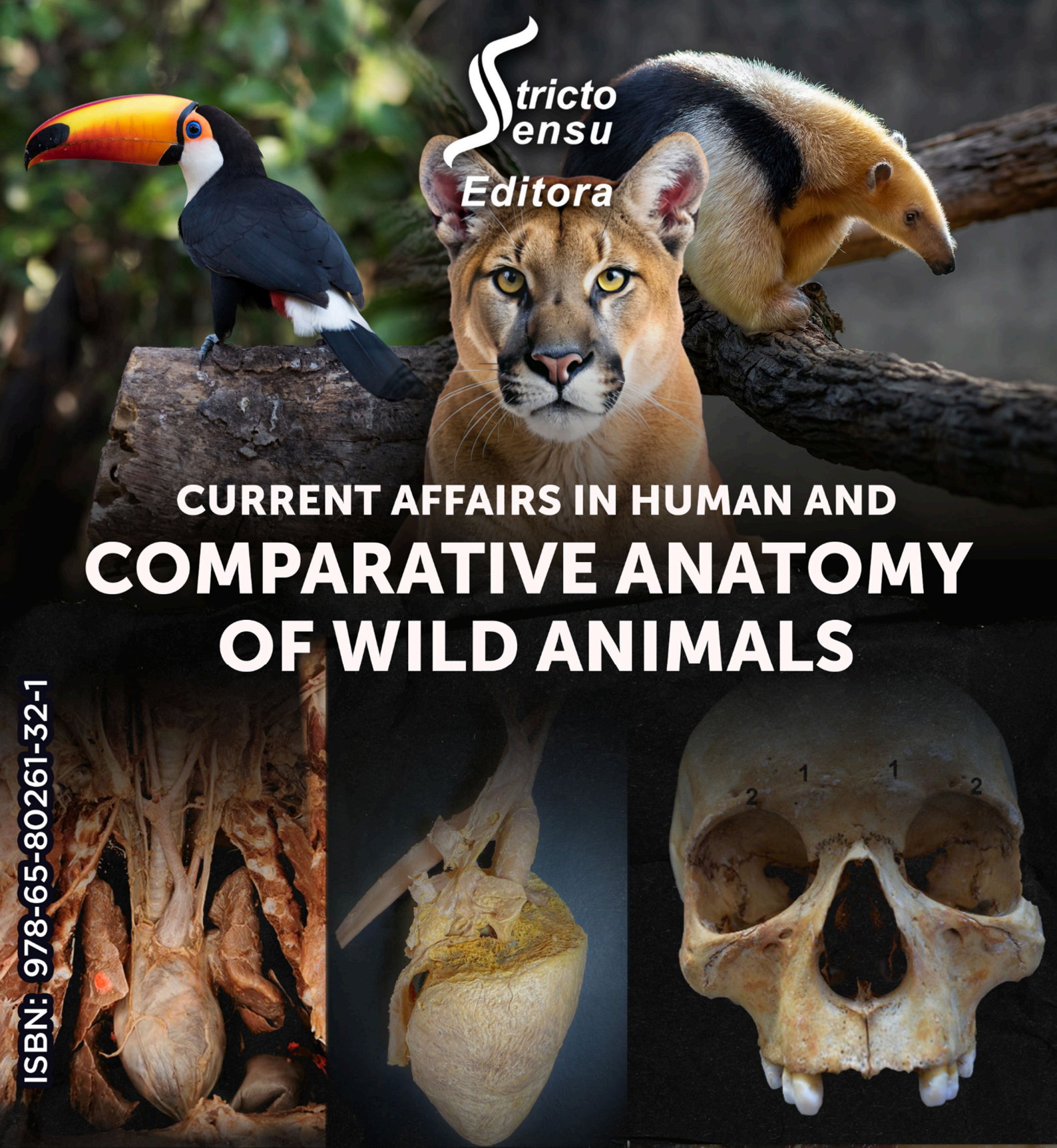

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2024

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APRESENTAÇÃO

The science that involves research in relation to Human and Animal Anatomy focuses on the study of description, comparison, alteration and variation ofhuman and animal morphology, in addition to presenting technologies and new forms of studies and teaching. Human and Animal Anatomy is one of the oldest and mosttraditional courses providing support and basis for various courses in health sciences and biological.

The book “Current Affairs in Human and Comparative Anatomy of Wild Animals: Volume 2” has 18 chapters with different areas of study in Human and Animal Anatomy, containing original research works that may be used as research and studies by students, professors and researchers ofdifferent areas of health and biological sciences.

The studies shown in this work encompass original and new research study trends or ways of teaching in anatomy that are not normally used or demonstrated in undergraduate courses.

We hope that this work provides new knowledge and is a sourceof inspiration to stimulate different types of research in the area of Human an Animal Anatomy.

Why use a descriptive and comparative anatomy book? When we compare and describe anatomical structures and functionality between different mammals, their recognition and learning becomes more easy.

All images were taken from carefully dissected carried out by professors with experience in the area, precisely so that there would be the highest level of fidelity to science possible, and, in this way, the reader would enjoy impeccable anatomical comparisons and description during his studies.

We wish you all a great read.

SUMÁRIO

ORIGIN AND BRANCHING OF THE CELIAC ARTERY IN THE TOCO TOUCAN (*Ramphastos toco albogularis* Cabanis, 1862)

Ludmila Angélica da Fonseca[1], Thiago Sardinha de Oliveira[2], Renata Anastácia de Oliveira Batista[2], Lázaro Antonio dos Santos[3], Lucas de Assis Ribeiro[1], Romeu Paulo Martins Silva[4], Roseâmely Angélica de Carvalho Barros[4], Zenon Silva[4] and Frederico Ozanam Carneiro e Silva[1]

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ABSTRACT
Tucanuçu is considered a typical representative of South American avian diversity, constituting one of the symbols of American rainforests and one of the oldest strains of birds with living representatives. In view of the importance of the Digestive and Circulatory systems, this study aims to study the origin and branching of the Celiac artery, the largest visceral branch of a. Descending aorta, in Tucanuçu, with a descriptive focus. Eight specimens of Tucanuçu (*Ramphastos toco albogularis* Cabanis, 1862) are used, however, with no defined age, from CETAS- Catalão - GO, which have the arterial system injected with latex “Artecola”, stained with pigment “Wandalar” Red. The preparation of the anatomical specimens follows the usual dissection procedures in Macroscopic Anatomy. The analysis of the data obtained, with regard to the origin and destination of the a. Celiac, in Tucanuçu, indicate striking similarities with those of other species of domestic and wild birds including a wide variability in the number of final branches of this vessel, an aspect not analyzed here, given the little or no practical importance.
Keywords: Anatomy, Birds and Arteries.

1. INTRODUCTION

Descriptive and comparative studies of wild animal anatomy are fundamental for acquiring data on their biology and for the conservation and preservation of ecosystems.

Furthermore, the knowledge of the circulatory system anatomy in wild animals has been sought by researchers worldwide, focusing on organs or specific body segments. Research on the anatomy of the circulatory system in wild birds, including the Toco Toucan, is rare.

The Toco Toucan is considered a typical example of the avian diversity of the South American continent. It is a member of the family Ramphastidae, which symbolizes the South American tropical forests, constituting one of the oldest lineages of birds with living descendants (SILVA NETO et al., 2013). Around 40 species of this group are recognized today (GALETTI et al., 2000; PIAZERA; CARVALHO JUNIOR, 2005; PIRES 2008). Here, the focus is on the Toco Toucan (*Ramphastos toco albogularis*).

Therefore, considering the importance of the circulatory system for animal anatomy, this study aims to investigate and describe the origin and distribution of the celiac artery in the Toco Toucan (*Ramphastos toco albogularis* - Cabanis 1862), intending to provide a foundation for understanding the biology of this species and contributing as support for conservation programs, and it may also be useful in clinical and surgical practices in veterinary medicine.

2. MATERIAL AND METHODS

This is a study aimed at describing the Anatomy of the Celiac arteries of the Toco Toucan (*Ramphastos toco albogularis*), focusing on their origins and branches, comparing the data with citations in other domestic and wild birds. Eight adult Toco Toucan cadavers were used, without defined ages or gender discrimination, donated by the Wildlife Research Institute - IPEVIS and by the Center for Rehabilitation of Wild Animals - CETAS of Catalão or collected on the margins of highways in Goiás and Minas Gerais under Authorization - SISBIO 37072-2.

This research was carried out at the Laboratory of Comparative Anatomy of Wild Animals of the Department of Biological Sciences of the Federal University of Catalão (LACAS-UFCAT). The specimens had their arterial system injected with "Artecola" Latex, stained with "Wandalar" red pigment, and fixed in a 10% aqueous solution of formalin by immersion for two weeks. Dissection followed usual techniques in Macroscopic Anatomy, but when necessary, a magnifying glass with 10X magnification was used for better preservation of the structures. Each specimen had the area of interest photographed, using a 7.2 mp

Cybershot camera. The structures were named according to the recommendations of the Handbook of avian anatomy (1993 avian anatomical nomenclature) and/or Nickel, Shummer, Seiferle (1977). The work is part of the project: Study of Comparative Anatomy of Wild Animals, which is approved by CEUA/UFU under Nº. 067/12.

3. RESULTS AND DISCUSSION

According to the observations in this research, in the Toco Toucan, the celiac artery is the first major branch of the descending aorta and irrigates most of the abdominal viscera, along with the cranial mesenteric artery and caudal mesenteric artery, (Figure 1) in accordance with citations by Baumel (1986), who states that the irrigation of the digestive tract is carried out by the celiac, cranial mesenteric, and caudal mesenteric arteries.

According to Bhaduri; Biwas; Das (1957), in domestic pigeons, the celiac artery is a large, single vessel that originates from the right face of the descending aorta, citations that are in agreement with the observations in Toco Toucan, (Figure 1), as well as with Silva et al. (1996) in broiler breeders; Nickel (1977) in domestic birds; Baumel (1986), also in domestic birds; Silva et al. (1997) in *Gallus gallus*, Ross breed; Perissotto et al. (2001) in *Gallus gallus*, Label Rouge breed; Rafael et al. (2005) in *Gallus gallus*, Arbor Acres breed; Miranda et al. (2006) in *Gallus gallus*, Redbro Plumé breed; Sacilotti de Carvalho et al. (2011) in *Gallus gallus*, Coob Avian 48 breed; Vasconcelos et al. (2012) in ostriches; Neira et al. (2014) in ostriches; Barbosa et al. (2016) in Canary. On the other hand, there are authors who do not refer to the celiac artery as the first branch of the descending aorta, although they affirm that it is a branch of it (SCHWARZE; SCHRÖDER 1970, in domestic birds; SILVA et al. 2005, in *Gallus gallus*, Coob 500 breed; GONÇALVES et al. 2010, in Mutum; GEEVERGHESE et al. 2012, in domestic pigeons; SILVA NETO 2013, in Toco Toucan and TOSTA et al. 2018, in *Gallus gallus*, AP 95 breed) (Figure 1).

Regarding the site of emergence of the celiac artery in the Toco Toucan, it arises from the right ventrolateral face, (Figure 1), while Silva et al. (1997) mention that in *Gallus gallus*, Ross breed, its origin is from the ventral face of the aorta, in agreement with the data from Perissotto et al. (2001) in *Gallus gallus*, Label Rouge breed; Miranda et al. (2006) in *Gallus gallus*, Redbro Plumé breed, and Geeverghese et al. (2012) in domestic pigeons. Still in this

regard, Silva et al. (2005), in *Gallus gallus*, Coob 500 breed, mark its origin from the right face of the aorta, in accordance with Tosta et al. (2018) in *Gallus gallus*, AP 95 breed (Figure 1).

The observations in the Toco Toucan show that the first branch of the celiac artery is an esophageal artery (Figure 1), in accordance with the literature in other birds (BHADURI; BIWAS; DAS 1957, SCHWARZE; SCHRÖDER 1970, NICKEL 1977, PERISSOTTO et al. 2001, RAFAEL et al. 2005, MIRANDA et al. 2006, SACILOTTI DE CARVALHO 2011, GEEVERGHESE et al. 2012, NEIRA et al. 2014, TOSTA et al. 2018). On the other hand, Silva et al. (1996) claim that in broiler breeders of the Hubbard lineage, the first branch of the celiac artery is the ventral proventricular artery, which in turn gives rise to an esophageal artery, in agreement with citations by Baumel (1986), Gonçalves et al. (2010), Vasconcelos et al. (2012), Silva Neto et al. (2013), and Barbosa et al. (2016).

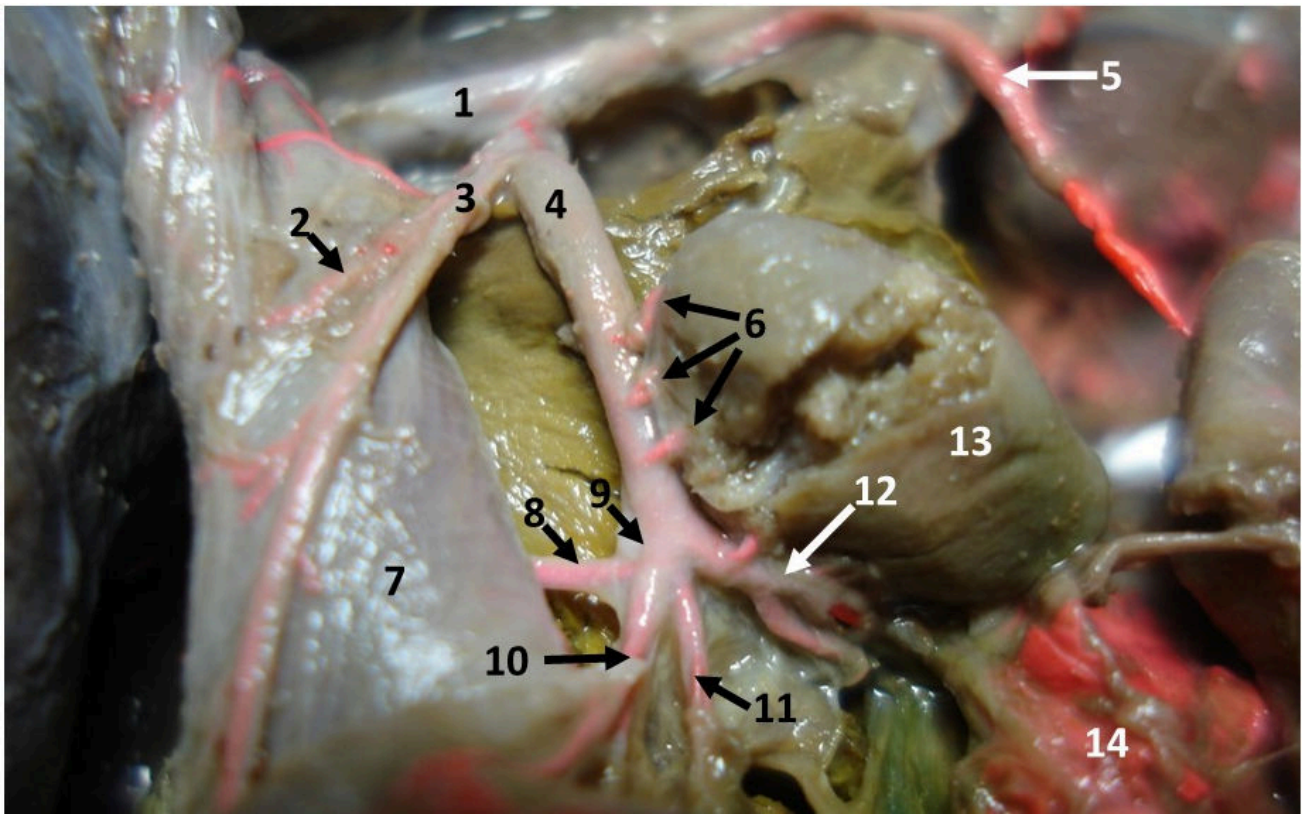

Figure 1. Ventral view of the abdominal cavity of Toco Toucan (*Ramphastos toco albogularis*).
1- aorta, 2- esophageal artery, 3- ventral proventricular artery, 4- celiac artery, 5- cranial mesenteric artery, 6 – Splenic arteries, 7- proventriculus, 8- dorsal proventricular artery, 9 – right branch of celiac artery, 10- ventral ventricular artery, 11- dorsal ventricular artery, 12- left branch of celiac artery, 13- spleen, 14- small intestine.

According to Schwarze; Schröder (1970), in domestic birds, the second branch of the celiac artery is the ventral proventricular artery, in accordance with the observations in the Toco Toucan, (Figure 1), as well as with the statements of Perissotto et al. (2001), Rafael et

al. (2005), Neira et al. (2016), Tosta et al. (2018), citations that are in agreement with the observations in the Toco Toucan.

The observations in the Toco Toucan reveal that immediately after the emergence of the ventral proventricular artery, the celiac artery divides into a Left branch and a Right branch (Figure 2), in agreement with the assertions of Baumel (1986), Silva et al. (1997), Perissotto et al. (2001), Miranda et al. (2006), Silva et al. (2005), Gonçalves et al. (2010), Vasconcelos et al. (2012), Geeverghese et al. (2012), Silva Neto et al. (2013), Barbosa et al. (2016), and Tosta et al. (2018).

Observations in the Toco Toucan reveal that the left gastric artery arises from the right branch, with the gastroduodenal artery being an individual branch of the celiac artery, (Figure 2). However, according to citations by Bhaduri; Biwas, Das (1957), the left branch gives rise to the left gastric artery, ventral proventricular artery, and left hepatic artery, while Baumel (1986) considers the hepatic artery and ventral proventricular artery as divisions of the left branch. On the other hand, Silva et al. (1997) mention that the left branch gives rise to the ventral gastric artery, left gastric artery, ventral proventricular artery, left hepatic artery, and esophageal artery. Perissotto et al. (2001) enumerate divisions of the left branch as the ventral gastric artery, left gastric artery, ventral proventricular artery, ventricular artery, and left hepatic artery. Similarly, Miranda et al. (2006) relate the ventral gastric artery, left gastric artery, and left hepatic artery as divisions of the left branch. Gonçalves et al. (2010) state that in Mutum, the left branch, after emitting several collaterals, terminates as the left gastric artery, ventral gastric artery, and left hepatic artery. Sacilotti de Carvalho et al. (2011) describe that the left branch of the celiac artery emits the ventral proventricular artery, ventricular artery, and left hepatic artery, while Vasconcelos et al. (2012) mention that the left branch emits the ventral proventricular artery, left gastric artery, ventral gastric artery, esophageal arteries, and left hepatic artery. Geeverghese et al. (2012) state that in domestic pigeons, the left branch divides into ventral ventricular artery, dorsal ventricular artery, splenic arteries, and left hepatic artery, terminating as the ventral gastric artery and left gastric artery.

