



Topographic and morphological aspects of the spleen of brown-throated sloth *Bradypus variegatus* (SCHINZ, 1825)

[Aspectos topográficos e morfológicos do baço de preguiça-de-garganta-marrom
Bradypus variegatus (SCHINZ, 1825)]

"Scientific Article/Artigo Científico"

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Abstract

Sloths are wild animals with arboreal habits, with slow metabolism, found in tropical forests from South America to Central America. However, the lack of knowledge of their anatomy does not favor the conservation of the species in veterinary care centers, due to its peculiar anatomy. Therefore, the objective of this study was to describe the topography and morphology of the spleen of the species *Bradypus variegatus*, in order to collect more information to assist in the clinical-surgical processes of the species. Eight corpses of *B. variegatus*, previously fixed with 20% formaldehyde and preserved in 30% saline solution, were dissected for the macroscopic study of the spleen. A healthy animal, living in semi-captivity, was underwent to a tomography of the abdominal region, for observation of the spleen, while two specimens were destined for the microscopic study of the organ immediately after death. Based on the data obtained, the spleen presented a topography and tissue composition similar to other mammals, but its morphology, absence of lienal hilum and anatomical arrangement in the abdominal cavity differed from most domestic and wild animals.

Palavras-chave: Anatomy; Bradypodidae; lymphoid organ; clinic; surgery.

Resumo

Preguiças são animais silvestres de hábitos arbóreos, de metabolismo lento, encontrados em florestas tropicais desde a América do Sul até a América Central. Porém, o desconhecimento de sua anatomia não favorece a conservação da espécie em centros de atendimento veterinário, devido a sua anatomia peculiar. Portanto, o objetivo deste estudo foi descrever a topografia e morfologia do baço da espécie *Bradypus variegatus*, a fim de coletar mais informações para auxiliar nos processos clínico-cirúrgicos da espécie. Oito cadáveres de *B. variegatus*, previamente fixados em formaldeído a 20% e preservados em solução salina a 30%, foram dissecados para estudo macroscópico do baço. Um animal saudável, vivendo em semi-cativeiro, foi submetido a uma tomografia da região abdominal, para observação do baço, enquanto dois espécimes foram destinados ao estudo microscópico do órgão imediatamente após a morte. Com base nos dados obtidos, o baço apresentou topografia e composição tecidual semelhantes a outros mamíferos, porém sua morfologia, ausência de hilo lienal e disposição anatômica na cavidade abdominal diferem da maioria dos animais domésticos e silvestres.

Keywords: Anatomia; Bradypodidae; órgão linfoide; clínica; cirurgia.

Introduction

Sloths are wildlife and arboreal animals, spending most of their time on top of trees like *Ficus* sp. and *Cecropia* sp., where they feed on its leaves. Often associated with their slow metabolism, they have additional joints between the lumbar vertebrae, eight to nine cervical vertebrae, internal testicles in males and a duct common to the genital and urinary systems in females (Montgomery and Sunquist, 1978; Wetzel, 1982; Nowak, 1999; Barreto et al., 2013; Fonseca Filho et al., 2018).

Bradypus variegatus is the best known species of the genus, also called the common sloth or brown-throated sloth, it can be found in tropical forests from southeastern Brazil to Honduras. It has brown fur on the throat and sides of the face, so that the male can be distinguished from the female by an orange stain on the back hair, with long limbs, short body, short and thick tail. Even though it is not yet an endangered species, the reduction in its population is remarkable, directly associated with the destruction of its natural habitat, along with illegal exploitation and trade (Hayssen, 2009; Xavier et al., 2010).

Despite the continuous advances in science, the spleen is still an organ of which little is known, especially regarding its role in the functioning and maintenance of the organism's homeostasis. Until then, it is known that this organ performs functions involved with the immune and circulatory systems, participating in hematopoiesis and contributing to the removal of microorganisms and senescent cells (König and Liebich, 2016).

