

https://doi.org/10.31533/pubvet.v17n11e1490

New morphological aspects of the hyoid apparatus of the giant anteater (*Myrmecophaga tridactyla*) living in a human-wildlife interface in the Atlantic Forest biome, Brazil

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Abstract. The aim of this study was to identify the anatomical structures in the cervical region of the giant anteater (*Myrmecophaga tridactyla*). For this, three adult female giant anteater corpses (head/neck) were used. The digital radiography analysis was performed in the sagittal direction. Subsequently, the heads of these animals were anatomically sectioned in anatomical sagittal plane. Radiography images were compared with anatomical sections. The association between digital radiography and anatomical sections allowed the visualization of anatomical structures found in the pharyngeal cavity. The new structure, never described before, was found and its topography revealed a close relation to the pharynx/larynx area. The findings of this study suggest that the giant anteater has structures morphologically adapted to its feeding habits, such as an elongated skull, tongue, styloid bones, mandible and a new structure serving as a feed reservoir connected directly to the esophagus (cervical part) described here. In addition, it was possible to establish anatomical parameters as an important tool for the veterinary routine of wild animals, since they allow for the precise identification of anatomical structures.

Keywords: Bone, giant anteater, larynx, morphology

Novos aspectos morfológicos do aparelho hióide do tamanduábandeira (Myrmecophaga tridactyla) vivendo na interface homemfauna no bioma Mata Atlântica, Brasil

Resumo O objetivo deste estudo foi identificar as estruturas anatômicas da região cervical do tamanduá-bandeira (*Myrmecophaga tridactyla*). Para isto, foram utilizados dois cadáveres de tamanduá-bandeira (cabeça/pescoço), ambos de idade adulta e fêmeas. A análise da radiografia digital foi realizada no sentido sagital. Posteriormente, as cabeças desses animais foram seccionadas anatomicamente no plano sagital anatômico. As imagens radiográficas foram comparadas com os cortes anatômicos. A associação da radiografia digital com os cortes permitiu a visualização das estruturas presentes na cavidade faríngea. A estrutura cavitaria, nunca antes descrita, foi documentada e sua topografia revelou estreita relação com a região da laringe. Os resultados do presente estudo sugerem que o tamanduá-bandeira possui estruturas morfologicamente adaptadas aos seus hábitos alimentares, como um crânio alongado, língua, ossos estiloides, mandíbula e um reservatório de alimento aqui descrito. Além disso, é possível estabelecer parâmetros anatômicos como uma ferramenta importante para a rotina veterinária de animais selvagens, uma vez que permite a identificação precisa de estruturas anatômicas.

Palavras-chave: Osso, tamanduá-bandeira, laringe, morfologia

Introduction

The giant anteater (*Myrmecophaga tridactyla*) is considered member of the *Myrmecophagidae* family and appears in all Brazilian biomes, from the Amazon to the southern fields, including Atlantic Forest biome (<u>Aparicio & Plana, 2021</u>; <u>Medri & Mourão, 2005</u>). The suborder Xenarthra is divided into two orders, Cingulata consisting of armadillos, carapace animals and order Pilosa with five genera and ten species, where eight of them occur in Brazil (<u>Barragán-Ruiz et al., 2021</u>). In addition, Anteaters have characteristics in relation to their eating habits given their adaptations to seize food and have cursorial foraging activity, carried out on the ground, with ability to climb trees (<u>Gaudin et al., 2018</u>).

Giant anteaters exhibit a low metabolic rate, which is understood as an adaptation possibly associated with their low-calorie diet with ants (*Hymenoptera*) and termites (Isoptera), avoiding higher temperatures in tropical areas (Cáceres et al., 2010). Moreover, anteaters regulate their metabolism in accordance to environmental temperatures, presenting greater activity in hours of low temperature (Medri & Mourão, 2005; Naples, 1999). Meanwhile, it is the largest species among geotropically vermilingua in a rural area belonging to Atlantic Forest biome, distributed in Northeastern of São Paulo State, Araçatuba city (Passos et al., 2017; Rodrigues et al., 2008; Sartori et al., 2021).

The mammalian hyoid apparatus consists of an articulated series of skeletal elements located in the laryngeal-pharyngeal topographical region (Gomes et al., 2019; Smith., 2007; Springer & Murphy, 2007). It functions mainly to control the airway, but also for supporting and maintaining the position of the tongue between the mandibular rami in the procurement, processing, and deglutition of food (pharyngeal region). It also participates indirectly in the modulation of sounds produced by the vocal cords (laryngeal region). Comparative analyses of the hyoid apparatus of living and extinct xenarthrans may provide important information for the determination of feeding behaviours described previously in a fossil (Bertassoli et al., 2013). This study was realized to provide an anatomical, radiography and image design view of *Myrmecophaga tridactyla* hyoid apparatus and other intrinsic structures in order to elucidate the regional topography.

Material and methods

Animals

The studied region is localized 21°12′32″S 50°25′58″W under agricultural economy (Fig. 1). Moreover, usually Federal Road Police collect dyed animals and bring to CERETAS (Selvages Animals Recovery and Trial Center Care) localized in University of São Paulo State, UNESP, and Veterinary Medicine College. These animals, sent to Veterinary Anatomy Laboratory, conserved under formaldehyde solution at 10% (v/v) used in this study has unknown origin, only heads were found, and belong to Laboratory of Morphological Studies localized at Veterinary Medicine College. All procedures were performed under all applicable institutional guidelines for the care and ethical use of animals were followed as recommended by Animal Brazilian Experimentation Committee protocol. The anatomical species were prepared under supervision of technical personal responsible for the laboratory in 2022 year.