According to Silva Netto et al. (2013), in Green-billed Toucan, the left branch of the celiac artery produces the ventral proventricular artery and left gastric artery as ramifications, terminating as the gastroduodenal artery. Neira et al. (2014) assert that the left branch supplies the ventral proventricular artery and ventricular artery. Barbosa et al. (2016) mention the ventral proventricular artery, gastroduodenal artery, ventral gastric artery, and left hepatic artery as collaterals of the left branch, terminating as the left gastric artery. Tosta et al. (2018)

enumerate left hepatic artery, ventral proventricular artery, ventricular artery, duodenal artery, left gastric artery, and jejunal artery as divisions of the left branch.

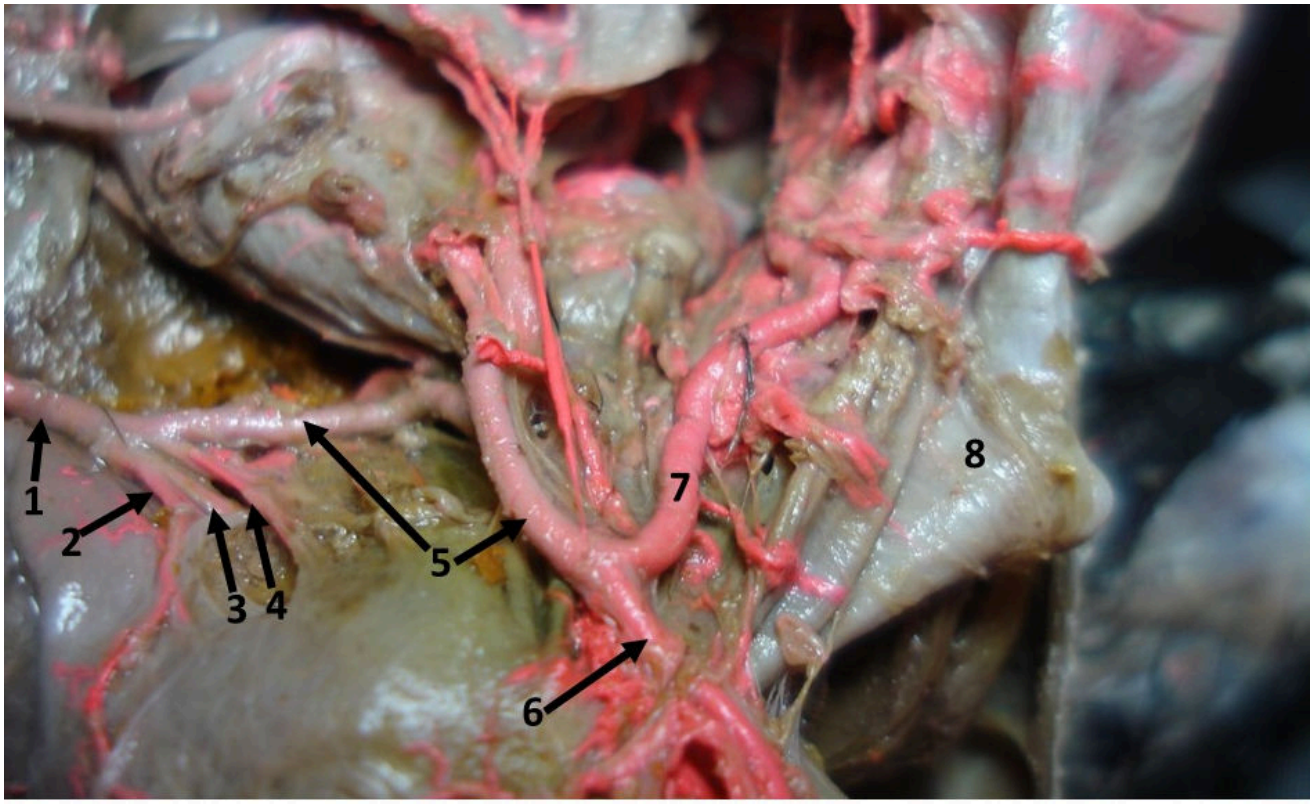

Figure 2. Ventral view of the abdominal cavity of Toco Toucan (*Ramphastos toco albogularis*).
1- celiac artery, 2- ventral ventricular artery, 3- dorsal ventricular artery, 4- duodenal artery, 5- left branch of celiac artery, 6- jejunal artery, 7- ileoceco-colic artery, 8- ileum

Regarding the right branch of the celiac artery, Baumel (1986) describes that in domestic birds, the right branch emits the splenic arteries, right hepatic artery, right gastric artery, and gastroduodenal artery, terminating as the pancreaticoduodenal artery and ileal artery. Silva et al. (1997), in *Gallus gallus*, Ross breed, mention that the right branch produces the splenic arteries, right gastric artery, dorsal gastric artery, vesicular artery, pancreaticoduodenal artery, jejunal artery, and ileocecal artery as collaterals. Perissotto et al. (2001) point out the splenic arteries, right gastric artery, vesicular artery, pancreaticoduodenal artery, ileocecal artery, and jejunal artery as divisions of the right branch. Miranda et al. (2006), in *Gallus gallus*, Redbro Plumé breed, describe the collaterals of the right branch as splenic arteries, right gastric artery, dorsal gastric artery, right hepatic artery, vesicular artery, pancreaticoduodenal artery, duodenojejunal artery, and ileal artery. Similarly, Gonçalves et al. (2010) enumerate splenic arteries, duodenal artery, jejunal artery, right hepatic artery as

divisions of the right branch, terminating as the right gastric artery and pancreaticoduodenal artery. Sacilotti de Carvalho et al. (2011) mention in *Gallus gallus*, Coob Avian 48 breed, the splenic arteries, right hepatic artery, hepatoduodenal artery, right gastric artery, pancreaticoduodenal artery, and ileocecal artery. Geeverghese et al. (2012), analyzing the Anatomy of domestic pigeons, describe the collaterals of the right branch as right hepatic artery, ileal artery, gastroduodenal artery, terminating as the pancreaticoduodenal artery. Silva Neto et al. (2013) point out the splenic arteries, right hepatic artery, right gastric artery, duodenal artery, pancreatic artery, duodenojejunal artery, terminating as the pancreaticoduodenal artery. Meanwhile, Neira et al. (2014) cite that in ostriches, the right branch produces the hepatic artery, ventricular artery, pancreatic artery, duodenal artery, cecal artery, and ileal artery. Barbosa et al. (2016) name in Canary, the right hepatic artery, right gastric artery, pancreatic artery, and ileocecal artery as collaterals of the right branch. In Toco Toucan, the splenic arteries arise from the celiac artery before it divides into the right and left branches (Figure 1).

Therefore, it is observed that in both domestic and wild birds, there is a wide variation in the branching, number of branches, and destination of the arteries originating from the celiac artery and its branches, from the first to the last branches, including within the same species, as observed in the Toco Toucan. Establishing a pattern of branching and destination of the respective branches of the celiac artery is practically impossible and apparently devoid of any practical result.

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Stricto Ensu Editora

ANATOMY OF THE MAIN ARTERIES OF THE NECK AND THEIR BRANCHES IN THE TOCO TOUCAN (*Ramphastos toco albogularis* Cabanis, 1862)

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ABSTRACT

The study uses eight adult specimens of Toco Toucan, without defined age or gender. Four of these specimens are injected with latex colored with red pigment into the arterial system and fixed in a 10% aqueous formalin solution, while the other four are injected with vinyl acetate into the arterial system and subjected to corrosion in a 30% nitric acid solution. The resulting molds and dissected pieces are analyzed and photographed. The description of the structures follows the Nomina Anatomica Avium. In the anatomy of the Toco Toucan, the aorta originates at the base of the left ventricle and cranially ascends as the ascending part of the aorta before bending to the right and dorsally, forming the aortic arch. From the aortic arch, arise the two large brachiocephalic arteries, located ventrally to the esophagus and trachea. Blood supply to the head occurs through the carotid artery and the two vertebral arteries. The bird is levocarotid. There is no branching of the common carotid near its origin, but the trunk that originates the vertebral artery and other arteries destined for neck structures arise separately from the subclavian artery. The carotid artery divides into the right and left carotid arteries, which then branch out. The vertebral arteries arise from a large cranio-cervicothoracic trunk, with several anastomoses between them and the common carotid artery, including two large anastomoses between the left vertebral artery and the common carotid artery in the middle part of the neck, one between C_4 and C_5 and another between C_5 and C_6.

Keywords: Anatomy, Arterial Vascularization and Toco Toucan.

1. INTRODUCTION

Comparative studies in Animal Anatomy are essential for descriptions, comparisons, and understanding the biology of different taxonomic groups, especially of wild animals. Moreover, these studies can assist in clinical diagnosis, treatment, and surgical approaches in Veterinary Medicine (PFRIMER, 2018). The Toco Toucan (*Ramphastos toco albogularis*) belongs to the order Piciformes and the family Ramphastidae, essentially consisting of toucans and aracari (PALLINGER; APRILLE, 2015). It is among the oldest avian lineages, with living descendants still present, representing one of the symbols of American tropical forests (FERREIRA-JUNIOR, 2012). It has specific characteristics including a large yellow-orange bill with a black spot at the end, black plumage on the back and belly, as well as a yellow, naked skin border around the eyes. The eyelids are blue, the chest is covered with white feathers, and the plumage under the tail is reddish. It is included in the order Piciformes and Ramphastidae (RAGUSA-NETTO, 2006 and 2008). It is not listed on the red list of endangered animals and is considered the largest species among the members of the Ramphastidae family (SICK 2001; MARINI, GARCIA 2005). It is considered a medium-sized bird, measuring up to 56cm in length and weighing around 540g, using the tip of its beak as a clamp to capture fruits and other food items (PIACENTINI et al. 2015).

The Toco Toucan is widely distributed in South American territory, inhabiting fields and forest areas of Brazil, Paraguay, Bolivia, and Argentina. It is an exotic bird of rare beauty, thus desired as a pet, linking it to wildlife trafficking (FECCHIO, 2010). The Toco Toucan is considered a typical example of avian diversity on the South American continent, which may be threatened, mainly by anthropogenic actions, including wildlife trafficking (PADULA 2017; REDFORD 1992). This bird generally lives alone or in pairs, at the tops of trees. Two subspecies are recognized: *Ramphastos toco toco* Muller, 1776, and *Ramphastos toco albogularis* Cabanis, 1862 (PALLINGER; APRILLE 2015). It is one of the major canopy frugivores, found in both continuous forest and semi-open environments, being common in landscapes ranging from closed to open environments (FRANÇA; RAGUSA-NETTO; PAIVA, 2009).

Regarding the anatomical knowledge of this species, including the circulatory system, the literature is scarce, if not absent. Thus, considering the importance of knowing the biology of the components of a biome, this work, associated with the research project entitled:

Comparative Anatomy of Wild Animals, aims to dissect, analyze, and describe the Anatomy of the carotid artery and its branches in the Toco Toucan (*Ramphastos toco albogularis*).

2. MATERIAL AND METHODS

This is a descriptive research, and for its development, 8 adult specimens of Toco Toucan, without defined age and without gender distinction, donated by the Wild Animal Screening Center of Catalão/Goiás (CETAS - Catalão/GO), under authorization SISBIO 37072-2, are used for the Laboratory of Comparative Anatomy of Wild Animals at the Federal University of Catalão-GO (LACAS-UFCAT).

The specimens are plucked, and 4 specimens are injected, in the Arterial System, with Latex Arte Cola, colored with red checkered pigment, in order to enhance the arteries to be dissected. After this procedure, they are fixed in a 10% aqueous solution of formalin and preserved in it. For the preparation of anatomical pieces, usual techniques of dissection in Macroscopic Anatomy are employed, namely: the opening of the thoracic and abdominal cavities, in the median plane, from the ventral aspect, to expose the circulatory system, starting from the heart. After the section, the soft tissues adjacent to the heart are removed so that the vessels of the base can be identified. Next, the skin of the ventral aspect of the neck is sectioned and removed, and the arterial vessels are dissected from the heart to the base of the skull. The carotid artery and its branches, as well as the vertebral arteries, are analyzed, described, and schematized. The other 4 specimens are injected into the arterial system with Vinilite (vinyl acetate) and then subjected to corrosion in a 30% nitric acid solution. The resulting molds, as well as the dissected pieces, are analyzed and photographed using a Sony Cybershot 7.2-megapixel camera.

The experimental protocols are carried out in accordance with the recommendations of the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee on Animal Use of the Federal University of Catalão (CEUA/UFCAT nº 01/22), and the description of the structures follows the Handbook of Avian Anatomy: Nomina Anatomica Avium (BAUMEL, 1993).

3. RESULTS AND DISCUSSION

The aorta, the common origin of all arteries comprising the systemic circulation, in the Toco Toucan, originates at the base of the left ventricle, extends cranially for a short distance as the Ascending Aorta before bending to the right and dorsally, forming the Aortic Arch.

From the Aortic Arch, the two large Brachiocephalic Arteries arise, located ventrally to the esophagus and trachea, which is consistent with the citations of Glenny (1951), Nickel; Schummer; Seiferle (1977), in birds in general, and Baumel in Sisson; Grossman (2008), in domestic birds. Piciformes, the taxonomic group to which the Toco Toucan belongs, are Levocarotid birds, meaning they possess a single carotid artery (GLENNY, 1951). This citation is in agreement with observations in the Toco Toucan (Figures 1 and 3).

Observations in the Toco Toucan indicate that blood supply to the head occurs through the Carotid Artery and two Vertebral Arteries, consistent with Glenny (1951) in Levocarotid birds and inconsistent with the considerations of Nickel; Schummer; Seiferle (1977), in birds in general, and Baumel in Sisson; Grossman (2008), in domestic birds, even though these belong to other taxonomic groups considered Bicarotid birds (Figure 1).

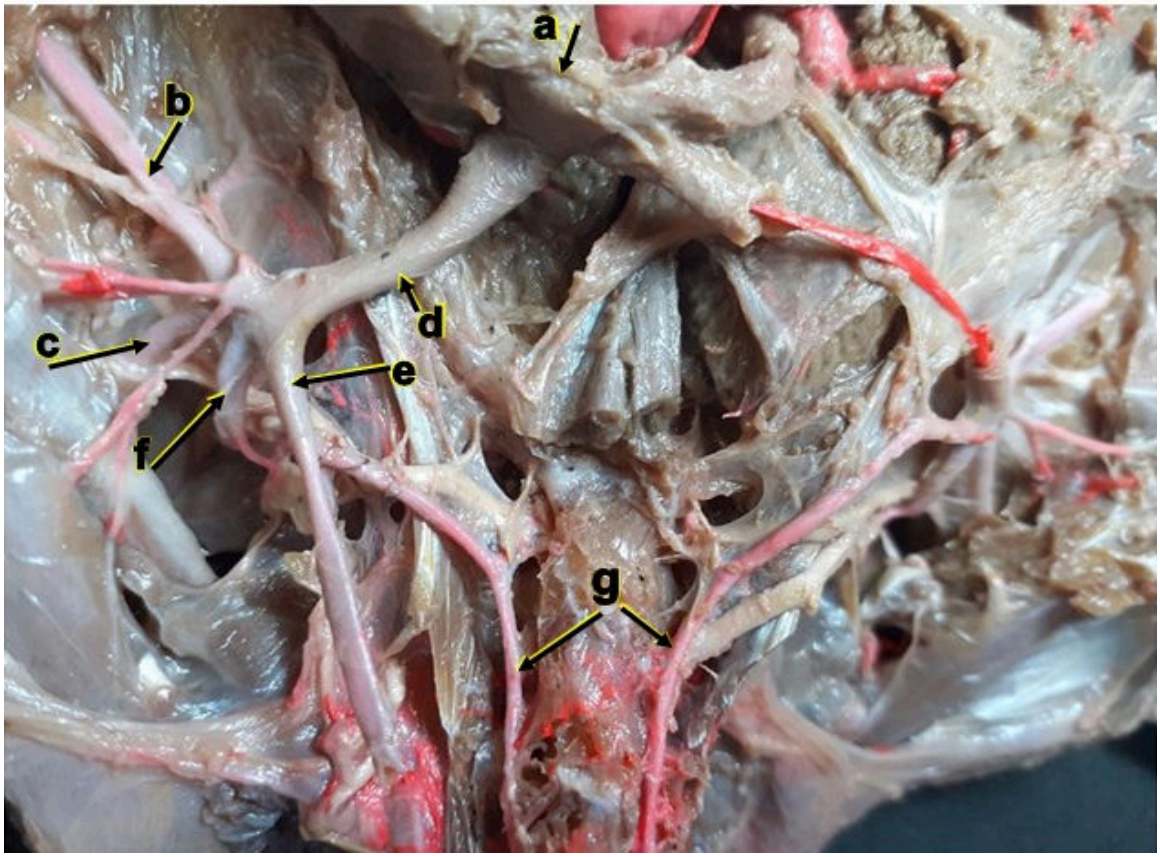

Figure 1. Ventral view of the cervicothoracic region of the Toco Toucan.
a- Aortic arch; b- Left pectoral artery; c- Left subclavian artery; d- Left brachiocephalic trunk; e- Common carotid artery; f- Craniocervicothoracic trunk; g- Vertebral arteries.

According to Nickel; Schummer; Seiferle (1977), in birds in general, the Common Carotid Arteries originate from the respective Brachiocephalic Trunks and shortly after their origins, they give off several branches: the Vertebral artery as the main branch, the Cranial Thyroid artery, the Caudal Thyroid artery, a small Bronchial artery, and the Ascending Esophageal artery, following cranially as the Common Carotid artery. Conversely, Baumel in Sisson; Grossman (2008) states, in domestic birds, that the Common Carotid artery emits a Vagovertebral Trunk and continues to converge towards the median plane still as the Common Carotid artery. Both authors affirm that the Common Carotid Arteries converge towards the median plane, following cranially over the ventral surface of the vertebral bodies in a deep osteofibromuscular canal, between the ventral vertebral processes. Observations in the Toco Toucan indicate that there is no branching of the Common Carotid artery near its origin, but rather the trunk that originates the Vertebral artery and other arteries destined for the structures of the neck root arise separately from the Subclavian artery. The Common Carotid artery arises independently and converges towards the median plane, following cranially in the osteofibromuscular groove, between the ventral vertebral processes (Figure 1). Santos et al. (2006) mention that in domestic geese, the main characteristic of the arterial pattern is the presence of two carotids, consistent with the statements of Nickel; Schummer; Seiferle (1977), in birds in general, and Baumel in Sisson; Grossman (2008), in domestic birds, which leads to the belief that they only studied bicarotid birds. Since they never refer to levocarotid birds. Observations in the Toco Toucan are inconsistent, as only one Common Carotid artery is found, also because, according to Glenny (1951), toucans, as Piciformes, are included in the Levocarotid group.