The understanding of its functions and the morphophysiological knowledge of this organ are essential for veterinary clinic and surgery. Based on this information, the veterinarian can develop the best strategy to preserve the animal's life, such as performing procedures that preserve splenic tissue as much as possible, such as partial splenectomy in the case of initial treatment of splenomegaly, for example (Fossum and Caplan, 2014).

Thus, this study aimed to describe and discuss the topography and macroscopic and microscopic morphology of the spleen of the *B. variegatus* species sloth, hoping to generate data to contribute to veterinary medical practice and development of appropriate procedures aimed at treating animals injured or sick, favoring the preservation of the species.

Materials and Methods

For the research development, 10 corpses of adult sloths of the *B. variegatus* species were used, coming from the Center for Triage of Wild Animals of Pernambuco (CETAS-TANGARÁ), after natural death and taken to the Anatomy Area of the Department of Morphology and Physiology Animal from UFRPE, to obtain the data. In addition to a healthy adult specimen, living in semi-captive condition at the Recife Zoo, at the Preguiça-de-Garganta-Marrom Institute.

The topographic and morphological description of the spleen was performed in eight cadavers, four males and four females, fixed in 20% formaldehyde and preserved in a tank containing 30% saline solution. After conservation, they were dissected through a midsagittal incision of the abdominal wall, with the aid of a scalpel, forceps and scissors, and subsequent retraction of the skin and musculature, in order to observe the spleen *in situ*. Its relationship with other organs and adjacent structures was analyzed, considering the abdominal limits and their regional subdivisions, based on the recommendations of Merighi (2010): The cranial limits of the abdomen were obtained by tracing oblique planes to the median plane, following the xiphoid process of the sternum to the last pair of ribs, while the caudal limits were acquired from planes that obliquely tangent the caudal margin of the iliac bones until the pubis. The dorsal limit was reached by delineating a longitudinal plane that passed from the spine of the scapula to the coxal tuberosity of the iliac bone and the ventral limit determined by a longitudinal line that passes through the condyle of the humerus to the cranial margin of the iliac bone. Then, a transverse plane was traced bordering the last pair of ribs, obtaining the cranial region of the abdomen, subdivided into xiphoid, left and right hypochondriac regions. Similarly, a transverse plane was designed between the coxal tuberosities of the pelvis to obtain the mid-abdominal region, divided into umbilical, left and right lateral abdominal regions. Between this transverse plane and the caudoventral oblique plane of the ilium, the caudal abdominal region was obtained, where the right and left inguinal and pubic regions were obtained.

Subsequently, in each of the animals, the spleen was dissected and removed from the cavity to identify its morphology. Measurements of length, width and thickness were performed. Morphometric data were obtained with a steel

caliper (150mm/0.02mm), calculating the mean of the values for males and females and their respective standard deviations.

The live specimen used was a male, designated for tomography of the abdominal region, in a veterinary clinic, in order to obtain parameters of the spleen from an image exam, through single-channel GE model tomography, the sedative Dexmedetomidine alpha-2 at a dosage of 10 micrograms being administered in the animal.

For the microscopic study, two spleens were collected immediately after the animals died. The organs were removed from the abdominal cavity and placed in buffered formaldehyde for 24 hours and transferred to a 70% alcohol solution. After that, the technique of embedding in paraffin was performed (Tolosa et al., 2003). The slides obtained were stained with hematoxylin-eosin and analyzed by light microscopy.

The steps were photo documented for analysis of the results and the International Committee on Veterinary Gross Anatomical Nomenclature, Nomina Anatomica Veterinária of 2017 (ICVGAN, 2017) was used for nomenclature purposes.

Results

Skeletonpia

The dissection showed the spleen of *B. variegatus* located in the left antimer of the middle region of the abdomen, positioned caudally to the left diaphragmatic pillar, cranial to the left kidney and transverse colon, latero ventrally to the costal arches and intercostal muscles of the 13th to 15th ribs, laterally to the musculature internal abdominal in the limits between the 15th rib and 2nd lumbar vertebra and latero dorsally to the prepylori I and II of the stomach and pancreas (Figures 1A and 1B). In a regional topography, it was located only in the left hypochondriac region in 75% of the females and 50% of the males and it was found between the left hypochondriac and left lateral abdominal regions in 25% of the females and 50% of the males. The tomographic examination corroborated these findings, where this organ was located in the left lateral abdominal region in a laterodorsal disposition to the prepylori of the stomach, in the limits between the 15th rib and 2nd lumbar vertebra, in the region of the paralumbar fossa (Figures 2A and 2B).

