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Morphological analysis of the sloth tongue: *Bradypus variegatus* (Schinz, 1825)

Análise morfológica da língua da preguiça: Bradypus variegatus *(Schinz, 1825)*

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ABSTRACT: Sloths are arboreal folivores and rely on an efficient digestive system to thrive in Neotropical forests. This study was performed to evaluate the morphological aspects of the tongue of the brown-throated sloth (*Bradypus variegatus*). Five adult specimens (three males and two females) were obtained from the collection of the Anatomy Laboratory at the Academic Center of Vitória de Santo Antão, Universidade Federal de Pernambuco. The tongues were dissected and analyzed for their macroscopic characteristics. They were also subjected to histological analysis, in which sections were stained using hematoxylin–eosin, 1% toluidine blue, Masson's trichrome, picrosirius–hematoxylin, and mucicarmine. The average tongue length was 4.12 ± 0.17 cm, width was 1.50 ± 0.05 cm, and thickness was 2.00 ± 0.11 cm. The tongue consisted of an apex, body, and root. Numerous filiform and fungiform papillae were present on the dorsum of the tongue, and only two vallate papillae were observed. Microscopic examination revealed that the dorsal surface of the tongue was lined with keratinized stratified squamous epithelium, with melanin in the basal layer. The tongue predominantly consisted of skeletal striated muscle fibers. Occasional mast cells were scattered in the connective tissue. Numerous mucous glands were observed, but no serous glands were found. These morphological findings of the tongue of *B. variegatus* are partially unique to this species and are influenced by its diet and feeding habits.

KEYWORDS: digestive system; oral cavity; papillae; morphometry; Bradypodidae.

RESUMO: Os bichos-preguiça são folívoros arborícolas e dependem de um sistema digestivo eficiente para prosperar nas florestas neotropicais. Este estudo foi realizado para avaliar os aspectos morfológicos da língua da preguiça-de-garganta-marrom (*Bradypus variegatus*). Cinco espécimes adultos (três machos e duas fêmeas) foram obtidos da coleção do Laboratório de Anatomia do Centro Acadêmico de Vitória de Santo Antão, Universidade Federal de Pernambuco. As línguas foram dissecadas e analisadas quanto às suas características macroscópicas. Também foram submetidas à análise histológica, com cortes corados utilizando hematoxilina-eosina, azul de toluidina a 1%, tricrômico de Masson, picrosirius-hematoxilina e mucicarmim. A média de comprimento das línguas foi de 4,12 ± 0,17 cm, a largura foi de 1,50 ± 0,05 cm e a espessura foi de 2,00 ± 0,11 cm. A língua era composta por ápice, corpo e raiz. Numerosas papilas filiformes e fungiformes estavam presentes no dorso da língua, e apenas duas papilas valadas foram observadas. O exame microscópico revelou que a superfície dorsal da língua era revestida por epitélio pavimentoso estratificado queratinizado, com presença de melanina na camada basal. A língua era composta predominantemente por fibras musculares esqueléticas estriadas. Mastócitos ocasionais foram encontrados no tecido conjuntivo. Numerosas glândulas mucosas foram observadas, mas não foram encontradas glândulas serosas. Esses achados morfológicos da língua de *B. variegatus* são parcialmente únicos para a espécie e são influenciados por sua dieta e hábitos alimentares.

PALAVRAS-CHAVE: sistema digestivo; cavidade oral; papilas; morfometria; Bradypodidae.

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INTRODUCTION

The superorder Xenarthra comprises sloths, armadillos, and anteaters. Sloths are divided into two genera: Choloepus, which includes two-toed sloths, and Bradypus, which encompasses three-toed sloths (Ruiz-Garcia *et al.*, 2020). The brown-throated sloth, *Bradypus variegatus*, is notable within the Bradypodidae family for its extensive geographical distribution, encompassing regions from Central America to the Amazon and Atlantic Forest in Brazil (Hayssen, 2010).

The anatomical and physiological characteristics of *B. variegatus* have been the subject of considerable research and are well documented. The coat exhibits various shades ranging from brown to gray. The body support of these small animals is maintained by a skeleton composed of 8 or 9 cervical vertebrae, 14 or 15 thoracic vertebrae, only 4 lumbar vertebrae, and 6 vertebral bodies fused to form a sacrum. The thoracic limbs are modestly larger than the pelvic limbs (Hautier *et al.*, 2010).