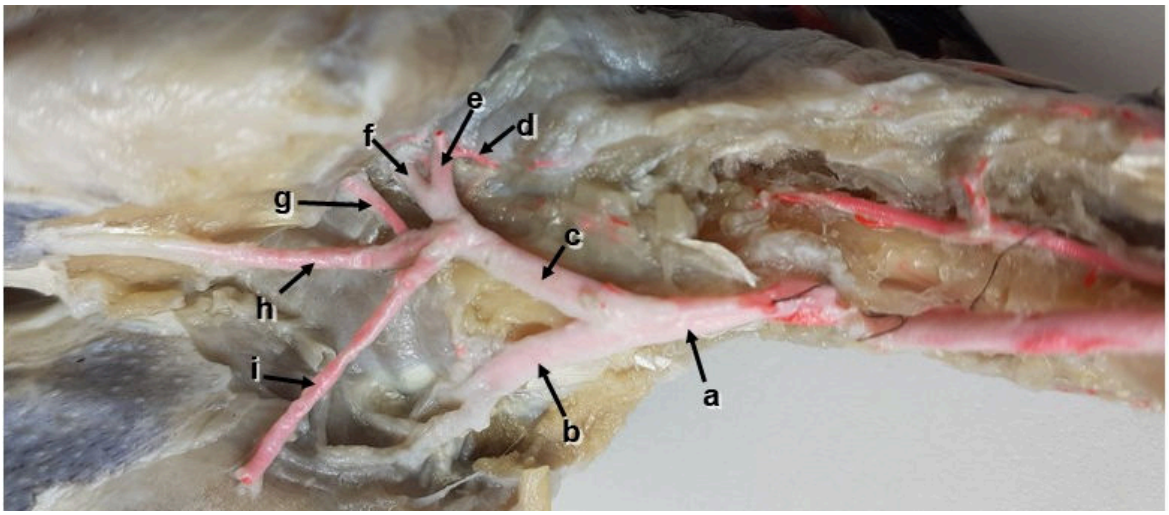

Figure 2. Ventral view of the cervical region of the Toco Toucan.
a- Common carotid artery, b- Right carotid artery, c- Left carotid artery, d- Cervical branch, e- Occipital artery, f- Internal carotid artery, g- Palatine artery, h- Maxillary artery, i- Mandibular artery.

According to Baumel, in Sisson; Grossman (2008), the arterial supply of the chicken's head is characterized by multiple large-caliber anastomoses between arteries destined for the head, consistent with observations in the Toco Toucan.

Near the base of the skull, each Common Carotid artery divides into: the External Carotid artery, which branches into head structures, and the Internal Carotid artery, which proceeds into the skull without supplying branches to surrounding structures (Nickel; Schummer; Seiferle, 1977), in birds in general, and Baumel in Sisson; Grossman (2008), in domestic birds. In the Toco Toucan, the single Carotid artery, near the base of the skull, divides into the right and left Carotid arteries, which are very short and soon divide into the External Carotid artery, Palatine artery, Mandibular artery, Occipital artery, and Internal Carotid artery, whose destinations are similar to those in other birds (Figure 2).

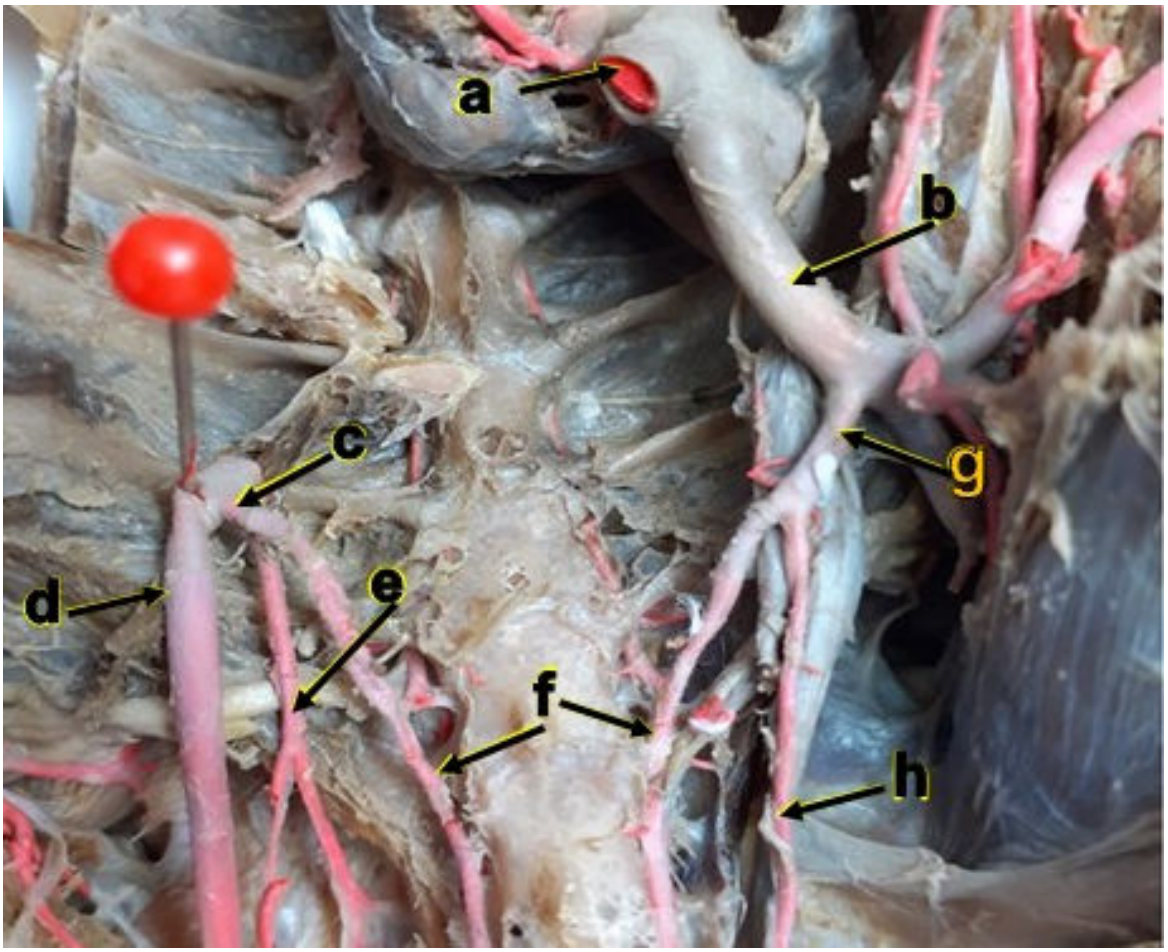

Figure 3 - Ventral view of the cervicothoracic region of the Toco Toucan.
a- Left brachiocephalic trunk ruptured; b- Right brachiocephalic trunk; c- Left craniocervicothoracic trunk; d- Common carotid artery; e- Left cervicobrachial artery; f- Vertebral arteries; g- Right craniocervicothoracic trunk; h- Right cervicobrachial artery.

In the Toco Toucan, the Vertebral arteries arise from a large Craniocervicothoracic trunk on each side, as they supply the vertebral arteries and several branches to structures of the neck and thorax, with the Vertebral artery being the main branch (Figure 3). Both Vertebral arteries converge towards the median plane until the 12th cervical vertebra when they penetrate the transverse foramen and then successively follow through the transverse foramina of subsequent vertebrae up to the base of the skull, at the level of C_1.

In this context, Nickel; Schummer; Seiferle (1977) are discordant, stating that in birds, the two Vertebral arteries are branches of the Common Carotid arteries, both left and right. Additionally, they do not mention anything about the Vagovertebral arteries, which are also not observed in the Toco Toucan but are enumerated in domestic birds, by Baumel in Sisson; Grossman (2008). Along their course, the Vertebral arteries in the Toco Toucan provide a series of collateral branches to structures of the neck, in accordance with the citations of Nickel; Schummer; Seiferle (1977), in birds in general, and Baumel in Sisson; Grossman (2008), in domestic birds.

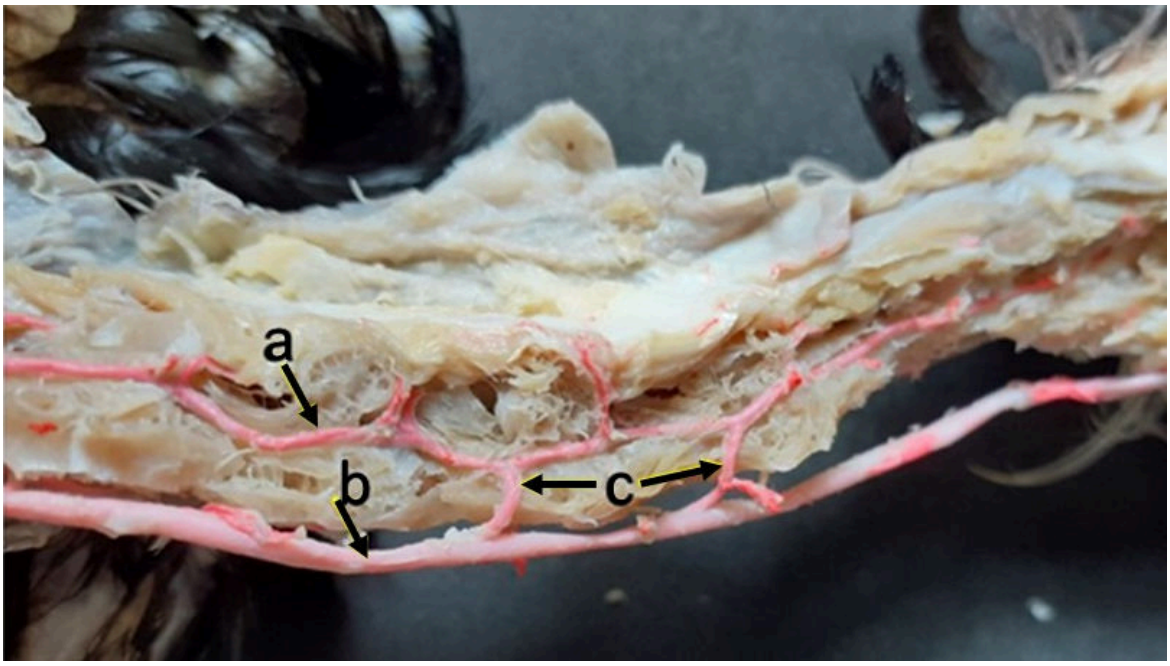

Figure 4 - Ventral view of the cervical region of the Toco Toucan.
a- Right vertebral artery; b- Common carotid artery; c- Right carotid-vertebral anastomoses.

Observations in the Toco Toucan show several anastomoses between the Vertebral arteries and the Common Carotid artery, including two large anastomoses between the left Vertebral artery and the Common Carotid artery, as well as between the right Vertebral artery

and the Common Carotid artery in the middle part of the neck, one between C_4 and C_5 and another between C_5 and C_6 (Figures 4 and 5).

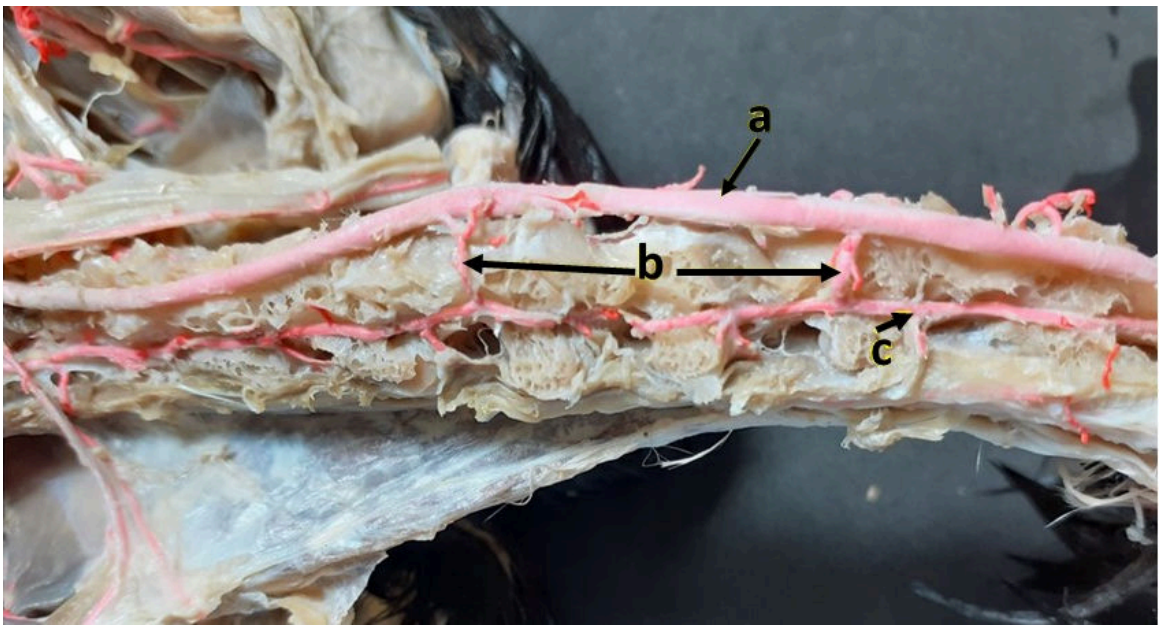

Figure 5 - Ventral view of the cervical region of the Toco Toucan.
a- Common carotid artery; b- Left carotid-vertebral anastomoses; c- Left vertebral artery.

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Stricto Sensu Editora

ANATOMY OF THE AORTA ARTERY AND ITS BRANCHES IN THE PREHENSILE-TAILED PORCUPINE (*Coendou prehensilis* Linnaeus, 1758)

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ABSTRACT

Understanding the form and function of the Circulatory System in animals found in the Brazilian fauna is of great importance for understanding the biology of the animal and providing support for the creation of preservation and sustainable exploitation programs for the biome. The objective is to study the macroscopic aspects of the Abdominal Aorta and its branches in the Prehensile-tailed Porcupine, focusing on the identification and description of the Visceral and Parietal Branches originating from this important arterial vessel, without delving into the details of the distribution of each branch, as well as statistical analysis, mainly due to the difficulty in accessing a larger number of specimens, given that the studied specimens are carcasses collected on the roadside or donated by CETAS - Catalão/GO. Experimental protocols are conducted following the recommendations of the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee on Animal Use of the Federal University of Catalão - CEUA/UFCAT nº. 01/22, and the naming of structures follows the Veterinary Anatomical Nomenclature. The Abdominal Aorta in the Prehensile-tailed Porcupine originates at the aortic hiatus, descends caudally over the vertebral bodies, between the diaphragmatic pillars, but is slightly displaced to the left due to the presence of the Caudal Vena Cava. Its branches include the Celiac Trunk or Celiac Artery, the Cranial Mesenteric Artery, the Renal Arteries, and the Lumbar Arteries. The Abdominal Aorta

terminates at the level of the abdominopelvic transition, trifurcating into the Right and Left Common Iliac Arteries and the Median Sacral Artery.
Keywords: Anatomy, Wild Animals and Arterial Vascularization.

1. INTRODUCTION

Comparative Anatomy is the science that studies the morphological similarities and differences among different species of animals. Macroscopic analysis of the body is a crucial tool for describing a species or for comparing species with similar morphological characteristics. These similarities lead to the classification of species into the same taxonomic group (STORER et al., 2000; RIBEIRO 2002).

The prehensile-tailed porcupine (*Coendou prehensilis*) belongs to the order Rodentia and the family Erethizontidae. Also known as the Brazilian porcupine or common porcupine, it is a mammal that inhabits agricultural lands in Brazil. This animal, which mainly lives in tropical forests and jungles, is found in almost all regions of Brazil. When adult, it can weigh from 2 to 5 kilograms and measure from 30 cm to 1 meter in length, being found in Argentina, Bolivia, Brazil, Colombia, French Guiana, Guyana, Paraguay, Peru, Suriname, Trinidad and Tobago, and Venezuela. They can live up to 20 years. The species is classified as Least Concern (LC) on the International Union for Conservation of Nature (IUCN) Red List (MARINHO-FILHO; EMMONS, 2016).

Porcupines are arboreal animals that live on tree branches and shrubs, which they cling to with the help of their strong tails. They rarely descend to the ground and mainly eat leaves and fruits. They are rarely seen because they are mostly shy and nocturnal. It is native to South America and has nothing in common with its Eurasian and African relatives except for how it defends itself with sharp spines up to 5 cm long on the back and sides of the body (HADDAD; VIEIRA; CORTES, 2010; MARINHO-FILHO; EMMONS, 2016).

The circulatory system plays a fundamental role in the functioning of the body, being responsible for the transport of nutrients, oxygen, and hormones to the body's cells, as well as the removal of waste and carbon dioxide. Therefore, studying the circulatory system is of utmost importance for understanding how a body functions. Additionally, understanding how blood is pumped by the heart, how it circulates through the body, and the different functions of blood vessels is also crucial for animal biology.

In general, the circulatory system of mammals presents more similarities than differences, as the course of nature preserves well-adapted aspects and slowly induces changes necessary for the preservation of life and perpetuation of a species. Therefore, significant differences in the Circulatory System Anatomy of the prehensile-tailed porcupine and other taxonomic groups that coexist in similar environments are not expected.

The aim of this research is to study the macroscopic aspects of the Abdominal Part of the Aorta and its branches, focusing on the identification and description of the Visceral and Parietal Branches originating from this important arterial vessel, without, however, delving into the details of the distribution of each branch, as well as statistical analysis, because, regarding wild animals, the specimens studied are carcasses collected on the margins of highways and/or donated by Wild Animal Screening Center of Catalão/Goiás (CETAS - Catalão/GO).

The literature relevant to the Anatomy of Wild Animals is relatively scarce; therefore, comparisons prioritize literature already well-established in domestic animals, although studied wild species may also contribute to the discussions.

2. MATERIAL AND METHODS

For the development of the present research, four specimens of Prehensile-tailed Porcupine (*Coendou prehensilis* Linnaeus, 1758) are utilized, consisting of two males and two females, all adults but without defined age, donated by the Wild Animal Screening Center of Catalão/Goiás (CETAS - Catalão/GO), under authorization SISBIO 37072-2, to the Laboratory of Comparative Anatomy of Wild Animals at the Federal University of Catalão (LACAS-UFCAT).

The specimens have their spines removed using tweezers or scissors to minimize potential accidents. The arterial system is injected with red-colored Latex Arte Cola, mixed with chess pigment, through the common carotid artery. After 24 hours, the venous system is injected with 10% formalin solution for fixation and, when necessary, manually injected and preserved in the same solution. Dissection and preparation of specimens for analysis follow the standard protocol in Macroscopic Anatomy. For photographic documentation, a Samsung Galaxy A10 cellphone camera is used. As this is a descriptive, non-quantitative research, statistical analysis is not employed.

Experimental protocols are conducted following the recommendations of the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee on Animal Use of the Federal University of Catalão - CEUA/UFCAT Nº. 001/22, and the naming of structures follows the Veterinary Anatomical Nomenclature (International Committee on Veterinary Gross Anatomical Nomenclature, 2017).