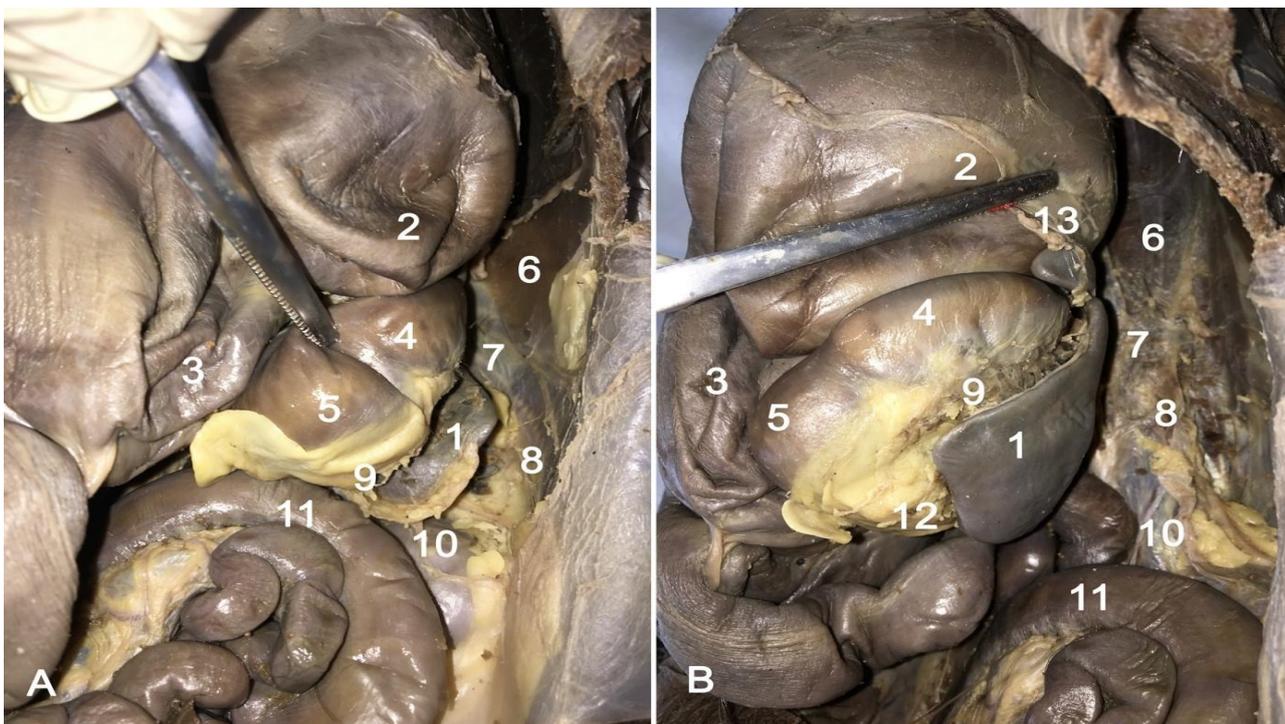


Figure 1. Photomacrographs of the anatomical position and topography of the spleen of the *Bradypus variegatus* sloth. (A) Ventral view; (B) Side view: 1. Spleen; 2. Left heart chamber of the stomach; 3. Central stomach chamber; 4. Prepylorus I; 5. Prepylorus II; 6. Diaphragm; 7. Left diaphragm pillar; 8. Intercostal muscles; 9. Pancreaticocolic ligament; 10. Left kidney; 11. Transverse colon; 12. Pancreas; 13. Gastrosplenic ligament.