Sloths generally have a low metabolic rate, considered to be one of the lowest among mammals. Their metabolic rate is approximately 74% of the expected value for their body weight, and their body temperature ranges from 30°C to 34°C (Nagy & Montgomery, 1980). With regard to the digestive system, studies indicate that *B. variegatus* has 18 teeth, all of which are molariform with cusps and interdental spaces (Albuquerque *et al.*, 2016). This dental characteristic may be associated with the process of maceration of their folivorous diet, facilitating enzymatic action in the stomach (Azarias *et al.*, 2006).

The stomach is a large organ situated within the abdominal cavity. The main portion of the stomach is divided into four distinct compartments, and a diverticulum and two prepyloric regions are also present (Mesquita *et al.*, 2020). Fermentation of ingested food appears to occur within these gastric compartments, facilitated by symbiotic microorganisms including bacteria and fungi (Foley *et al.*, 1995). The small and large intestines of sloths are notably shorter than those of most mammals (Carvalho *et al.*, 2014). The length of the small intestine in males is 163 ± 1.65 cm, while that in females is 151 ± 2.30 cm. The large intestine has a length ranging from 43 ± 1.7 to 44 ± 3.1 cm (Fonseca Filho *et al.*, 2018). However, detailed information on other vital organs within the digestive system of sloths remains insufficient to fully comprehend their digestive physiology.

In this context, we conducted a morphological investigation of the mucosa and interface between the epithelium and connective tissue of the tongue of *B. variegatus*. The objective was to expand the current knowledge of the anatomy, histology, and physiology of the digestive system of this species and thereby contribute to future research and conservation of these animals.

MATERIAL AND METHODS

The study utilized five adult *B. variegatus* specimens (three males and two females). All animals had died of natural causes, and the research team obtained authorization from the Brazilian Institute of Environment and Renewable Natural Resources to conduct the study. The animals were subsequently incorporated into the collection of the Anatomy Laboratory at the Academic Center of Vitória de Santo Antão, Universidade Federal de Pernambuco. Thereafter, the specimens were fixed in a 10% formaldehyde solution and preserved in a tank containing the same preservative solution.

Macroscopic Analysis

The animals were placed in the dorsal decubitus position, and the tongue was accessed through a median longitudinal incision in the ventral region of the neck. The skin, subcutaneous tissue, and muscles were then folded back. The mandibular area was sectioned to remove the tongue, facilitating its morphological study. After individualization, the tongues were identified and subjected to a comprehensive examination for anatomical description, including the acquisition of external morphological characteristics, utilizing a trinocular stereomicroscope (Tecnival).

Morphometry

Morphometric data were collected using a steel caliper with a measurement range of up to 150 mm and accuracy of 0.02 mm, which was used to measure the length, width, and thickness of the tongue. The length was defined as the distance between the root and the apex of the tongue. The width was measured as the distance between the lateral edges of the organ. The thickness was defined as the distance between the dorsal and ventral surfaces of the tongue.

Microscopic Analysis

Each tongue was sectioned in the transverse plane along the rostrocaudal axis, resulting in six fragments that spanned the apex, body, and base of the tongue. The fragments were then immersed in a solution of 10% neutral buffered formaldehyde for 24 hours. Next, the fragments were dehydrated in ascending concentrations of ethyl alcohol, cleared with xylene, and impregnated and embedded in paraffin. For each tongue fragment, semi-serial sections with a thickness of 5 µm were produced. The sections were then placed on slides that had been greased with albumin and subjected to a 24-hour drying process in an oven at 37°C. The sections were stained with hematoxylin–eosin for preliminary histomorphological analysis, as well as with 1% toluidine blue, Masson's trichrome, mucicarmine, and picrosirius–hematoxylin.

RESULTS

Macroscopic Aspects

The tongue of *B. variegatus* had a root, an elongated body with a slightly rounded apex, and a median groove near the root (Figure 1A). Macroscopically, three types of papillae could be distinguished on

the dorsal surface: fungiform (Figure 1B), filiform (Figure 1C), and vallate (Figure 1D). The two vallate papillae were situated in two shallow grooves in close proximity to the root of the tongue.