3. RESULTS AND DISCUSSION

The abdominal aorta, the largest artery in mammalian bodies, is renamed the Abdominal Aorta or Abdominal Part of the Aorta shortly after crossing the aortic hiatus of the diaphragm. In the Prehensile-tailed Porcupine, it begins at the aortic hiatus, descends caudally over the vertebral bodies, between the diaphragmatic pillars, but is slightly displaced to the left due to the presence of the Caudal Vena Cava, ending at the abdominopelvic transition, consistent with the compiled literary citations (MILLER; CHRISTENSEN; EVANS 1964, in domestic dogs; CULAU; AZAMBUJA; CAMPOS 2008, in Nutria; GOSHAL in SISSON; GROSSMAN 2008, in domestic animals; BAVARESCO; CULAU; CAMPOS 2012, in New Zealand white rabbits; MACEDO et al., 2013, in Giant anteater; PINHEIRO et al., 2014, in Ocelot; FARIA; BRANCO; LIMA 2016, in Night monkey; SILVA et al., 2018, in Hoary fox; ASSUNÇÃO et al., 2019, in Coati, and OLIVEIRA et al., 2019, in Six-banded armadillo).

In the Prehensile-tailed Porcupine, the first branch of the Abdominal Aorta (Figure 1a) is the Celiac Trunk or Celiac Artery (Figure 2a), which arises from the ventral aspect of the aorta, very close to the second branch, the Cranial Mesenteric Artery (Figure 2b), practically forming a common trunk with it. The compiled authors' citations are unanimous regarding the Celiac Trunk being the first branch of the Abdominal Aorta; however, they do not refer to the proximity between the two vessels (MILLER; CHRISTENSEN; EVANS 1964), in domestic dogs; CULAU; AZAMBUJA; CAMPOS 2008, in Nutria; GOSHAL in SISSON; GROSSMAN 2008, in domestic animals; BAVARESCO; CULAU; CAMPOS 2012, in New Zealand white rabbits; MACEDO et al., 2013, in Giant anteater; PINHEIRO et al., 2014, in Ocelot; FARIA; BRANCO; LIMA 2016, in Night monkey; SILVA et al., 2018, in Hoary fox; ASSUNÇÃO et al., 2019, in Coati, and OLIVEIRA et al., 2019, in Six-banded armadillo). On the other hand, the Celiac Trunk in the Prehensile-tailed Porcupine is quite long and divides into the Hepatic Artery, Splenic Artery, and Left Gastric Artery. These observations are consistent with the

compiled authors regarding branching but differ regarding the length of the Celiac Trunk, referred to as short, whereas in the Prehensile-tailed Porcupine, it is quite long (MILLER; CHRISTENSEN; EVANS 1964, in domestic dogs; CULAU; AZAMBUJA; CAMPOS 2008, in Nutria; GOSHAL in SISSON; GROSSMAN 2008, in domestic animals; BAVARESCO; CULAU; CAMPOS 2012, in New Zealand white rabbits; MACEDO et al., 2013, in Giant anteater; PINHEIRO et al., 2014, in Ocelot; FARIA; BRANCO; LIMA 2016, in Night monkey; ASSUNÇÃO et al., 2019, in Coati, and OLIVEIRA et al., 2019, in Six-banded armadillo). On the other hand, Silva et al., (2018) describe a fourth branch of the Celiac Trunk in the Hoary fox, a Dorsal Gastric Artery that enters the dorsal aspect of the stomach, and Macedo et al. (2013) assert that, in the Giant anteater, the Hepatic Artery may arise in common trunk with the Cranial Mesenteric Artery.

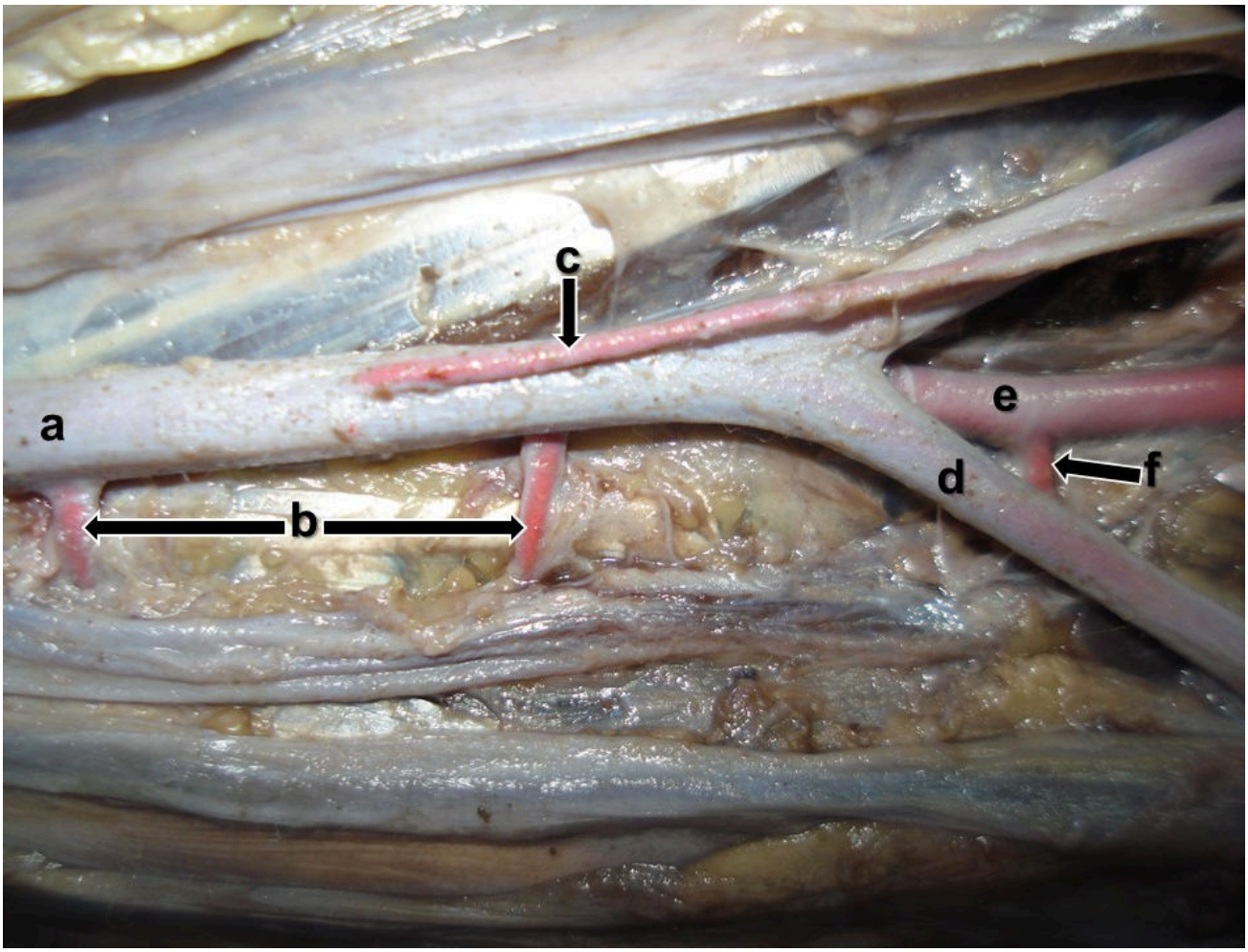

Figure 1. Ventral view of the abdominal part of the abdominal cavity of the Prehensile-tailed Porcupine.
a- abdominal aorta, b- lumbar arteries, c- caudal mesenteric artery, d- right common iliac artery, e- median sacral artery, f- deep circumflex iliac artery.

The second major branch of the Abdominal Aorta in the Prehensile-tailed Porcupine is the Cranial Mesenteric Artery (Figure 2b), originating from the ventral aspect of the Aorta, caudally to the origin of the Celiac Trunk but very close to it, distributing to the small intestine and most of the colon, in accordance with the citations of the compiled authors, except regarding the proximity between both vessels (MILLER; CHRISTENSEN; EVANS 1964, in domestic dogs; CULAU; AZAMBUJA; CAMPOS 2008, in Nutria; GOSHAL in SISSON; GROSSMAN 2008, in domestic animals; BAVARESCO; CULAU; CAMPOS 2012, in New Zealand white rabbits; MACEDO et al., 2013, in Giant anteater; PINHEIRO et al., 2014, in Ocelot; FARIA; BRANCO; LIMA 2016, in Night monkey; SILVA et al., 2018, in Hoary fox; ASSUNÇÃO et al., 2019, in Coati, and OLIVEIRA et al., 2019, in Six-banded armadillo).

The Renal Arteries (Figure 2c) are two large arterial vessels that arise from the lateral aspects of the Aorta, caudally to the origin of the Cranial Mesenteric Artery, always singular on each side but dividing into a variable number of branches before penetrating the renal hilum, with the left Renal Artery presenting a greater number of branches, consistent with the compiled literature (MILLER; CHRISTENSEN; EVANS 1964, in domestic dogs; CULAU; AZAMBUJA; CAMPOS 2008, in Nutria; GOSHAL in SISSON; GROSSMAN 2008, in domestic animals; BAVARESCO; CULAU; CAMPOS 2012, in New Zealand white rabbits; MACEDO et al., 2013, in Giant anteater; PINHEIRO et al., 2014, in Ocelot; FARIA; BRANCO; LIMA 2016, in Night monkey; SILVA et al., 2018, in Hoary fox; ASSUNÇÃO et al., 2019, in Coati, and OLIVEIRA et al., 2019, in Six-banded armadillo). However, Miller; Christensen; Evans (1964) assert that in domestic dogs, duplication of the Renal Artery, especially the left one, is relatively common, and Faria; Branco; Lima (2016) mention that in Night monkeys, the Renal Arteries arise in a common trunk from the ventral aspect of the Abdominal Aorta.

The Caudal Mesenteric Artery (Figure 1c) in the Prehensile-tailed Porcupine is a long but slender branch of the Abdominal Aorta that originates from the ventral aspect, close to the terminal branches, irrigating the caudal part of the sigmoid colon and cranial part of the rectum, in agreement with the citations of (MILLER; CHRISTENSEN; EVANS 1964, in domestic dogs; GOSHAL in SISSON; GROSSMAN 2008, in domestic animals; MACEDO et al., 2013, in Giant anteater; PINHEIRO et al., 2014, in Ocelot; FARIA; BRANCO; LIMA 2016, in Night monkey; SILVA et al., 2018, in Hoary fox; ASSUNÇÃO et al., 2019, in Coati, and OLIVEIRA et al., 2019, in Six-banded armadillo).

The Lumbar Arteries (Figure 2d) in the Prehensile-tailed Porcupine are present in a number of four impar arteries, arising from the dorsal aspect of the Aorta and immediately penetrating the structures adjacent to the vertebral column. The compiled literature is highly

variable concerning the lumbar arteries. Thus, Miller; Christensen; Evans (1964) mention 5 lumbar arteries, arising from the dorsal aspect of the Aorta in domestic dogs; Goshal in Sisson; Grossman (2008) cite 5 pairs in domestic animals, which may arise separately or form trunks among themselves; Bavaresco; Culau; Campos (2012) point out, in New Zealand white rabbits, the first lumbar artery as a pair and others 6 impar arteries; Macedo et al. (2013) assert that in Giant anteater, there are 3 pairs of lumbar arteries; Pinheiro et al. (2014) mention 6 pairs of lumbar arteries in Ocelot; Silva et al. (2018) indicate 5 pairs in Hoary fox; Assunção et al. (2019) show 6 pairs in Coati while Oliveira et al. (2019) describe 5 pairs in Six-banded armadillo.

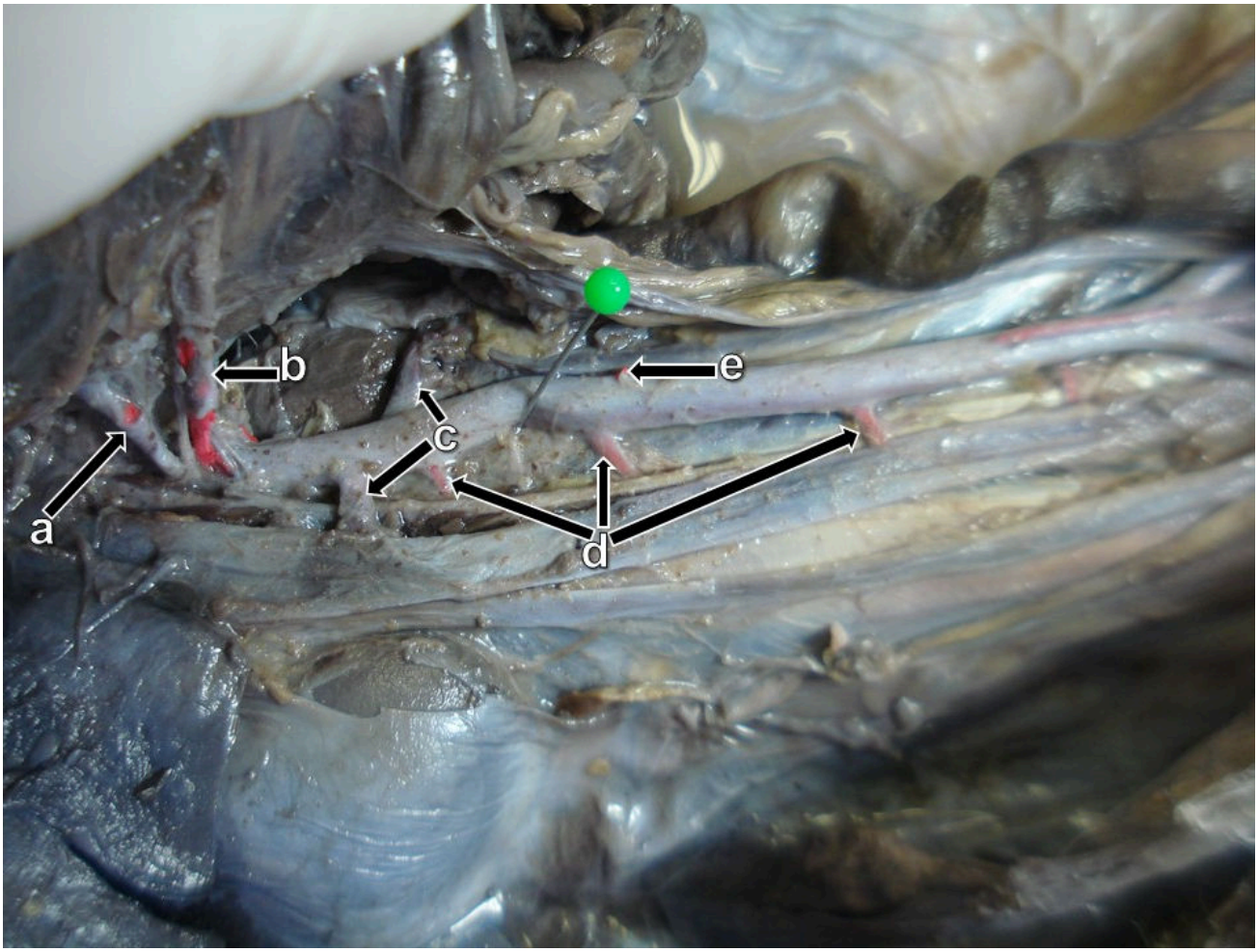

Figure 2. Ventral view of the abdominal part of the abdominal cavity of the Prehensile-tailed Porcupine.
a- celiac trunk, b- cranial mesenteric artery, c- renal arteries, d- lumbar arteries, e- left gonadal artery.

The Abdominal Aorta in the Prehensile-tailed Porcupine terminates at the level of the abdominopelvic transition, trifurcating into the Right and Left Common Iliac Arteries (Figure 1d) and the Median Sacral Artery, originating at the same level, but from the dorsal aspect,

in agreement with the citations of Bavaresco; Culau; Campos (2013) in New Zealand white rabbits; Macedo et al. (2013) in Giant anteater; Silva et al. (2018) in Hoary fox. On the other hand, the terminal branches of the Aorta are sometimes described as the Internal Iliac Arteries and the Median Sacral Artery, since the External Iliac Arteries arise earlier, from the lateral aspect, and head towards the pelvic limbs (MILLER; CHRISTENSEN; EVANS 1964, in domestic dogs; GOSHAL in SISSON; GROSSMAN 2008; ASSUNÇÃO et al., 2019, in Coati; OLIVEIRA et al., 2019, in Six-banded armadillo).

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Stricto Ensu Editora

ANATOMY OF THE LUMBOSACRAL PLEXUS OF COATIS

(*Nasua nasua* Linnaeus, 1766)

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ABSTRACT

The coati is a widely distributed animal in Brazilian territory, a natural inhabitant of the Cerrado biome, but its biology is still poorly understood. As an important component of the Cerrado fauna, the coati has attracted interest from researchers interested in promoting sustainable development of the Cerrado biome. The aim of this research is to dissect and describe the Anatomy of the Lumbosacral Plexus of the coati (*Nasua nasua*). Six adult specimens, three males and three females of coati, donated by IBAMA/GO or collected dead due to roadkill in highways of Southeast Goiás, were used. The specimens were fixed in a 10% aqueous solution of formaldehyde and preserved in the same solution. The preparation of the anatomical specimens followed usual procedures in macroscopic anatomical studies. Observations resulting from meticulous analysis reveal that the Lumbosacral Plexus of the coati has a relatively simple structure with few intercommunications between its components. There is no typical loop formation, but the roots or their branches converge to form nerves destined for the pelvic limb. The components of the Lumbosacral Plexus involve the spinal segments L_5, L_6, L_7, S_1, and S_2, with occasional contributions from L_4 (Pre-fixed Plexus) or S_3 (Post-fixed Plexus). In most cases, there is communication between the Lumbar and Sacral Plexuses, resulting in the Lumbosacral Plexus, which occurs through a "trifurcated" nerve, usually L_6. The Lumbosacral Plexus gives rise to several short nerves destined for pelvic structures and simultaneously originates three long nerves: femoral, obturator, and sciatic nerves, destined for the pelvic limb.

Keywords: Anatomy, Lumbosacral plexus and Coati.

1. INTRODUCTION

The Anatomy of wild animals is a science that has been gaining space in teaching and research laboratories worldwide, as it provides adequate references to evaluate morphological and functional relationships between different taxonomic groups (OLIVEIRA; TEIXEIRA; CONCHALO, 2004).

On the other hand, knowledge of animal biology will always provide useful information, including for organizing and implementing sustainable development, conservation, and protection programs for species, especially those at risk of extinction.

The Cerrado, the second largest Brazilian biome, covers about two million km² and houses a rich fauna, including the coati (*Nasua nasua*), the focus of our research.

The concept of biome is broad and diverse, and according to Dajoz (2005), it is the set of physiognomies and homogeneities of a region, regardless of the floristic formation that composes it.

In the Brazilian Cerrado, mammals constitute the second most diversified group, accounting for approximately 15% of the known species. Although most of the Brazilian Cerrado is located in the Central Plateau, the components of its fauna can occupy different geographical patterns, forming a mosaic of physiognomies that includes fields, savannas, and forests (BOCCHIGLIERI; MENDONÇA; HENRIQUES, 2010).

The coati (*Nasua nasua*) is found in all three environments, although it seems more at ease in typical Cerrado formations, such as sandbanks and small forests, where it appears to be well adapted. It is worth mentioning that the knowledge of the biology, including the Anatomy of this taxonomic group, is not at the same level of development as that of other wild animals, perhaps because they are native to the South American continent and have only recently attracted the attention of researchers.