Syntopy

At the dorsal extremity, there is syntopy cranially with the diaphragmatic pillar through the

phrenicocolic ligament and with the left heart chamber of the stomach through the gastrosplenic ligament, while its ventral extremity maintains a

caudal relationship with the cranial pole of the left kidney and transverse colon (Figures 1A and 1B). Most of its parietal surface is syntopy dorsally with the ribs and internal intercostal muscles of the 13th and 15th ribs and laterally with the abdominal muscles between the 15th rib and 2nd lumbar vertebra. Its visceral surface is in ventral syntopy with the pancreas through the pancreaticolienal ligament and with the pre-pylori I and II of the stomach, to which it remains attached by the gastrolienal ligament (Figure 1A and 1B).

Macroscopy

It is a dense, irregularly shaped organ with an enlarged ventral extremity, tapering to its dorsal

extremity, the latter presents a constriction, which inclines it in a caudal direction, giving it an atonic shape or a large “comma”. It has two margins, a slightly concave cranial one in contact with the pre-pylori I and II of the stomach and the other is slightly convex caudal and directed towards the pancreas. It revealed a smooth and convex parietal surface and a concave visceral surface (Figures 3A and 3B). On the parietal face, two impressions were observed, one gastric in the cranial portion and one pancreatic impression caudally. However, the presence of hilum was not observed on the visceral surface, since its lienal vessels presented extraparenchymal branches to three independent regions of the organ.

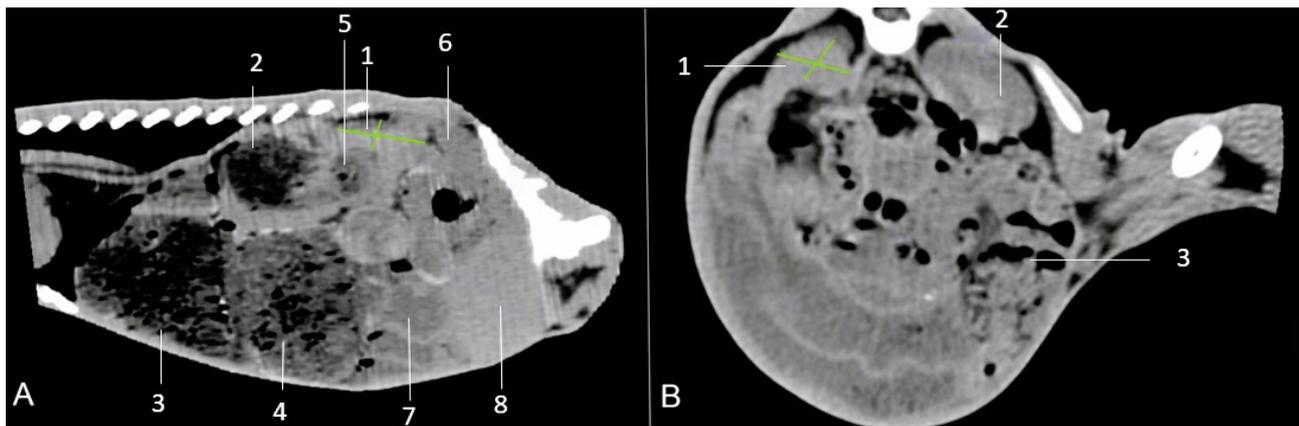


Figure 2. Identification of *Bradypus variegatus* spleen by tomography of the abdominal region. (A): 1. Spleen; 2. Left heart chamber of the stomach; 3. Cranial sac stomach; 4. Central stomach chamber; 5. Pre-pylorus; 6. Left kidney; 7. Small intestine; 8. Bladder. (B): 1. Spleen; 2. Right kidney; 3. Small intestine.