The filiform papillae were distributed throughout the dorsal surface of the tongue, while the fungiform papillae were preferentially arranged in the dorsolateral region, interspersed with the filiform papillae. Additionally, small numbers of papillae were observed in the lateral region of the tongue, whereas none were present on the ventral surface. In all animals, the apex of the tongue exhibited dark pigmentation with an irregular distribution pattern.

Morphometric Aspects

The average length, width, and thickness of the tongue of the brown-throated sloth (*B. variegatus*) were 4.12 ± 0.17, 1.46 ± 0.05, and 2.00 ± 0.11 cm, respectively, as shown in Table 1.

Microscopic Aspects

The dorsal surface of the tongue was lined with a keratinized stratified squamous epithelium exhibiting basal, spinous, granular, and horny layers. However, the lucid layer was absent. By contrast, the epithelium on the ventral surface was thicker and non-keratinized. Melanin was deposited in the basal layer of the dorsal epithelium at the apex of the tongue (Figure 2A). The underlying epithelium was surrounded by loose connective tissue, with a large-caliber vein situated immediately below it (Figure 2B). Additionally, a variable amount of dense, unshaped connective tissue was observed (Figure 2C). The body of the tongue was primarily composed of skeletal striated muscle tissue, with numerous skeletal striated muscle fibers intersecting in various planes and exhibiting strong adherence to the connective tissue (Figure 2D). The fibers exhibited a gradation in thickness, with the thinnest occurring at the apex of the tongue and the thickest at the middle third and root.

The muscle fibers contained numerous sets of mucous glands with excretory ducts, which comprised more than two layers of cubic cells (Figures 3A and 3B). No serous or seromucous glands were observed. Mast cells were occasionally identified in the connective tissue of all three sections of the tongue (Figure 3C), with a higher prevalence near nerves and blood vessels. The cells exhibited various shapes ranging from round to slightly fusiform.

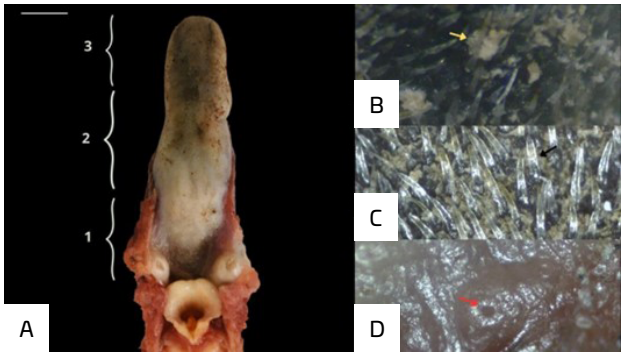

Figure 1. (A) View of the dorsal surface of the tongue of *Bradypus variegatus*: 1: root, 2: body, 3: apex, scale bar 2 cm. (B) Fungiform papillae (yellow arrow), 80x magnification; (C) Filiform papillae (black arrow), 90x magnification; (D) Vallate papillae (red arrow).

DISCUSSION

In accordance with the classification proposed by Doran and Baggett (1971), the tongue morphology of *B. variegatus* is

Table 1. Morphometric data from the tongue of *Bradypus variegatus*.

Animals	Length	Width	Thickness
I	4.1 cm	1.4 cm	1.9 cm
II	4.0 cm	1.4 cm	1.9 cm
III	4.4 cm	1.5 cm	2.1 cm
IV	4.1 cm	1.5 cm	2.1 cm
V	4.0 cm	1.5 cm	2.0 cm
Mean	4.12 cm	1.46 cm	2.0 cm
Standard Deviation	0.17	0.05	0.11