The sustainable development of a region involves the organization and implementation of preservation and conservation programs for the members of the biome, and when the focus is on the Cerrado, these aspects are more intense, as it is a complex environment that houses a wide variety of species, many of which are still in the adaptation phase, and therefore more exposed and susceptible to the effects of human intervention.

The present study aims to dissect, analyze, and describe the Anatomy of the lumbosacral plexus of the coati (*Nasua nasua*), with a comparative focus on human and other well-established animal literature.

2. MATERIALS AND METHODS

For this research, six specimens of coati (*Nasua nasua* Linnaeus, 1766), adults, comprising three males and three females, were used. These specimens were either donated by the Wild Animals Screening Center of the Catalão-GO (CETAS-Catalão/GO) or collected dead on the margins of highways in Brazilian Southeast of Goiás, under authorization – SISBIO 37072/2 (Authorization and Information System on Biodiversity).

The experimental procedures were conducted at the Laboratory of Comparative Anatomy of Wild Animals of the Federal University of Catalão (LACAS-UFCAT).

The specimens were fixed in a 10% aqueous formaldehyde solution, through perfusion via the femoral vein, and subsequently preserved immersed in the same solution.

The preparation of anatomical specimens followed the standard process in Macroscopic Anatomy, which involved carefully dissecting the area of interest, either with the naked eye or under a magnifying glass at 10X magnification.

Dissection began by opening the abdominal cavity through the Alba line and the pelvis through the pubic symphysis. Once the abdominal and pelvic cavities were exposed, the viscera were sectioned and moved aside enough not to hinder the dissection of the dorsal wall.

The vertebral bodies were exposed from the first cervical vertebra to the last lumbar vertebra to verify the number of vertebrae in each region. After this procedure, the dorsal wall of the abdomen and pelvis were carefully dissected, removing muscles and other soft structures with a scalpel and scissors. After exposing the extra-vertebral neural roots, the vertebral bodies were carefully removed using an osteotome until the spinal cord, surrounded by the dura mater, was fully exposed.

Photographic documentation was done using a Sony Cyber Shot 7.2-megapixel digital camera.

The nomenclature adopted for the description of the results generally followed that of the Nomina Anatomica Veterinaria (2017).

All procedures were conducted in accordance with ethical principles and approved by the Institutional Ethics in Research Committee at the Federal University of Uberlândia (CEUA/UFU nº 067/12).

3. RESULTS AND DISCUSSION

Anatomical variations of the lumbosacral plexus in coatis (*Nasua nasua*) are frequently observed both inter- and intra-specifically. Even when analyzing the same specimen, differences between the right and left plexuses are evident. Therefore, the results of this research are presented bilaterally.

To facilitate the analysis and description of the constituent roots of the lumbosacral plexus, it is necessary to verify the number of thoracic and lumbar vertebrae to name the respective roots. Thus, some brief considerations will be made regarding the number of thoracic and lumbar vertebrae of the animal under study.

In coatis (*Nasua nasua*), the subject of investigation in this study, the number of thoracic vertebrae is 15, while the lumbar region comprises six vertebrae. No variation in these numbers is observed, perhaps due to the small number of specimens used.

Verification of the number of vertebrae in these regions of the vertebral column is important due to the neural alterations accompanying the modifications of the vertebral column.

Therefore, Howell and Straus (1932) cited by Hartman and Straus (1932) affirm that the number of lumbar nerves determining a pre-fixed or post-fixed plexus is intimately related to the number of pre-sacral vertebrae, that is, the shortening or lengthening of the trunk produces modifications in the adjacent neural system. The coati is a species that exhibits a relatively elongated vertebral column, with 15 thoracic vertebrae and six lumbar vertebrae, consequently displaying a caudally displaced lumbosacral plexus, mainly at its cranial limits (L_5), whereas in animals with a shorter spine, such as orangutans, there is a cranial displacement of the plexus to L_1 or even T_{12}, and in lemurs with a longer spine, opposite conditions occur (HOWELL; STRAUS, 1932 *apud* HARTMAN; STRAUS 1932).

In coatis (*Nasua nasua*), the number of lumbar nerves is seven (L_1-L_7), and the sacral ones are six (S_1-S_6), however, only the last three, L_5, L_6, L_7, and the first two sacral nerves, S_1 and S_2, comprise the formation of the Lumbosacral Plexus, with occasional contribution from S_3. The incorporation of S_3 into the Sacral Plexus seems to be due to the apparent absence of a coccygeal plexus.

According to Chang and Ruch (1947), the Spider Monkey has five lumbar nerves. In coatis, there are seven nerves, showing a significant shortening of the lumbar spinal cord between a Procyonid (coati) and a primate.

Hill (1953) mentions a close relationship between the Lumbosacral Plexus of Humans and that of Rhesus Monkeys, even though there is no numerical correspondence, because both are primates.

Similarly, as observed in Strepsirhini (Lemuridae), considered primitive primates compared to Humans, the same applies to coatis (Procyonidae), in line with Hill's assertion (1953) that there is a tendency for caudal displacement of the Lumbosacral Plexus in all groups below Hominids in the phylogenetic scale, characterizing a post-fixed plexus.

In *Tarsius* (Haplorhini), although occupying a lower position in the phylogenetic scale compared to Humans (primates), the ilioinguinal and iliohypogastric nerves originate from L_1; the genitofemoral from L_1 and L_2, the lateral cutaneous nerve of the thigh from L_2 and L_3, while the femoral and obturator nerves emerge from L_2, L_3, and L_4, with the Sacral Plexus formed by sacral nerves and the last lumbar nerve (Hill, 1955).

In coatis, the cranial limit of the Lumbosacral Plexus is L_5, although there are cranial intercommunications at this level, they are independent of the plexus. On the other hand, the caudal limit is L_6, occasionally L_7.

In *Tarsius*, according to Hill (1955), the cranial limit is L_1, like in Humans, but the same Author considers the iliohypogastric, ilioinguinal, and lateral cutaneous nerve of the thigh as components of the Lumbar Plexus, whose caudal limit is marked by the last lumbar nerve (Hill, 1955), while in coatis, generally, it is the penultimate lumbar nerve (L_6), occasionally the last (L_7).

In the domestic dog, not far removed from the coati in the phylogenetic scale, as both are carnivores, the Lumbosacral Plexus consists of intercommunications of the last five lumbar nerves and the first three sacral nerves (MILLER; CHRISTENSEN; EVANS, 1964). In the coati, the participation of the last three lumbar nerves (L_5, L_6, and L_7) and the first two sacral nerves (S_1 and S_2) seems to be the rule, with L_5 being the cranial limit in 75% and S_2 the caudal limit.

On the other hand, El-Assy (1966) states that in primates, the Lumbosacral Plexus is composed of the ventral branches of all lumbar nerves and the first sacral nerve, with occasional contribution from the last thoracic nerve. In the coati, the cranial limit of the Lumbosacral Plexus is the root of L_5, but L_4, L_3, and L_2 may form anastomoses or individually constitute the subcostal, iliohypogastric, and ilioinguinal nerves, which are often considered components of the Lumbar Plexus.

According to Bolk (1898) cited by El-Assy (1966), there are differences regarding the cranial limit of the Lumbosacral Plexus for Humans and Anthropoids, with the first roots

involved being T_{12} or L_1 in Humans, and L_1 or L_2 in Anthropoids. Similarly, Testut and Latarjet (1979) consider that in humans, it is difficult to establish an upper limit of the Lumbar Plexus valid for all cases, due to existing variations, but they consider the classical form as one in which there is participation of L_1-L_4. In the coati, the cranial limit is L_5, since cranial nerves to L_5 only rarely establish intercommunications between themselves, except between L_2-L_3, almost always present, in accordance with Sonntag (1923) in *Chimpanzees*, and Eisler (1890) in *Gorillas*, both cited by El-Assy (1966).

In *Mandrillus phenix*, there are eight lumbar nerves, and seven in *Macaca nemestrina* (UTSCHNEIDER, 1892), while in *Macaca mulatta*, there are seven lumbar nerves (HOWELL; STRAUS, 1933), both cited by Hill (1966).

Hill (1966) himself reports in *Cercopithecus petaurista*, seven lumbar nerves, but only the last five participate in the formation of the Lumbar Plexus, while in the coati, there are seven lumbar nerves, but only the last three (L_5, L_6, and L_7) compose the mentioned plexus.

Analyzing the citations from Urbanowicz and Zaluska (1969), it is observed that in Humans, the Lumbar Plexus receives contributions from L_1-L_4, and in *Rhesus* and *Cynomolgus* monkeys, from L_1-L_5, indicating a shortening of the Lumbar Plexus in Humans compared to non-human primates.

In *Rhesus*, *Cynomolgus*, and Humans, the thinnest branch of the Lumbar Plexus is the subcostal nerve, and the diameter of the nerves increases from cranial to caudal (URBANOWICZ; ZALUSKA, 1969). These statements take into account a plexus with a high cranial limit, at the level of L_1, which would lead us to consider, in the coati, a level at L_2 and not L_5 as previously thought in this study. However, according to Urbanowicz and Zaluska (1969), in Prosimians, the subcostal nerve does not contribute to forming the Lumbar Plexus, rarely does so in lower Catarrhines, with relative frequency in Platyrrhines, and always in Anthropoids and Humans.

The L_2 nerve preserves its autonomy in all primates, meaning it travels alone without establishing anastomoses.

In turn, L_3 is always present in the Lumbar Plexus, as L_4 is in monkeys, except in Anthropoids and Humans. L_5 makes its contribution always in Prosimians, lower Catarrhines, relatively in Platyrrhines, and in Humans. L_6 is present in 25% of Prosimians and lower Catarrhines and rarely in Platyrrhines (WOOD JAMES, 1910, cited in URBANOWICZ; ZALUSKA, 1969). In the coati, L_5 is always present, along with L_6 in the formation of the Lumbar Plexus, with L_4 contributing rarely.

Furthermore, according to Wood James (1910) cited in Urbanowicz and Zaluska (1969), some monkeys have only four lumbar nerves, therefore, in addition to the cranial displacement of the plexus, there is a reduction in the number of nerves in the lumbar segment.

For Wood James (1910) cited in Urbanowicz and Zaluska (1969), L_2-L_3 are joined in 42% of humans, however, in the coati, this connection appears in four antimers, 25% of cases.

Analyzing the citations from Hill (1972), it is noted that in *Brachyteles*, several loops form near the emergence of the nerves, a characteristic absent in coatis (*Nasua nasua*). Furthermore, according to the same author, L_2 releases a small bundle that constitutes the cranial root of the obturator nerve. In coatis, the most cranial root of the obturator nerve originates from L_5. As described by Hill (1972), in *Ateles*, the Lumbar Plexus is relatively simple, generally composed of five nerves, with most of them not establishing anastomoses, with the most common interconnection being between S_2- S_3. In coatis, the Lumbar Plexus is equally simple, formed by the last three lumbar nerves and the first two sacral nerves (L_5, L_6, L_7, S_1, and S_2), with occasional contribution from S_3 and rarely from L_4.

Krechowiecki et al. (1972) consider the cranial limit of the Lumbar Plexus in *Macaca mulatta* to be at T_{12}, with the iliohypogastric nerve originating from L_1 and L_2 contributing to form the obturator and femoral nerves. Thus, the Lumbar Plexus itself can receive contributions from L_2, with the caudal limit of the Sacral Plexus being the third sacral nerve (S_3), and the bifurcated nerve is often missing. The Lumbar Plexus is larger in animals whose limbs are both prehensile and transporters (such as monkeys) than in animals that are solely transporters (like humans) (Krechowiecki et al., 1972).

The pelvic limb of the coati may be considered merely a transporter, as it lacks prehensile ability, although it is essential for climbing trees and other obstacles, making it a possessor of an intermediate Lumbar Plexus. Differences in the osteomuscular domain influence the adjacent neural system, so a decrease in the number of vertebrae leads to a shortening of the Lumbar Plexus (Bolk et al., 1934, cited in Piasecka-Kacperska; Gladykowska-Rzeczycka, 1972). In coatis, a relatively short Lumbar Plexus is observed, composed of L_5, L_6, L_7, S_1, and S_2, despite their considerably long vertebral column, containing 15 thoracic and six lumbar vertebrae.

According to Piasecka-Kacperska and Gladykowska-Rzeczycka (1972), differences in the structure of the Lumbar Plexus in different taxonomic groups are closely associated with limb development and body posture. After analyzing the Lumbar Plexus of the coati, it is

possible to support this idea, as the plexus of this animal is large yet simple, with few interconnections among its branches, consistent with animals that prioritize strength over pelvic limb abilities, such as for grasping functions.

For the same authors, establishing an exact cranial limit for the Lumbar Plexus is difficult due to variations in the presacral column, a citation shared by Testut and Latarjet (1979), Dyce; Sack and Wensing (2010), Bergman et al. (2001), Carvalho-Barros (2003), and Anloague et al. (2009).

On the other hand, there is consistency among various authors in stating that the Lumbar Plexus is formed by roots from L_1-L_4 (Hepburn 1892, Hill 1955, Bolk cited *in* El-Assy 1966, Urbanowicz; Zaluska 1969, Wood James cited *in* Urbanowicz; Zaluska 1969, Hill 1972; Krechowiecki et al., 1972; Testut; Latarjet 1979, Anloague 2009, Matejcík 2010, Guérin 2012, Patton; Warman 2012, Yasar et al. 2012). However, observations in coatis show a Lumbar Plexus structure shifted caudally, as the cranial limit seems to be L_5, although in 50% of cases, there is intercommunication between L_5-L_4 and 33% between L_4- L_3, while L_5-L_6 are involved in 100% of cases. Anastomoses between L_2- L_1 are absent in coatis, with these roots forming spinal nerves destined for structures outside the pelvic limb individually. Therefore, it may be possible to consider the cranial limit of the Lumbar Plexus of the coati at L_4 or L_3 in these cases if the ilioinguinal nerve is considered part of the plexus, as it may receive contributions from L_5.

The sixth lumbar nerve of the coati is the caudal limit of the Lumbar Plexus and the cranial limit of the Sacral Plexus, as it contributes to the formation of both plexuses. The boundary nerve between the two plexuses, typically called the "bifurcated nerve," is not present in the coati; instead, there is a trifurcated nerve, usually L_6 and occasionally L_5, whose branches incorporate the obturator, femoral, and sciatic nerves. According to Ihering (1878) cited by Hill (1960), the bifurcated nerve in cebids is L_3. Zuckerman (1938), referred to by Hill (1966), states that the bifurcated nerve, the boundary between the Lumbar and Sacral Plexuses, is L_3 in *Macaca mulatta*, *Papio papio*, *Papio ursinus*, and *Semipithecus*, but in *Cercopithecus sabaeus*, *Macaca nemestrina*, *Mandrillus leucophaeus*, and *Papio cynocephalus*, this boundary is L_4.

In humans, the Lumbar Plexus connects to the Sacral Plexus in 94% of cases, mostly through L_4, which is the bifurcated nerve. In *Rhesus* monkeys, this connection occurs in 87% of cases, and in *Cynomolgus* monkeys, it occurs in 100% of cases, but with L_5 instead of L_4 (Urbanowicz; Zaluska, 1969).

Preuschoft (1961), cited by Krechowiecki et al. (1972), considers that in *Gorillas*, the bifurcated nerve is always present, but only in 60% of cases in *Macaca mulatta* does L_4 participate in the constitution of both the Lumbar and Sacral Plexuses, hence the name "bifurcated nerve." Occasionally, L_3 may behave similarly (Bergman, 2001). In coatis, the trifurcated nerve is almost always L_6, but occasionally it may occur at L_5. Conversely, Iczi et al. (2005) found no connection between the Lumbar and Sacral Plexuses in humans.

Castro et al. (2009) consider that in sea lions, the cranial limit of the Lumbar Plexus is L_3, as observed in Marsh deer (Teixeira et al., 2003), *Gibbons* (Oliveira et al. 2003), and *Capricornis crispus* (Atoji et al. 1987). Matejcík (2010) asserts that the human Lumbar Plexus is formed by L_1- L_4, with occasional participation from T_{12}, and the Sacral Plexus by S_1-S_3, with a small contribution from S_4 or L_5. According to Guérin et al. (2012), the human Lumbar Plexus encompasses L_1-L_4, with occasional contribution from T_{12}. Patton and Warman (2012), on the other hand, consider the involvement of L_1-L_3 and sometimes T_{12}.

In coatis, the Lumbar Plexus is primarily formed by L_5 and L_6, with occasional participation from L_4 or L_3. The Lumbar Plexus of coatis is always connected to the Sacral Plexus by a branch from L_6 or L_5. In *Rhesus* monkeys, it is convenient to consider the two plexuses as a unit, as they are formed by neural branches that are often common (Howell; Straus, 1932, cited in Hartman; Straus, 1932). In *Lemurs*, the cranial limit of the Lumbar Plexus is L_5 or L_6, with four component roots. In *Ateles*, this limit is L_3- L_4; in *Cercopithecus* and *Macaca mulatta*, it is L_5-L_6; while in humans and *Chimpanzees*, L_3 is the limit (Piasecka-Kacperska; Gladykowska-Rzeczycka, 1972).

Some authors consider the Lumbar Sacral Plexus of primates to be typically formed by L_4-S_3, with the number of nerves varying between five and nine (Baldeen; Elting 1910, Bocheneck; Reicher 1965, Loth 1931, Paterson 1894, cited in Zaluska; Urbanowicz 1972). In coatis, the Lumbar Plexus is fundamentally constituted by L_5 and L_6, occasionally L_4, while the Sacral Plexus is formed by L_7, S_1, and S_2, occasionally S_3.

Swindler and Wood (1982) assert that there is considerable variability in the number of nerves contributing to the Lumbar Sacral Plexus of primates, and according to Goshal in Getty (2018) and Dyce; Sack and Wensing (2010), the number of nerves depends on the number of vertebrae in the spinal column.

According to Carvalho-Barros (2003), to consider the Lumbar and Sacral Plexuses as a single entity, there must be a connection between them. In coatis, there is always a branch from L_6 or L_5 that makes the connection with L_7.

Regarding the nerves originating from the Lumbar Sacral Plexus, this varies in coatis, but only concerning the short nerves destined for muscles and other adjacent anatomical structures, as the obturator, femoral, and sciatic nerves are always present.

