratory pattern, which may differ between genders (Slavit, 1999; Ximenes Filho *et al.*, 2003). In the three-toed sloths case, the aspect of vocalization is directly linked to the condition of the animal, so that when the young emit a squeak, the males usually release a short and sharp cry in case of agonistic interactions, and females have a high-pitched and strong cry during reproductive periods (Dünner; Pastor, 2017; Greene, 1989; Lara-Ruiz; Srbek-Araujo, 2006). Bearing the uniformity of the sloths thyroid cartilage in mind, it is speculated that the discrepant timbres of these animals are associated with the supraglottic vocal tract.

The epiglottis cartilage, in the sloth, was demonstrated without macroscopic particularities, while the arytenoids presented an evident corniculate process, which was also described in the dog, horse and nine-banded armadillo (Fonseca *et al.*, 2017; König; Liebich, 2021), however, differs from several other species, considering that they are the cartilages with the most variable morphology among mammals (Evans; Lahunta, 2013). The presence of the corniculate process helps to reduce the risk of bronchoaspiration during food or liquid ingestion (Souza Júnior *et al.*, 2016).

From the microscopic point of view, all laryngeal cartilages of *B. variegatus* were of the hyaline type, which is common when dealing with cricoid, thyroid and arytenoid. However, it is different from the elastic epiglottis, which is more commonly found among mammals (Fonseca *et al.*, 2017; König; Liebich, 2020; Souza Junior et al., 2016).

The process of the laryngeal cartilages ossification has already been described, especially in birds, in this class, this characteristic is an important indicator of the age of the animal (Abidu-Figueiredo *et al.*, 2012; Onuk *et al.*, 2010). Based on this assumption, the histology of such sloth cartilages indicated endochondral thyroid synostosis, even being well advanced in some animals, which would therefore be the oldest adults. Calcification and ossification of the laryngeal cartilages have also been described in the giant anteater (*Myrmecophaga tridactyla*), being identified in the arytenoid, cricoid and thyroid (Oliveira, 2020) and in the nine-banded armadillo, seen exclusively in the thyroid, similar to that recorded in the common sloth (Fonseca *et al.*, 2017).

Hyaline cartilage promotes greater resistance to pressure and works as a more stable insertion point for muscles (Harrison, 1995). The contraction of the intrinsic and extrinsic musculature in the larynx, in turn, promotes tension that favors the cartilaginous ossification process (Strek *et al.*, 1993). Thus, through the unanimity of the hyaline cartilage in the sloth's larynx, it can be speculated that this characteristic is related to the need to frame the laryngeal structures and keep its skeleton firm, guaranteeing the integrity of the airway even when the animal is carrying out its activities in peculiar positions, including against gravity.

CONCLUSION

The larynx of the *B. variegatus* sloth is a median organ located at the level of the first cervical vertebra, composed of unpaired cartilages, cricoid, thyroid and epiglottis and pairs, the arytenoids. According to the macroscopic findings, sexual dimorphism factors were not seen. The cricoid cartilage presented two layers, one ventral and the other dorsal, while the arytenoids showed well-developed corniculate processes. The thyroid and epiglottis followed patterns common to mammals. Microscopically, all the cartilages were of the hyaline type, and an endochondral synostosis process was observed in the thyroid cartilage.

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