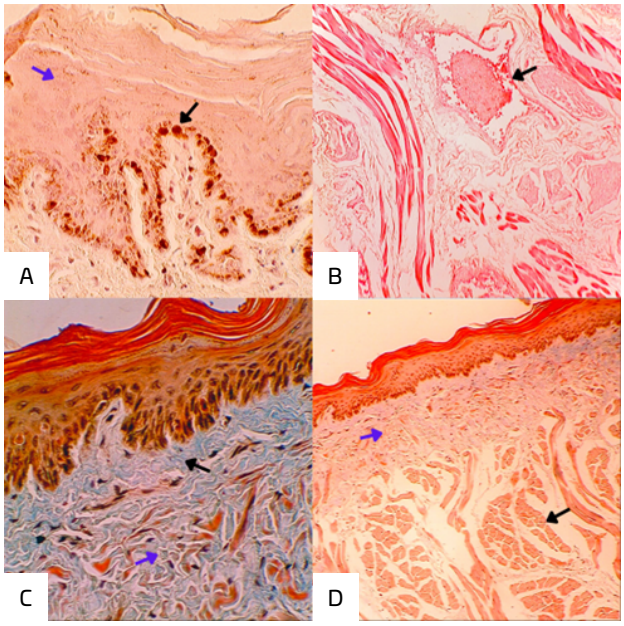

Figure 2. Photomicrograph of the tongue of *Bradypus variegatus*. A) Longitudinal section of the tongue apex, where the keratinized stratified sidewalk epithelial tissue (blue arrow) shows different layers of cells, and the basal layer is intensely stained due to the presence of melanin (black arrow). Stained with Hematoxylin-Eosin (HE), 400x magnification. B) Longitudinal section of the middle third of the tongue, highlighting a large vein (black arrow). Hematoxylin-Eosin (HE) stain, 400x magnification. C) Longitudinal section of the tongue, showing loose connective tissue (black arrow) and non-modeled dense connective tissue (blue arrow) stained with Masson's trichrome, magnification 400x. D) Longitudinal section of the tongue, showing the distribution of connective tissue (blue arrow) below the epithelium and between the muscle fibers (black arrow), stained with Masson's trichrome, magnification 100x.

consistent with type I, which is defined by an elongated body and a rounded apex. The presence of a median furrow near the root of the tongue is consistent with the description provided for *B. torquatus* (Benetti *et al.*, 2009). However, it differs from the findings of Martins *et al.* (2014), who described the presence of a median furrow extending from the root of the tongue to the apex of the tongue in the brown-throated sloth. The morphometric values obtained in the present study also demonstrated similarities with the measurements recorded by Benetti *et al.* (2009) for the tongue of *B. torquatus*, which were a length, width, and thickness of 4, 2, and 3 cm, respectively.

The dorsal surface of the tongue contained numerous filiform papillae throughout its length, a feature that differs from previous reports of the tongue of cingulate xenarthrans, such as *Euphractus sexcinctus* (Silva *et al.*, 2015) and *Dasypus novemcinctus* (Morais *et al.*, 1994), as well as vermilingua xenarthrans, which exhibit filiform papillae more densely distributed in the rostral and middle regions (Casali *et al.*, 2017). These discrepancies may be associated with the distinct dietary specificities of the Xenarthra. Cingulates are opportunistic feeders, ranging from insectivores to carnivores-omnivores (Ramsey *et al.*, 1981), while vermilinguas are strictly insectivores (Miranda *et al.*, 2003). By contrast, sloths have a specialized diet consisting primarily of leaves, although their diet can also include shoots, flowers, stems, and fruits (Dill-McFarland *et al.,* 2016).

Filiform papillae are regarded as mechanical structures. Their configuration endows the tongue with a surface that is conducive to its movements and to grasping food (Dyce *et al.*, 2019). In sloths of the genus Bradypus, the tongue has a rounded apex with lateral edges that are almost parallel for the majority of its length. The tongue's cross section is oval, resulting in a compact and thick structure (Benetti *et al.*, 2009). A higher density of filiform papillae may be associated with an increased capacity for intra-oral manipulation of resistant plant materials, which constitute the majority of sloths' diet.

In the present study, the fungiform papillae were predominantly located on the lateral margins of the tongue, a finding that is consistent with the descriptions provided in previous studies of rats (Miller & Preslar, 1975) and humans (Kutuzov & Sicher, 1951). However, these papillae were also interspersed with the filiform papillae on the dorsal surface, in a pattern similar to that observed in other herbivores such as wild ruminants (Machado *et al.*, 2016; Sari *et al.*, 2010) and domestic ruminants (Fonseca *et al.*, 2011). A previous study demonstrated that the fungiform papillae of *B. torquatus* possess approximately 10 taste pores (Benetti *et al.*, 2009). This indicates a gustatory function of these papillae in sloths, analogous to that observed in domestic animals (Dyce *et al.*, 2019).