According to Hepburn (1892), the Lumbar Plexus of anthropoid monkeys like *Gorillas*, *Chimpanzees*, *Orangutans*, and *Gibbons* presents numerous anastomoses forming many loops, characteristics not observed in coatis. Furthermore, according to Hepburn (1892), the ilioinguinal nerve originates from L_1 in *Gorillas*, *Gibbons*, *Chimpanzees*, and *Orangutans*. In coatis, L_1 never contributes to the formation of the ilioinguinal nerve, and although neural root anastomoses may occur, they do not form loops as in anthropoids. The lateral femoral cutaneous nerve originates from L_2 and L_3 in *Gorillas*, *Gibbons*, and *Chimpanzees*, but from L_1 and L_2 in *Orangutans*. In coatis, it originates from L_3 as the genitofemoral nerve, which sometimes may originate together with the ilioinguinal nerve. The obturator nerve originates from L_2- L_4 in *Gorillas* and *Chimpanzees*; from L_3-L_4 in *Gibbons*; and from L_1-L_2 in *Orangutans*. In coatis, it originates from L_5-L_6 in 75% of cases and from L_5, L_6, and L_7 in 25% of cases. The origin of the femoral nerve in coatis is highly variable, occurring in 41.5% of cases from L_5-L_6; in 24.9% from L_6-L_7; in 16.6% from L_4, L_5, and L_6; in 8.3% from L_6; and in 8.3% from L_5. Conversely, the sciatic nerve originates in 41.5% of cases from L_7-S_3; in 33.2% from L_6-S_1; and in 24.9% from L_6-S_2.

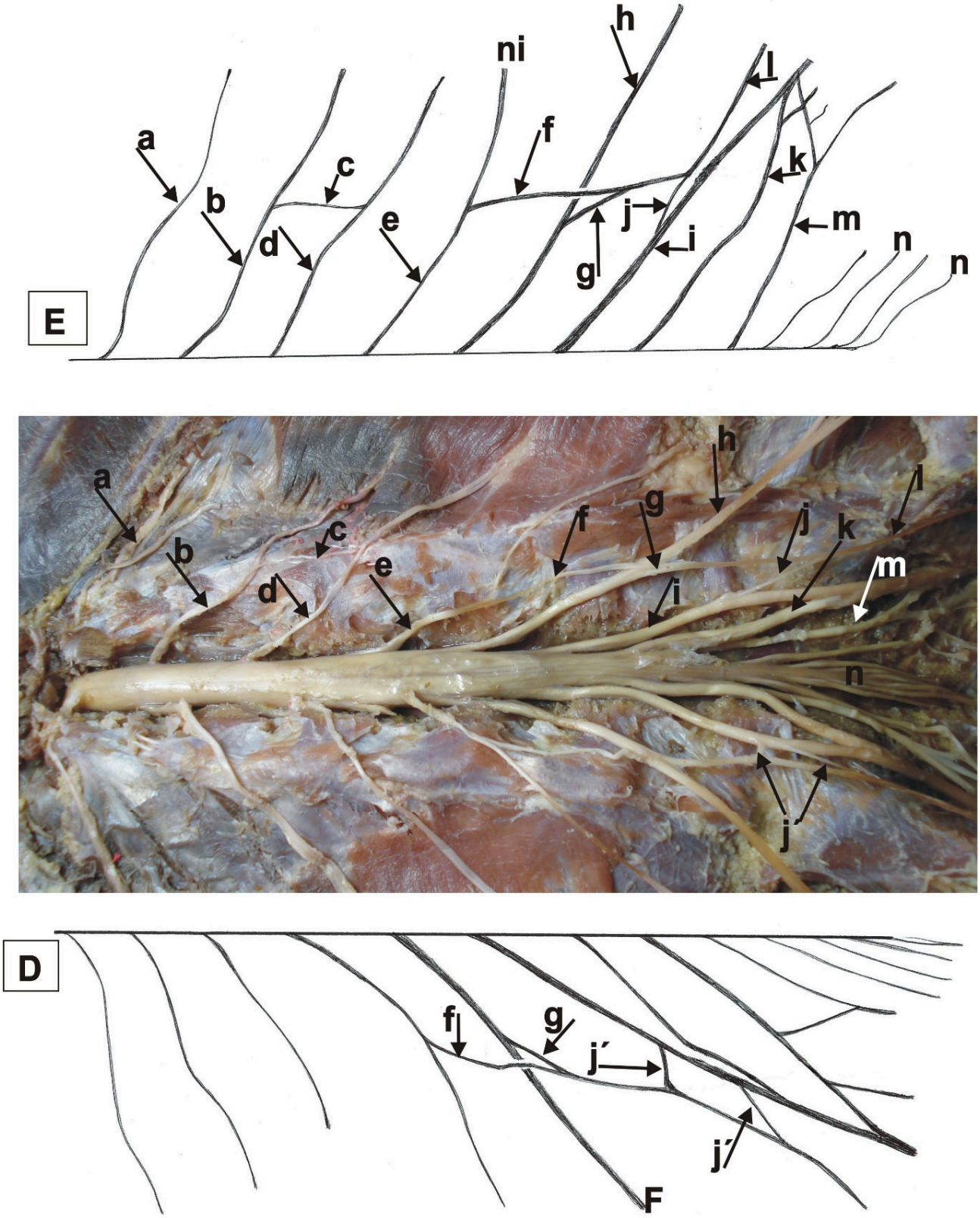

Figure 1. Ventral view of the abdominopelvic cavity of the coati (*Nasua nasua*).
E- left antimer, D- right antimer, a- L_2, b- L_3, c- L_3-L_4 anastomosis, d- L_4, e- L_5, f- cranial root of the obturator nerve, g- middle root of the obturator nerve, h- femoral nerve (L_6), i- cranial root of the sciatic nerve (L_7), j- caudal root of the obturator nerve (L_7), j'- caudal roots of the obturator nerve (L_7), k- middle root of the sciatic nerve (S_1), l- obturator nerve, m- S_2, n- sacral and caudal nerves, ni- ilioinguinal nerve, F- femoral nerve.

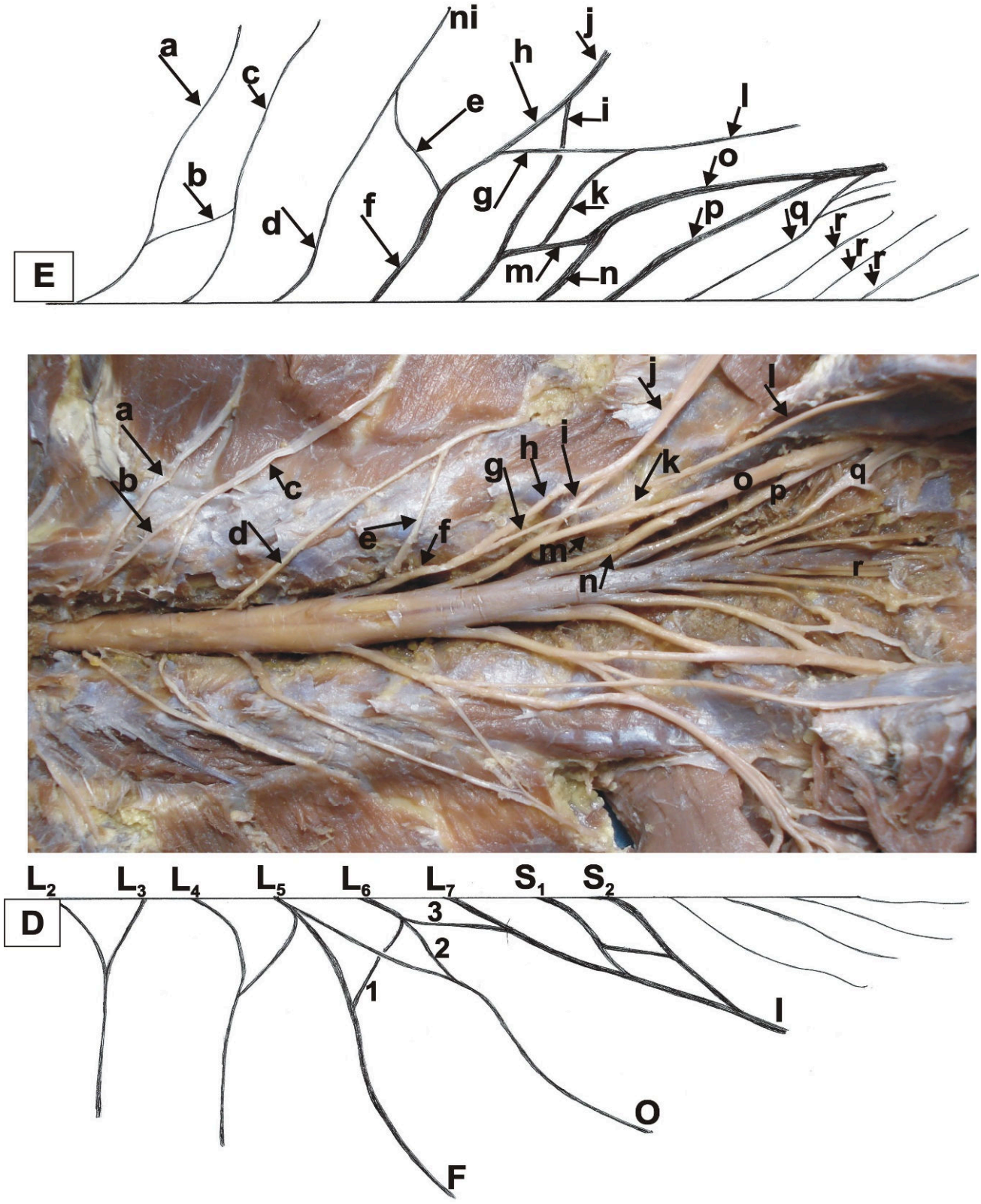

Figure 2. Ventral view of the abdominopelvic cavity of the coati (*Nasua nasua*).
E- left antimer, D- right antimer, a- L_2, b- L_3-L_4 anastomosis, c- L_3, d- L_4, e- L_4-L_5 anastomosis, f- L_5, g- cranial root of the obturator nerve, h- cranial root of the femoral nerve, i- caudal root of the femoral nerve, j- femoral nerve, k- caudal root of the obturator nerve, l- obturator nerve, m- cranial root of the sciatic nerve, n- middle root of the sciatic nerve, o- sciatic nerve, p- caudal root of the sciatic nerve, q- S_3, r- sacral nerves, F- femoral nerve, O- obturator nerve, I- sciatic nerve, ni- ilioinguinal nerve, 1- cranial branch of L_6, 2- middle branch of L_6, 3- caudal branch of L_6.

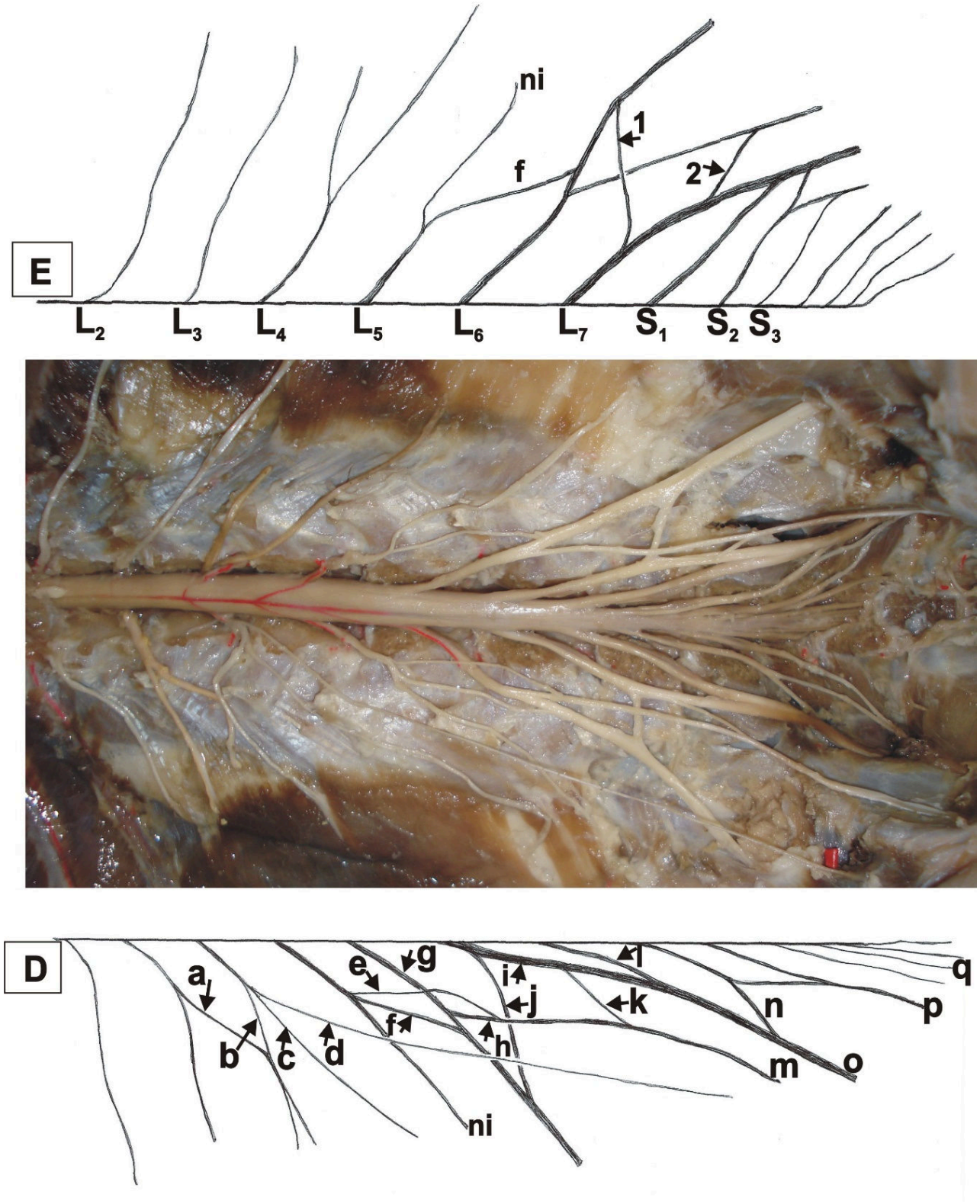

Figure 3. Ventral view of the abdominopelvic cavity of the coati (*Nasua nasua*).
E- left antimer, D- right antimer, a- L_3-L_4 anastomosis, b- cranial branch of L_4, c- middle branch of L_4, d- genitofemoral nerve, e- cranial root of the obturator nerve, f- cranial root of the femoral nerve, g- middle root of the femoral nerve, h- middle root of the obturator nerve, i- cranial root of the sciatic nerve, j- caudal root of the femoral nerve, k- caudal root of the obturator nerve, l- middle root of the sciatic nerve, m- obturator nerve, n- caudal root of the sciatic nerve, o- sciatic nerve, p- pudendal nerve, q- sacral nerves, ni- ilioinguinal nerve, 1- caudal branch of the femoral nerve, 2- caudal root of the obturator nerve.

4. CONCLUSION

The lumbosacral plexus originates from L_4 to S_3, and the femoral nerves from L_4 to L_5; the obturator from L_4 to L_6, and the sciatic from L_5 to S_3.

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Stricto Ensu Editora

ORIGIN, COURSE, AND DISTRIBUTION OF THE OBTURATOR NERVE IN COATIS (*Nasua nasua* Linnaeus, 1758)

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ABSTRACT
The aim of this study was to describe the origin, distribution and localization of the obturatorius nerve in the coati (*Nasua Nasua*) and compare with primates, domestic animals and wildlife species. Eight specimens of coatis, four males and four females, were prepared with 10% formalin solution and dissected using standard tools and techniques. The obturatorius nerve originated from the ventral rami of the third and fourth lumbar spinal nerves in 100% of specimens. That nerve showed symmetry between right and left sides, and there was no difference regarding sex. His path was through the psoas major muscle to the cranial edge of the lesser pelvis, where it divided into two branches, dorsal and ventral. The ventral branch distributed to the skin of the medial aspect of the thigh and the dorsal branch innervated the brevis and longus adductor muscles in 100% of antimeres. In 63% of cases, the dorsal branch of the obturatorius nerve innervated the pectineus muscle.
Keywords: Descriptive anatomy, Lumbosacral plexus and Morphology.

1. INTRODUCTION

The coati, an animal belonging to the phylum Chordata, class Mammalia, order Carnivora, family Procyonidae, and genus *Nasua*, is widely distributed in South America, ranging from Colombia and Venezuela to northern Uruguay and Argentina (BEISIEGEL,

2001). In Brazil, it is found in the Amazon, Caatinga, Cerrado, Pantanal, and Atlantic Forest biomes. It is a medium-sized animal with an elongated head and a trumpet-shaped snout, which provides it with great mobility to assist in digging for food in tree hollows, bird nests, and burrows. It has five toes on each limb, with the thoracic limb smaller than the pelvic limb, featuring long claws and highly mobile hands, giving it the ability to dig and climb, and it possesses a long tail used for balance (BEISIEGEL, 2001). According to Beisiegel (2001), coatis are diurnal, terrestrial, and arboreal animals that feed on insects and their larvae, fruits, and occasionally small vertebrates.

Despite being considered a widely distributed and relatively common species in Brazil, studies on the morphology of the coati are scarce, especially regarding the peripheral nervous system, although some research has been conducted on the respiratory system (OLIVEIRA et al., 2012), vessels at the base of the brain (BARREIRO et al., 2012), and male genital organs (FRANCIOLLI et al., 2007). According to Carvalho-Barros et al. (2003), the descriptive comparative study of nerve plexuses, such as the lumbar plexus and its branches, is extremely relevant as it represents the origin of nerves destined for the pelvic limbs, a segment of great importance in posture and locomotion.

Among the nerves of the lumbar plexus, the obturator nerve is one of the most important, originating from the ventral branch of the fourth lumbar nerve to the ventral branch of the first sacral nerve in all species (SCHARLLER, 1999). Injury to this nerve can result in dysfunction signs such as the inability to adduct the hip joint (SCHWARZE; SCHRÖDER, 1970). The affected limb may not effectively support weight, particularly when attempting sudden movement or turning. In such cases, there is a constant danger of serious rupture of the adductor muscles or coxofemoral, pelvic, or sacral dislocations (KNOTTENBELT; PASCOE, 1998).

Therefore, the aim of this study was to describe the origin, course, and distribution of the obturator nerve in coatis, which may contribute to clarifying clinical issues and species preservation.

2. MATERIAL AND METHODS

For the present study, eight specimens of coatis (*Nasua nasua*), comprising four males and four females, were used. These specimens were sourced from the collection of the Laboratory of Comparative Anatomy of Wild Animals at the Federal University of Catalão

(LACAS-UFCAT), donated by IBAMA MG/GO or collected deceased along highways. International parameters of bioethics and animal welfare were respected under authorization CEUA/UFU 067/12.

The specimens were depilated, fixed in 10% formalin solution via intravenous injection through the femoral artery, and kept immersed in the same solution. Dissection followed techniques commonly used in macroscopic anatomical studies, performed with the naked eye and, when necessary, under a 10x magnification loupe. Initially, an incision was made along the median sagittal plane in the abdominal region, and after the removal of connective and adipose tissue along with viscera, the lumbosacral plexus was exposed. The obturator nerve was dissected proximally to visualize its origin, along with the ventral roots contributing to its formation, highlighting the lumbar vertebrae. Subsequently, distal dissection of the obturator nerve was performed, observing its distribution to the thigh.

Photographic documentation was conducted using a Sony Cyber Shot 7.2-megapixel digital camera. The nomenclature adopted for the description of results followed the Veterinary Anatomical Nomenclature (ICGVAN, 2017), and the results were analyzed descriptively in terms of simple percentage.