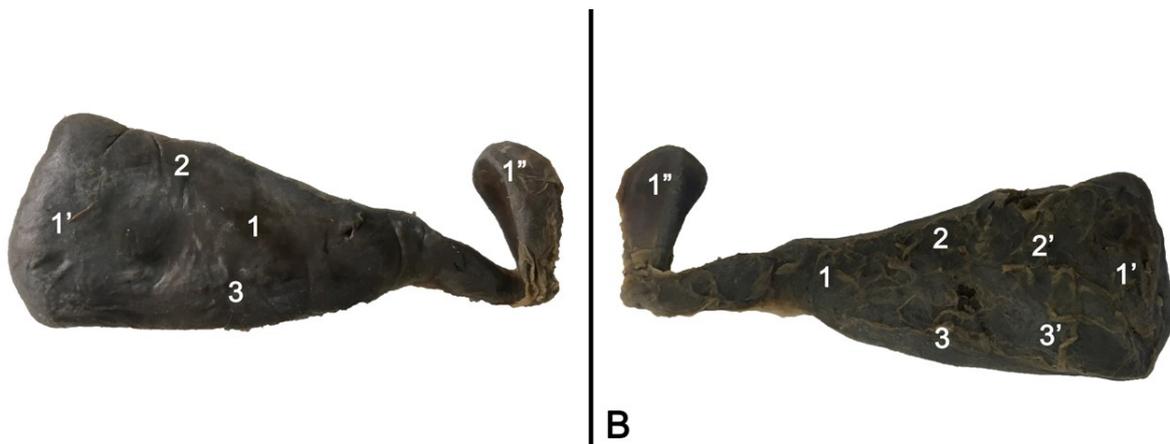


Figure 3. *Ex situ* photomacrographs of the *Bradypus variegatus* sloth spleen. (A) Parietal face: 1. Spleen; 1'. Ventral extremity; 1''. Dorsal extremity; 2. Cranial margin; 3. Flow margin. (B) Visceral Face: 1. Spleen; 1'. Ventral extremity; 1''. Dorsal extremity; 2. Cranial margin; 2'. Gastric impression; 3. Flow margin; 3'. Pancreatic impression.

Morphometry

As for the morphometric data, the spleen in females showed smaller measurements in the craniocaudal axis compared to males, mean of 5.15cm and 5.45cm, respectively. However, in relation to the laterolateral axis, the values were similar, with an average of 1.55cm for females and

1.60cm for males. Likewise, the measurement in the dorsoventral axis of this organ was similar for females and males, on average 0.40cm and 0.45cm, respectively. Despite the small variation observed between the analyzed models, there was no statistical difference for the analyzed parameters between females and males (Table 1).

Table 1. Morphometric measurements of the spleen of female and male *Bradypus variegatus* sloths.

Animals	Morphometrics Variables <i>ex situ</i>			
	Craniocaudal Axis*	Laterolateral Axis*	Dorsoventral Axis*	Volume**
Females	4,80	1,50	0,40	1,50
	5,10	1,80	0,30	1,44
	5,20	1,60	0,50	2,17
	5,30	1,50	0,40	1,66
Mean+DP	5,15±0,21	1,55±0,14	0,40±0,08	1,67±0,33
CV	0,042	0,091	0,204	0,199
Males	4,30	1,40	0,60	1,88
	5,80	1,70	0,40	2,06
	5,10	1,50	0,50	2,00
	6,20	1,70	0,40	2,20
Mean+DP	5,40±0,83	1,60±0,15	0,40±0,09	2,05±0,13
CV	0,153	0,094	0,213	0,064

DP: Standard deviation; CV: Coefficient of Variation; *cm; **cm³.

Microscopy

The microscopic analysis showed that the spleen does not have regions of cortex or medulla, but it was possible to identify the capsule, covered by a layer of dense non-modeled connective tissue, and still consisting of smooth muscle fibers from which trabeculae are emitted that divide the organ into incomplete compartments (Figure 4A).

It presents zones of red pulp and white pulp, being the main constituents of the internal structural organization of the organ. The arterial supply of the spleen was visualized in the germinal zones and in the white pulp, in the center of lymphoid nodules, while the marginal zone was constituted by dendritic cells, located between the red pulp and the white pulp of the spleen (Figure 4B and 4C).