Two papillae exhibiting a rounded shape and situated within deep grooves were also found in the present study. This is analogous to the findings reported for the sloth *B. torquatus* (Benetti *et al.*, 2009). The number of papillae on the tongue of the brown-throated sloth was comparable to that reported for other xenarthrans, including *Euphractus sexcintus* (Silva *et al.*, 2015), *Tamandua tetradactyla*, and *Myrmecophaga tridactyla* (Casali *et al.*, 2017). The positioning of the vallate papillae on the caudal third of the tongue exhibits a pattern consistent with that observed in the majority of mammals (Doran & Baggett, 1971).

Histochemical analysis revealed that the epithelial layer of the dorsal tongue mucosa of *B. variegatus* comprised multiple layers of epithelial cells, ranging from a basal layer with live and highly active cells to completely keratinized cells. This observation is consistent with the findings reported in other xenarthran species, including *B. torquatus* (Benetti *et al.*, 2009), *Myrmecophaga tridactyla*, *Tamandua tetradactyla*, *Cyclopes didactylus* (Casali *et al.*, 2017), and *Euphractus sexcinctus* (Silva *et al.*, 2015). Keratin is a polypeptide composed of amino acid units distinguished by their rigid conformation (Moore *et al.*, 2005). Therefore, this substance may confer resistance to the tongue, which is of particular significance given the herbivorous dietary habits of these animals.

The presence of melanin, microscopically observed in the rostral region of the tongue, has not been documented in prior studies of the genus Bradypus (Benetti *et al.*, 2009). The detection of this substance gives rise to questions regarding protection against ultraviolet radiation, traumatic damage, oxidative stress, extreme temperatures, and mechanical pressure. These phenomena have been described in other animal, plant, and fungal tissues (Freitas *et al.*, 2021; Tong *et al.*, 2023). However, the literature does not provide clear information on the specific function of melanin in the tongue of mammals. This is a characteristic that requires further investigation.

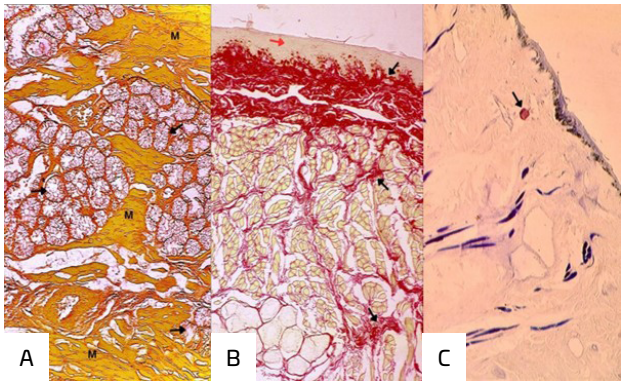

Figure 3. Photomicrograph of the tongue of *Bradypus variegatus*. A) Longitudinal section of the mid-rostral third, where the mucous glands (black arrow) are organized between the muscle fibres (M), stained in mucicarmine, magnification 100x. B) Longitudinal section of the mid-rostral third of the tongue, highlighting the abundant presence of connective tissue (black arrow) between the muscle fibers and the keratin layer of the epithelium (red arrow), stained in picrosirius–hematoxylin, magnification 100x. C) Longitudinal section of the apex of the tongue, where a mast cell stained red (black arrow) is immersed in the epithelium-conjunctive tissue interface, stained in toluidine blue, magnification 100x.

The tongue of *B. variegatus* is characterized by a high density of mucous glands, which may play a role in salivary secretion to support the herbivorous feeding habits of the sloth. This hypothesis was proposed by Quintarelli and Dellovo (1969), but the authors lacked confirmation. Given that mammalian salivary enzymes lack the capacity to digest cellulose, which is a significant component of the plant cell wall, saliva may play a crucial role in the feeding process. The moistening of food in the mouth may facilitate chewing, contributing to more efficient digestion in the stomach through the action of bacteria, as demonstrated in *B. tridactylus* by Foley *et al.* (1995).

CONCLUSIONS

The tongue of *B. variegatus* was shown to be keratinized and contain a high density of filiform papillae, which may provide resistance and aid in the manipulation of fibrous plant materials, which predominate in the sloth's natural diet. Additionally, the tongue was found to be rich in mucous glands, indicating that salivary secretion plays an important role in the eating habits of this animal. These findings should be considered in ex situ feeding protocols for this species. Further analysis of the functions of melanin within the tongue is warranted to clarify its morphofunctional contributions to these animals.

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