3. RESULTS

The obturator nerve of the coati originated from the ventral root of the third (L_3) and fourth (L_4) lumbar nerves in all studied antimers (100%). The cranial (L_3) and caudal (L_4) roots converged within the psoas major muscle to form the obturator nerve (Figure 1). This nerve then proceeded within the psoas major muscle until it emerged alongside the cranial margin of the lesser pelvis, dorsal to the external iliac vessels, as depicted in figure 2.

The proximal course of the obturator nerve was observed along the medial margin of the psoas major muscle and the tendon of the psoas minor muscle (Figure 3). Distally, the nerve proceeded through the lesser pelvis along its lateral margin, medially to the hypogastric vessels and ureter, until reaching the cranial margin of the obturator foramen (Figure 4).

Regarding the distribution of the obturator nerve, it was found that it divided into ventral and dorsal branches. The ventral branch supplied the skin of the medial aspect of the thigh, while the dorsal branch innervated the adductor brevis and adductor longus muscles in 100% of the antimers (Figures 5 and 6). In 63% of cases, the dorsal branch of the obturator nerve also innervated the pectineus muscle.

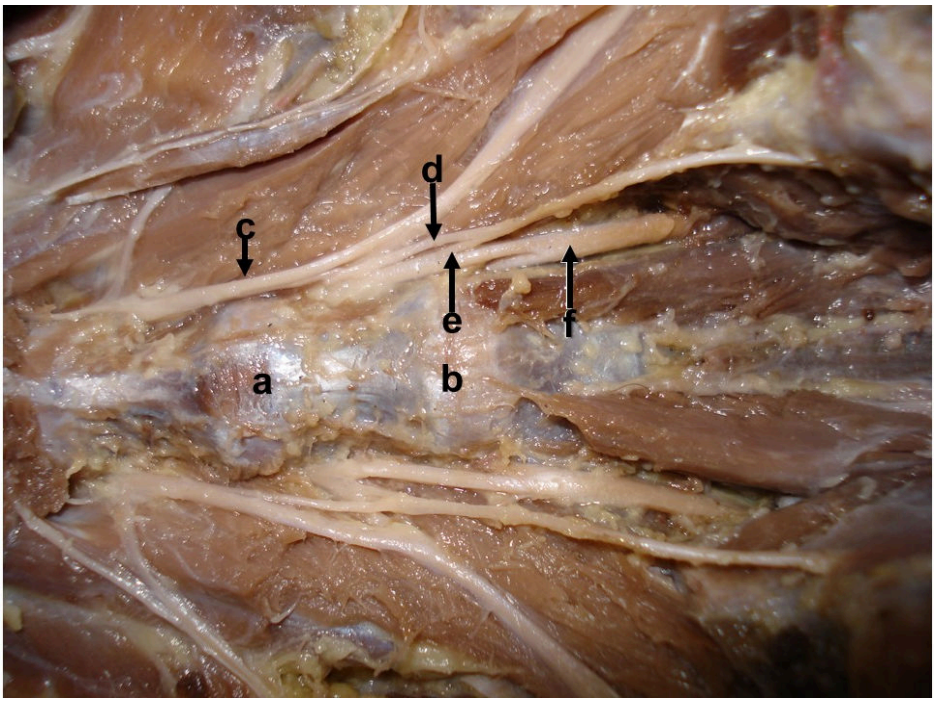

Figure 1. Ventral view of the abdominopelvic cavity of *Nasua nasua* (coati).
a- L_4, b- L_5, c- femoral nerve, d- cranial root of the obturator nerve (L_3), e- caudal root of the obturator nerve (L_4), f- sciatic nerve.

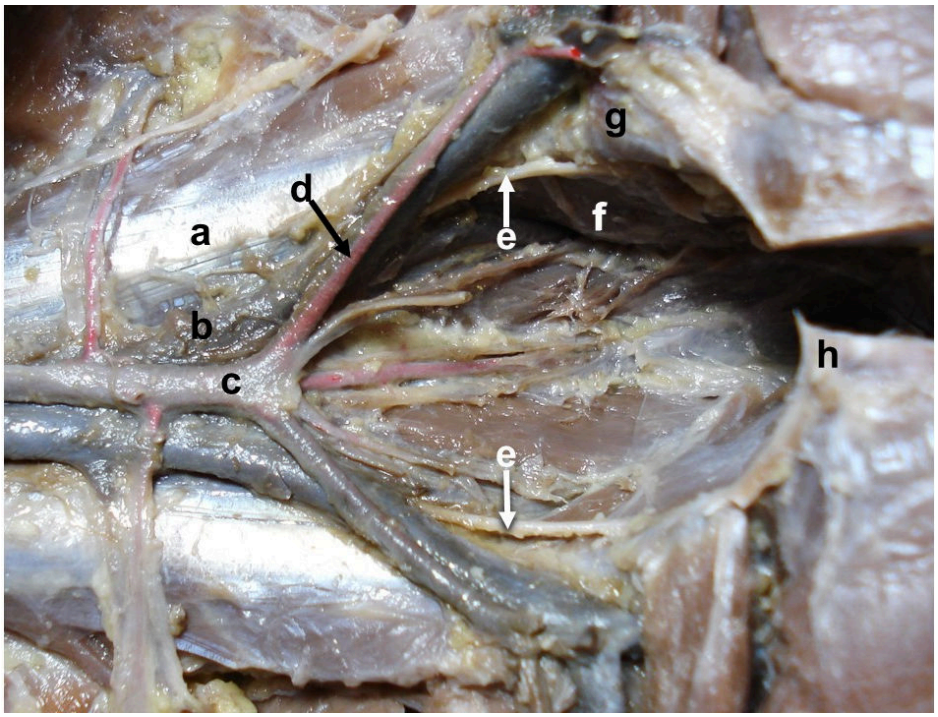

Figure 2. Ventral view of the abdominopelvic cavity of *Nasua nasua* (coati).
a- tendon of the psoas minor muscle, b- psoas major muscle, c- abdominal aorta artery, d- external iliac artery, e- obturator nerve, f- internal obturator muscle, g- lateral border of the lesser pelvis, h- distal border of the lesser pelvis.

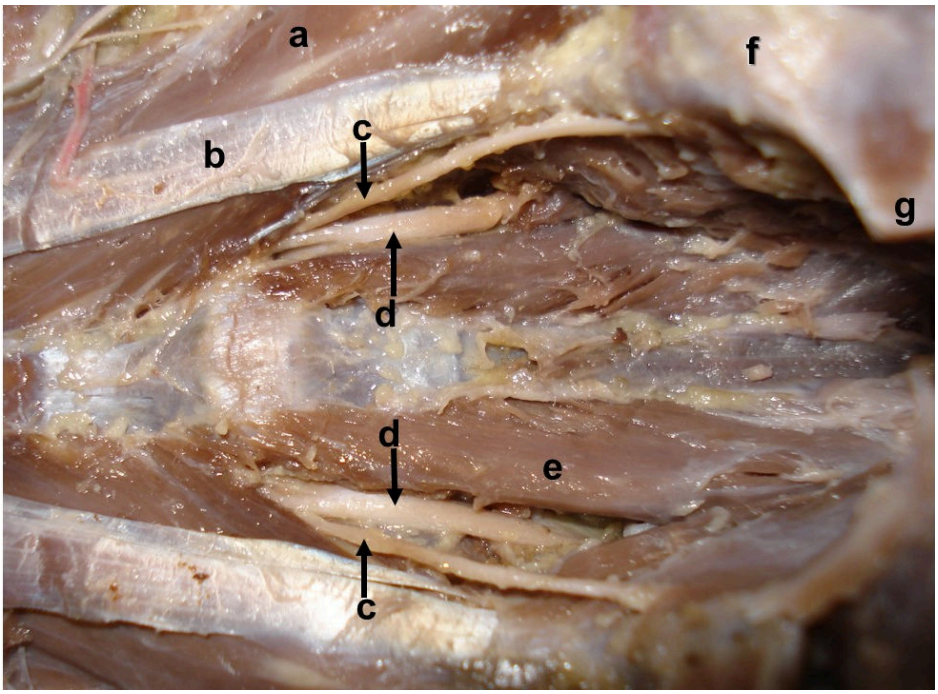

Figure 3. Ventral view of the abdominopelvic cavity of *Nasua nasua* (coati).
a- psoas major muscle, b- tendon of the psoas minor muscle, c- obturator nerve, d- sciatic nerve, e- ventral caudal muscle, f- iliac bone, g- pubic bone.

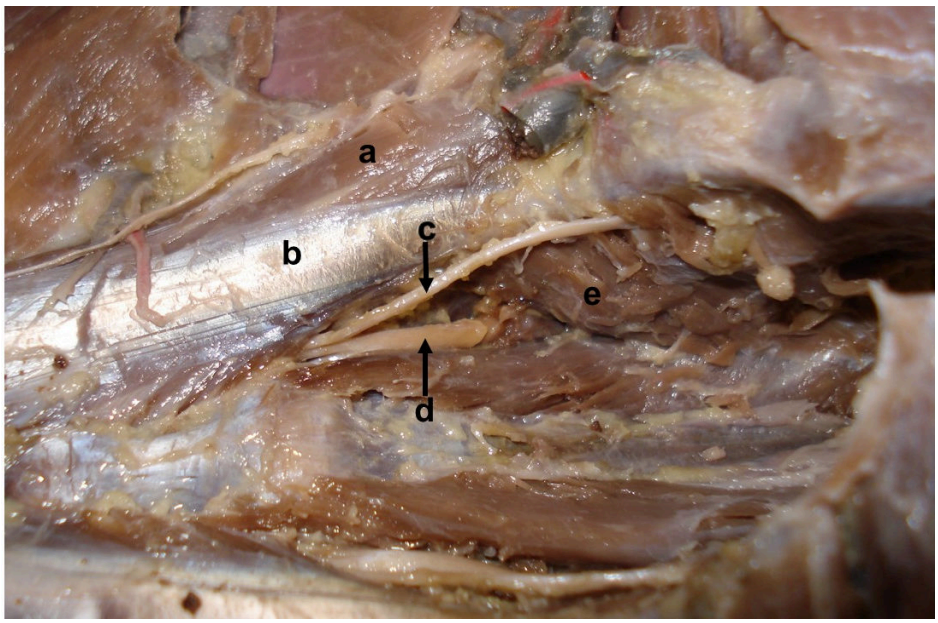

Figure 4. Ventral view of the abdominopelvic cavity of *Nasua nasua* (coati).
a- psoas major muscle, b- tendon of the psoas minor muscle, c- obturator nerve, d- sciatic nerve, e- internal obturator muscle.

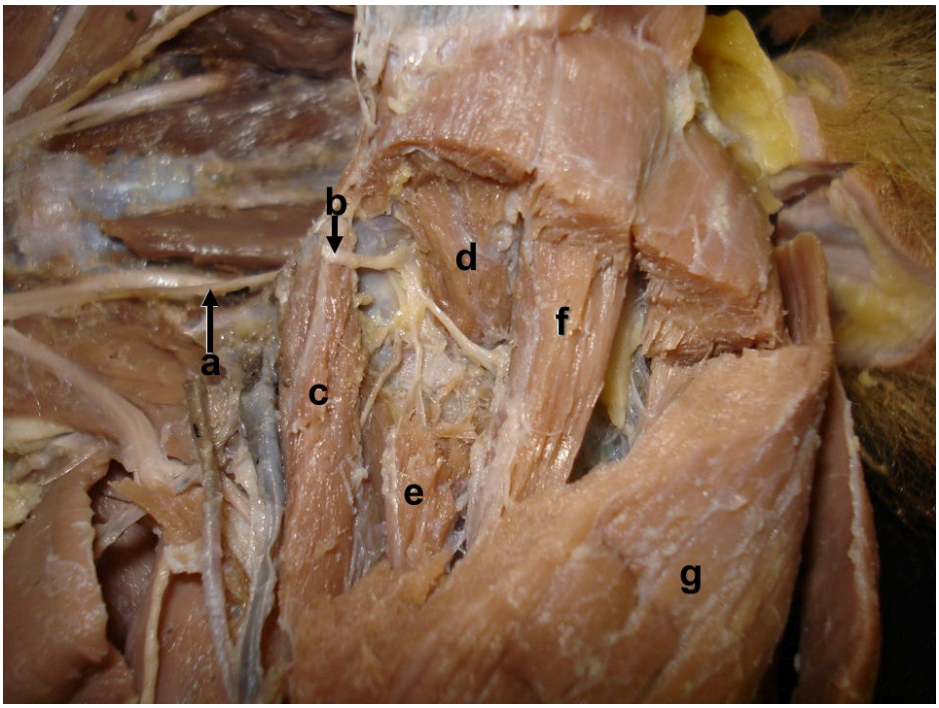

Figure 5. Ventral view of the abdominopelvic cavity of *Nasua nasua* (coati).
a- obturator nerve, b- ventral branch of the obturator nerve, c- pectineus muscle, d- external obturator muscle, e- adductor brevis muscle, f- adductor longus muscle, g- adductor magnus muscle.

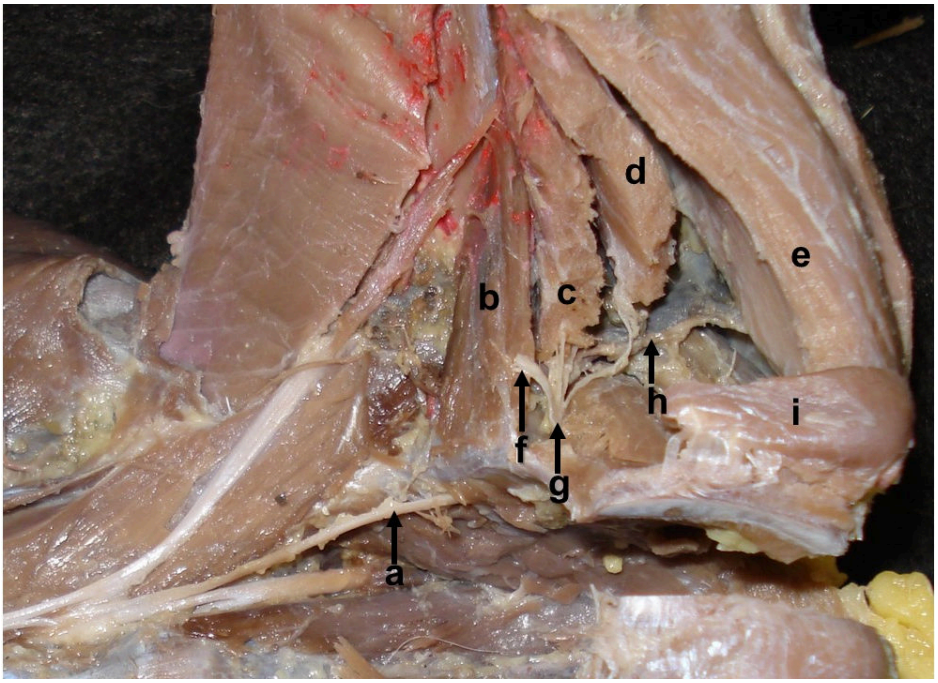

Figure 6. Ventral view of the abdominopelvic cavity of *Nasua nasua* (coati).
a- obturator nerve, b- pectineus muscle, c- adductor brevis muscle, d- adductor longus muscle, e- adductor magnus muscle, f- ventral branch of the obturator nerve, g- dorsal branch of the obturator nerve, h- obturator artery, i- gracilis muscle.

4. DISCUSSION

The origin of the obturator nerve in coatis (L_3 and L_4) was distinct from that in horses (DYCE et al., 2004; GETTY, 2018), ruminants (DYCE et al., 2004; GETTY, 2018), and dogs (MILLER, 1979; EVANS AND DELAHUNTA, 2001), where the origin of the obturator nerve was described at L_4, L_5, and L_6, although the contribution of L_4 was minimal. In human primates and some non-human primates (Gorilla and Chimpanzee), a divergence was also noted, with the obturator nerve originating at L_2, L_3, and L_4 (HEPBURN, 1891; GOSS, 1988). Getty (2018) described the origin of the obturator nerve in pigs from L_4 and L_5, and in cats from L_6 and L_7. Conversely, the origin of the obturator nerve in coatis was similar to that in primates such as Gibbons and *Cebus* (HEPBURN, 1892; HILL, 1960).

Thus, it is observed that in some groups of animals, the obturator nerve is formed from three roots, while in coatis, cats, pigs, and some primates, there is a contribution from only two roots. According to Machado (2007), the obturator nerve has a plurisegmental characteristic, being composed of two or more spinal nerves, a fact observed in the present study.

The cranially displaced origin of the obturator nerve, with the involvement of L_2, observed in humans, Gorilla, and Chimpanzee (HEPBURN, 1892; GOSS, 1988), could signify a higher phylogenetic origin of this nerve since it was not observed in any other group of animals.

The course of the obturator nerve, regarding the convergence of roots within the psoas major muscle to form the nerve and its displacement along the cranial margin of the lesser pelvis until it penetrates the obturator foramen, was similar to findings in horses (GETTY, 2018; DYCE et al., 2004), ruminants (GETTY, 2018; DYCE et al., 2004), dogs (MILLER, 1979; EVANS AND DELAHUNTA, 2001), cats, and pigs (GETTY, 2018), as well as in humans (GOSS, 1988).

The distribution of the obturator nerve to the skin of the medial aspect of the thigh and to the adductor brevis, adductor longus, and pectineus muscles was similar to data described in horses (GETTY, 2018; DYCE et al., 2004), dogs (MILLER, 1979; EVANS AND DELAHUNTA, 2001), pigs (GETTY, 2018), ruminants (GODINHO et al., 1987; GETTY, 2018; DYCE et al., 2004), and humans (GOSS, 1988). However, in these animals, the obturator nerve also innervated the hip joint, the external obturator, gracilis, and adductor magnus muscles.

Therefore, it can be concluded that the obturator nerve of coatis originates from the ventral branches of L_3 and L_4 and distributes to the adductor brevis, adductor longus, and pectineus muscles, as well as to the skin of the medial aspect of the thigh.

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Stricto Ensu Editora

ANATOMY OF THE SUPERFICIAL MUSCLES OF THE FOREARM OF THE HOARY FOX (*Lycalopex vetulus* Lund 1842)

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ABSTRACT

Anatomy is the science that studies the structural organization of the animal body, with the Muscular System being one of its focuses. The hoary fox (*Lycalopex vetulus*) is the only Brazilian canid species endemic to the cerrado and one of the least studied neotropical canids in the world. Therefore, the aim of this study is to describe, comparatively, the Anatomy of the superficial muscles of the forearm in hoary fox, discussing the results with data already existing in other wild animals, domestic animals and human literature, adding possible important information for the understanding the biology of this species, in addition to the health and conservation of wild species. The dissected structures are analyzed, described and discussed with the already well-established literature on domestic dogs and other species. The data are collected from the dissection, under authorization of CEUA/UFCAT, opinion nº 01/22 of carcasses of four specimens of hoary fox (*Lycalopex vetulus*), two males and two females, adults, but without defined age, donated by the Wildlife Research Institute – IPEVIS and the Wild Animal Screening Center of Catalão/Goiás (CETAS - Catalão/GO) and/or collected on the sides of highways in Goiás and Minas Gerais (Authorization - SISBIO 37072-2). The experimental protocols are carried out in accordance with the recommendations of the Brazilian College of Animal Experimentation (COBEA) guidelines, using usual techniques in Macroscopic Anatomy. The results indicate a muscular pattern similar to that of other mammalian species, regarding the presence and general characteristics of the muscles.