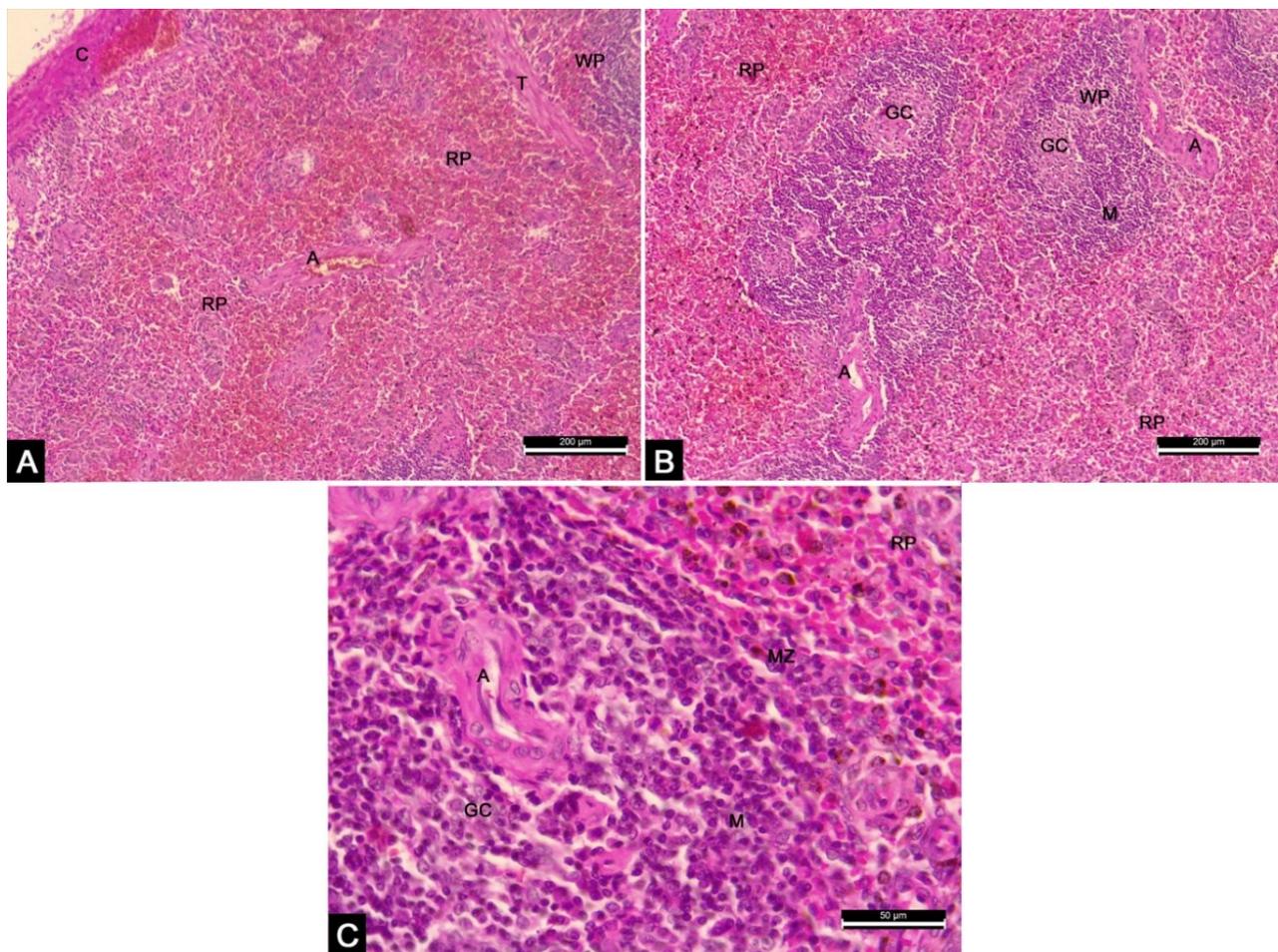


Figure 4. Photomicrographs of the spleen of *Bradypus variegatus* sloths. (A), (B) and (C): WP. white pulp; PR. red pulp; C. Capsule; T. Trabeculae; A. Arterial supply; GC. Germinal center; M. Mantle rich in B lymphocytes; MZ. Marginal zone rich in macrophages, B lymphocytes and some T lymphocytes.