Figure 1. Localization of studied area belonging to Atlantic Forest biome and the specimen studied.

Radiography and schematic design

In this study, only the animal head/neck was evaluated. The digital radiography was performed using Cassette 46 x 38.4 x 1.5 cm, Model KLX-1417 Shanghai PZ Medical Technology Co., Ltd. The images were captured by X-ray acquisition software Voyance. Features used: Drawings traced with brush of variable thickness, following the outline of the original anatomical pieces. Continuous lines for representation of the most prominent elements and fine hatches for representation of minor details and (or) suggestion of relief. The illustrations were made with pen and tablet, 4 model TPK-640 from Wacom.

Results and discussion

The ability to retract the elongated tongue is supported by the hyoid apparatus, in particular by the stylohyoid bones (Fig. 2A and B). The cranial portion of the stylohyoid bone presented no correlation to the crania structures according to previous study (Cunha et al., 2018) and were visible in the anatomic specie and radiographic images of this study (Fig. 2 A and B). The bones that comprised the hyoid apparatus in the giant anteater are formed by elements that have synovial joints between them (Oliveira et al., 2023; Thrall, 2019) and that appear at the level of the second to sixth cervical vertebrae as demonstrated in radiography analysis (Fig. 2B). The hyoid apparatus morphology as a whole, mobility among its elements, and its anatomical position and relationship to other skeletal features provide insight on procurement and processing of foods in the oral cavity (Borges et al., 2017). In parallel, apparatus have shorter elements, with marked angles between them, and with subparallel or oblique orientation to the anteroposterior axis of the oral cavity (Fig. 2A and B; Fig. 3).



Figure 2. Comparative analysis between anatomical (A) and radiographic images (B) of hyoid apparatus of giant anteater. In A panel colour, green represented stylohyal bone, colour blue epihyal bone, colour purple ceratohyal bone and colour red basyhyal bone. The v-bone was represented by epihyal, ceratohyal and basyhyal.

Probably, this typical arrangement of the hyoid apparatus is an anatomical adaptation to the food intake process in the giant anteater demonstrated in various studies (Borges et al., 2017; Mahdy & Zayed, 2020). The giant anteaters presented the soft palate and the nasal and oral parts of the pharynx extended to the caudal level of the cervical region demonstrated before (Oliveira et al., 2023). The nasal and oral parts of the pharynx extended until the transition between the fifth and sixth cervical vertebrae, with the laryngeal part of the pharynx starting at this level (Borges et al., 2017; Marques & Schimming, 2020). This is an anatomical adaptation to accommodate the elongated tongue (Smith, 2007). Studies reported that it is important to know these anatomical details to avoid incorrectly identifying the nasal part of the pharynx as the cervical part of the trachea as described here (Fig 2A and B).



Figure 3. Design of giant anteater hyoid apparatus complete.

The hyoid apparatus tends to form elongated and nearly perpendicularly oriented descending bars (Fig. 2A and B. Fig. 3), the apparatus tends to have shorter elements, with marked angles between them, and with subparallel or oblique orientation to the anteroposterior axis of the oral cavity (Fig. 2A and B). The thyrohyals and basihyals are fused (V-bone) and project dorsally, as is usual (Fig. 2A and B). The stylohyal, epihyal, and ceratohyal (anterior cornua) are oriented anteriorly (Fig. 2A and B; Fig. 4A and B).





Figure 4. Demonstration of an anatomical structure (A) in detail and (B) green cranial to V-bone;B) red thyroid gland, hyoid apparatus compounded by blue basyhyals, purple ceratohyals, brown epihyals, green stylohyals.

Herein, the muscle *sternohyoideus* was identified extending cranially in the neck, covering the ventral surface of the trachea, to be inserted on the basihyoid bone (Fig. 5A; a). The cranial portion of the muscle that is not covered by the muscle *sternocephalicus* is the most ventral muscle of that portion and was removed here (Fig. 5A, b). The muscle *sternothyrohyoideus* was identified close to thyroid cartilage (Fig. 5A; c) partially removed to identify the new structure characterized as *fundus ventriculi*. Moreover, a passage was detected between esophagus and this structure (Fig. 5B; a, b).



Figure 5. A – Photography of superficial plane of laryngeal region; (a) *sternohyoideus* muscle was identified extending cranially in the neck; (b) *sternocephalicus muscle*; (c) *sternothyrohyoideus* was identified close to thyroid cartilage; (d) *Thyreohyoideus* muscle; B – Photography of deeper plane of laryngeal region; (a) *fundus ventriculi* identify firstly in this study; (b) a passage detected between esophagus and this structure.

Conclusion

This study provide support to wild animal's care submitted to analgesia, surgeries, traumas and image diagnostic. Moreover, face to very limited studies in this topic and an increase of Veterinary Hospitals specialized on wild animal's care, our results may improve scientific investigations, in a near future, among wildlife animals.

Ethics Committee and biosafety

Protocol n. 2010/098740 (CEEA/FOA).

Acknowledgments

The authors thank Polícia Rodoviária Federal who capture of dead/injured animals. We thank members of the University of Sao Paulo State, FMVA, Araçatuba, Brazil, for technical support. Partially of this study was presented at *Congresso de Iniciação Científica* da UNESP in 2022.

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