Discussion

B. variegatus spleen was located in the left antimere of the abdominal cavity as in most domestic mammals (König and Liebich, 2016) and also in a close relationship with the stomach, similar to that described in other mammals (Dyce et al., 2010; Santos et al., 2013), however, it presented an oblique disposition, different from the spleen of rats and pigs, which are horizontally disposed towards the midline of the abdomen (Schanaider and Silva, 2004). In most domestic animals, the spleen is located in the left hypochondriac region (Sisson and Grossman, 1975). In this study, however, the organ extended to the left lateral abdominal region in some specimens.

B. variegatus sloth spleen had an irregular shape, being wider in the dorsal region and narrow in the middle region, similar to that reported for equids (Sisson and Grossman, 1975; Dyce et al., 2010) and capybaras (Germinaro et al., 1997), in which the spleen shows an enlarged base and a tapered apex, but there is no ventral region emitted in the craniodorsal direction. The spleens of bradypodids differ from those of rats, mice (Cesta, 2006; Treuting and Dintzis, 2011) and paca (Ribeiro et al., 2017) which are uniformly more elongated in their longitudinal axis, and even from cattle which are wide, triangular in shape in goats and sheep, and wide and short in cats (Dyce et al., 2010; König and Liebich, 2016). Its smooth, convex parietal face and concave visceral portion resembles other domestic mammals (Dyce et al., 2010), paca (Ribeiro et al., 2017) and agouti (Carvalho et al., 2017). However, no hilum was found on the visceral face, unlike most domestic species, which have a hilar region, with the exception of ruminants (König and Liebich, 2016).

As for morphometry, females and males showed similar spleen dimensions. However, it should be noted that these measures may not be conclusive, since some factors may interfere with the size of the organ of animals, especially splenic diseases (Morais et al., 2010), even if possible changes represent a normal response of the organ to its functions (Mahoney, 2011). Microscopy revealed structures similar to those reported in other mammals, with the presence of a capsular covering of dense connective tissue that emits trabeculae also made up of smooth muscle fibers into the parenchyma, dividing it into incomplete compartments. These muscle fibers in dogs, cats and horses are responsible for the contraction and expulsion of blood accumulated in the spleen (Junqueira and Carneiro, 2013). Despite this, smooth muscles are less concentrated than those found in rats and mice (Cesta, 2006) and other

xenarthrans such as armadillos (Galíndez et al., 2006).

In *B. variegatus* spleen, the parenchyma was structurally constituted by red pulp and white pulp. In the latter, the lymphoid nodules present throughout the parenchymal tissue surrounded the arterial supply, creating an island-shaped cellular arrangement, in accordance with that found in the paca, in which the lymphatic tissue formed periarterial lymphatic sheaths (Carvalho et al., 2017).

In turn, the red pulp, which covers a large part of the parenchyma, despite being constituted by reticular tissue and sinusoid capillaries, did not present surrounding muscle fibers as found in other species (Banks, 1999). The sinusoid branches found in the red pulp are directly related to liver functions related to filtration and maintenance of blood tissue in animals (Bachettini, 2009).

The arterial supply of the spleen was visualized in the germinal zones and in the white pulp, in the center of lymphoid nodules, as already explained. The lienal artery and its branches are responsible for the arterial supply of the spleen, in the same way that the splenic vein and its branches are responsible for the drainage of the organ also in domestic mammals (König and Liebich, 2016), capybara (Germinaro et al., 1997), agouti (Carvalho et al., 2017) and wild boar (Santos et al., 2013).

Conclusion

The spleen of sloths of the *B. variegatus* species has a topography and tissue composition similar to other mammals, but its morphology, the absence of lienal hilum and the anatomical arrangement in the abdominal cavity, differs from most domestic and wild animals.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics Committee

This study is authorized by the Ethics Committee for Animal Use and Experimentation of the Federal Rural University of Pernambuco (CEUA/UFRPE), nº 031/2019. And registration in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SinGen), nº ADA278D, in addition to a license from the Authorization and Information System in Biodiversity of the Chico Mendes Institute (SisBio/ICMBio), nº 46665-7.

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