Keywords: Comparative Anatomy, hoary Fox and Muscular System.

1. INTRODUCTION

The study of anatomy in wild animals is an area of great interest in many countries, particularly when seeking to understand the biology of endangered species. However, literature citations are scarce, especially concerning neotropical canids, including some endemic to the Brazilian Cerrado, such as the Hoary fox (*Lycalopex vetulus*), one of the least known canids in the world (BARROS et al., 2019).

Many species of neotropical mammals are utilized in scientific research; however, studies on the anatomy of mammals inhabiting endangered areas in Brazil are not common in the current scientific scenario (BARROS et al., 2019). In the Cerrado, the geographic distribution of endemic canids has been severely altered due to anthropogenic interventions. Among the endemic species affected are the crab-eating fox (*Cerdocyon thous*), bush dog (*Speothos venaticus*), maned wolf (*Chrysocyon brachyurus*), and the Hoary fox (*Lycalopex vetulus*) (SOUZA, 2022). These species, in addition to experiencing habitat modifications and loss, have their populations significantly affected by accidental deaths, particularly from roadkill. Although not categorized as critically endangered, they are considered vulnerable species (SANTEE et al., 2019).

The Hoary fox, *Lycalopex vetulus*, is the smallest Brazilian canid, weighing between 2 and 4 kilograms. It has a slender and delicate body, a small head, and a short, blackened muzzle (AZEVEDO; LEMOS, 2012). Its fur is short, with light gray coloration on the dorsal parts of the body and grayish-yellow on the ventral parts, although coloration may vary throughout the body. The ears and paws are slightly reddish, and its tail has long hairs (INSTITUTO PRÓ-CARNÍVOROS, 2023). It is a carnivorous-insectivorous-omnivorous species, with adaptations for a diet mainly composed of termites (ROCHA, 2008). It inhabits open field and Cerrado areas, displaying a crepuscular/nocturnal activity pattern (DALPONTE, 2009).

In the Triângulo Mineiro and Goiás, it is still easily spotted, but it already suffers from anthropogenic activities. It is common for specimens to be attacked by domestic dogs, and they can contract diseases through this contact. They are also frequently found run over on roads and railways (AZEVEDO; LEMOS, 2012). In this sense, studies on the anatomy of wild canids are important, both as subsidies for conservation research and for veterinary procedures (SOUZA et al., 2020).

The present study aims to dissect, analyze, and describe, with a comparative approach, the anatomy of the forearm muscles in the Hoary fox, comparing the data obtained with the well-established literature on domestic animals and wild animals of the Cerrado, in an attempt to identify evolutionary-adaptive aspects inherent in the anatomy of different taxon.

2. MATERIAL AND METHODS

This is a descriptive and comparative study where structures are dissected, analyzed, described, and compared with literary data in domestic and wild animals. Data were collected from the dissection of four Hoary fox carcasses, two males and two females, adults, but of unknown age, which were donated by the Wildlife Research Institute - IPEVIS and the Wild Animals Screening Center of the Catalão-GO (CETAS - Catalão/GO) or collected on the margins of highways in Goiás and Minas Gerais under Authorization - SISBIO 37072-2 and opinion of CEUA/UFCAT nº 01/22. All experimental protocols were followed in accordance with the recommendations and guidelines of the Brazilian College of Animal Experimentation (COBEA).

This research was conducted in the Laboratory of Comparative Anatomy of Wild Animals of the Department of Biological Sciences at the Federal University of Catalão (LACAS-UFCAT). The carcasses were fixed in a 10% aqueous formalin solution and preserved in it. The preparation of anatomical pieces was performed using usual techniques in Macroscopic Anatomy, involving the use of scalpels, scissors, and anatomical forceps.

The study of forearm muscles was conducted with emphasis on general characteristics such as shape, location, origin, and insertion. Considering the descriptive nature of this work, a reduced number of specimens were used, as it involves wild animals, and the use of a reduced number of specimens should be prioritized.

Documentation was carried out through photographs using a iPhone 11 smartphone camera (12 megapixel). The nomenclature adopted was based on the Veterinary Anatomical Nomenclature (International Committee on Veterinary Gross Anatomical Nomenclature, 2017).

3. RESULTS AND DISCUSSION

The macroscopic anatomy in the Hoary fox does not exhibit the same level of knowledge ascertained for other species of wild animals, although anatomical descriptions are of significant value, not only for understanding the biology of the species but also involving issues of animal health, reproduction, and conservation, including taxonomic groups on the verge of extinction. On the other hand, the comparative study of anatomy in the Hoary fox, with other wild species, domestic species, and with Human Anatomy, can provide valuable information as didactic and evolutionary subsidies.

In this context, this study proposes to describe and discuss the Anatomy of the superficial muscles of the forearm in the Hoary fox, with the literature consulted, on the same muscles in other wild species, domestic species, and Humans. In domestic animals, Miller et al. (1964) and Getty in Sisson and Grossman (2008) classified the superficial muscles of the forearm into two groups: Extensor and Flexor. The Extensor Group, located cranially, and the Flexor Group occupying the caudomedial face, in agreement with the observations in the Hoary fox and the citations of Moore, Dalley, and Agur (2019) in Humans.

In this context, the Superficial Extensor Group of the Forearm in the Hoary fox is formed by the muscles: Extensor Carpi Radialis, Common Extensor of the Toes, Lateral Extensor of the Toes, and Extensor Carpi Ulnaris, as described by Miller et al. (1964) in the domestic dog, and Moore, Dalley, and Agur (2019) in Humans, with the Extensor Carpi Radialis muscle in Humans being divided into two heads: Long Extensor Carpi Radialis and Short Extensor Carpi Radialis.

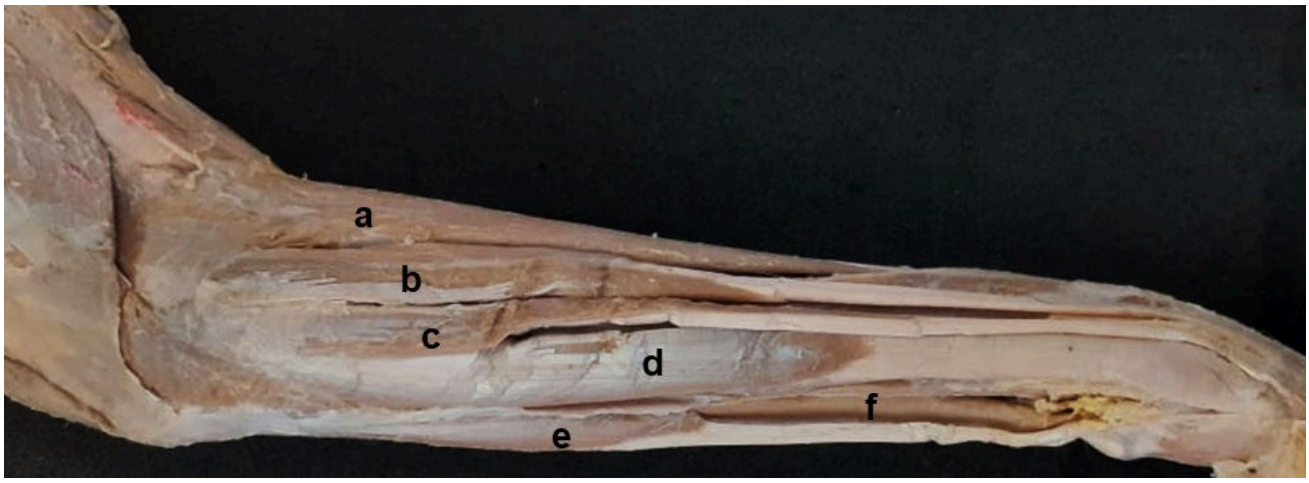

Figure 1. Lateral view of the right forearm of the Hoary Fox.
a- Extensor Carpi Radialis muscle; b- Common Extensor Digitorum muscle; c- Lateral Extensor Digitorum muscle; d- Extensor Carpi Ulnaris muscle; e- Superficial Digital Flexor muscle; f- Flexor Carpi Ulnaris muscle (Photo: Matheus Matutino, 2023).

Extensor Carpi Radialis Muscle - In the domestic dog, this muscle is described as the largest muscle of the Dorsolateral Group or Extensor of the forearm, being long, thick, and fleshy, located on the cranial-lateral face of the forearm, medially to the Common Extensor of the Toes (MILLER et al., 1964; GETTY in SISSON; GROSSMAN, 2008); in Short-eared dog and Crab-eating fox (VAZ et al., 2011); in Capuchin monkey (AVERSI FERREIRA et al., 2005); in Crab-eating Raccoon (SANTOS; BERTASSOLI; ROSA 2010); in Crab-eating Raccoon (LIMA et al., 2010); in Coati (SANTOS et al., 2010); in Maned wolf (PEREIRA et al., 2019), in agreement with observations in the Hoary fox. However, it is not clear whether, in the Hoary fox, it constitutes the largest muscle in this region. In other domestic species, in Horses, Getty in Sisson and Grossman (2008) only indicates it in figures but does not describe its general characteristics, and in Ruminants, it mentions that the Extensor Carpi Radialis muscle is by far the largest muscle of the Forearm Extensor Group and is located on the cranial face of the forearm. In Pigs, Getty in Sisson and Grossman (2008) states that the said muscle is strong, fleshy, and can be divided into two parts: Long and Short, as in Humans (MOORE; DALLEY; AGUR, 2019), a characteristic not observed in the Hoary fox, furthermore, it does not clearly refer to its location. In domestic Cats, the same author only indicates it in figures, without describing its general characteristics, however, he states that the said muscle is completely divided into two parts: Long and Short, as in Humans (MOORE; DALLEY; AGUR, 2019).

Observations in the Hoary fox reveal that the Common Extensor of the Toes muscle, concerning its general characteristics, closely aligns with descriptions in the domestic dog (MILLER et al., 1964 and GETTY in SISSON; GROSSMAN 2008), stating that the Common Extensor of the Toes muscle lies on the cranial-lateral surface of the Radius, between the Extensor Carpi Radialis muscle and the Lateral Extensor of the Toes, in agreement with Miller et al. (1964), consistent with descriptions in other wild animals, Vaz et al. (2011), in Short-eared dog and Crab-eating fox; Aversi Ferreira et al. (2005), in Capuchin monkey; Santos, Bertassoli e Rosa (2010); Santos et al. (2010), in Crab-eating Raccoon; Lima et al. (2010), in Crab-eating Raccoon; Santos et al. (2010), in Coati, and Pereira et al. (2019), in Maned wolf. However, in other domestic animals, Getty in Sisson and Grossman (2008) reports that, in Horses, the said muscle is located laterally to the Extensor Carpi Radialis muscle and, although less voluminous, it is similar to that one in its general characteristics, in line with the observations in the Hoary fox. In Ruminants, the Common Extensor of the Toes muscle is the most complex of the Extensor Group, divided into two very distinct bellies: a Lateral part,

thinner and more superficial, and a Deep part, bulkier. Both parts converge into their own tendon, which soon branches out, with resulting branches directed to each two corresponding toes (GETTY in SISSON; GROSSMAN, 2008). In Pigs, according to the same author, the Common Extensor of the Toes muscle divides into three parts, each converging into its own tendon, which, however, branch out and anastomose, forming a structure similar to a plexus, from which branches originate for each of the toes, consistent with the same authors in Carnivores and with Moore, Dalley, and Agur (2019) in Humans.

Observations in the Hoary fox indicate that the Lateral Extensor of the Toes muscle is long and slender but fleshy, located between the Common Extensor of the Toes muscles and the Extensor Carpi Ulnaris, consistent with the descriptions by Miller et al. (1964) and Getty in Sisson and Grossman (2008) in the domestic dog; Vaz et al. (2011) in Short-eared dog and Crab-eating fox; Aversi Ferreira et al. (2005) in Capuchin monkey; Santos, Bertassoli e Rosa (2010) in Crab-eating Raccoon; Lima et al. (2010) in Crab-eating Raccoon; Santos et al. (2010) in Coati; and Pereira et al. (2019) in Maned wolf, although its volume is smaller than in the domestic dog. In other domestic animals, in Horses, Getty in Sisson and Grossman (2008) describes that the Lateral Extensor of the Toes muscle is much smaller than the Common Extensor of the Toes but its location is similar. The same author states that, in Ruminants, the said muscle is the most caudal of the Carpal Extensor Group, but does not comment on its volume and location, while in Pigs, it has two distinct parts: Superficial, larger, whose tendon ends in the fourth toe, occasionally in the fifth, and Deep, which is smaller and ends in the fifth toe; On the other hand, Moore, Dalley, and Agur (2019) do not specifically mention the Lateral Extensor of the Toes muscle, since evolution led the human hand to a high degree of specialization, requiring deep specialization and individualization of the muscles that act on it.

In the domestic dog, according to Miller et al. (1964) and Getty in Sisson and Grossman (2008), the Ulnar Extensor of the Carpus Muscle is located on the caudolateral aspect of the Ulna, being the last muscle of the extensor group, from lateral to medial, on the dorsolateral surface of the forearm. It has a fleshy belly and its fibers converge to form a strong tendon that extends to the carpus. In the Hoary fox, the Ulnar Extensor of the Carpus Muscle appears as the most voluminous muscle of the Extensor Group, with its shape and extension being consistent with the descriptions by Miller et al. (1964) and Getty in Sisson and Grossman (2008). On the other hand, in other wild animals, several authors describe similar characteristics, Vaz et al., 2011), in Short-eared dog and Crab-eating fox, Aversi Ferreira et al. (2005), in Capuchin monkey; Santos, Bertassoli e Rosa (2010) in Crab-eating Raccoon;

Lima et al. (2010) in Crab-eating Raccoon; Santos et al. (2010), in Coati; and Pereira et al. (2019), in Maned wolf. In domestic animals, Getty in Sisson and Grossman (2008) does not refer to the aforementioned muscle in Horses, Ruminants, Pigs, and Cats, but in Dogs, states that it is the most caudal of the muscles of the dorsolateral aspect of the forearm. In Humans, Moore, Dalley, and Agur (2019) do not mention an Ulnar Extensor of the Toes muscle specifically but describe the Ulnar Extensor of the Carpus Muscle, which is fusiform and long, located on the medial margin of the forearm and exhibiting two heads: Ulnar and Humeral, whose tendons join those of the Toe Extensor Muscle under the Retinaculum of the toe extensor muscles.

In the domestic dog, according to Miller et al. (1964) and Getty in Sisson and Grossman (2008), the caudal group of superficial muscles of the forearm consists of the Flexor Carpi and the Flexor Digitorum muscles, with the superficial group composed of only three muscles: the Radial Carpal Flexor; Superficial Digital Flexor, and the Ulnar Carpal Flexor. The Radial Carpal Flexor originates from the medial epicondyle of the Humerus, behind the Elbow Collateral Ligament, between the Pronator teres and Deep Digital Flexor muscles, and extends distally under the skin and Antebrachial Fascia, between the Pronator teres and the Superficial Digital Flexor muscles. It has a voluminous, fusiform, but short belly, whose fibers converge in the middle part of the Radius to form a flat tendon, which becomes cylindrical more distally to insert into the II and III Metacarpals. Observations in the Hoary fox present general characteristics in close harmony with the citations of the aforementioned authors. In wild animals, Vaz et al. (2011), in Short-eared dog and Crab-eating fox; Aversi Ferreira et al. (2005), in Capuchin monkey; Santos, Bertassoli e Rosa (2010) in Crab-eating Raccoon; Lima et al. (2010) in Crab-eating Raccoon; Santos et al. (2010), in Coati; and Pereira et al. (2019), in Maned wolf. In domestic animals, Getty in Sisson and Grossman (2008) reports that in Horses and Ruminants, the Radial Carpal Flexor Muscle is located on the caudomedial surface of the forearm, displaying a fusiform shape, broader in its middle part; in Horses, it is located on the medial side of the forearm caudally to the edge of the Radius; in Pigs, it is well developed, and in Dogs and Cats, it is only indicated in figures. Moore, Dalley, and Agur (2019) indicate that the said muscle in Humans is fusiform, long, and situated medially to the Pronator teres Muscle. Its fibers converge to form a long, flattened tendon, which becomes cylindrical distally, serving as a good guide to find the Radial Artery, which is located immediately lateral to it.

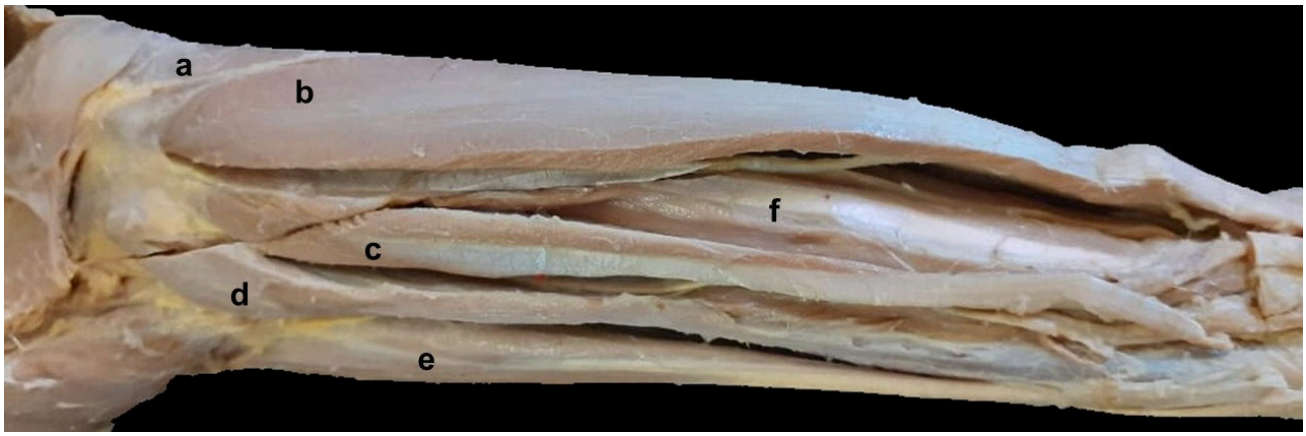

Figure 2. Medial view of the right forearm of the Hoary Fox.
a- Flexor Carpi Ulnaris muscle; b- Superficial Flexor Digitorum muscle; c- Flexor Radialis Carpi muscle; d- Pronator Teres muscle; e- Extensor Carpi Radialis muscle; f- Deep Digital Flexor muscle (Photo: Matheus Matutino, 2023).

According to Miller et al. (1964) and Getty in Sisson and Grossman (2008), in the domestic dog, the Superficial Digital Flexor is located on the caudomedial part of the forearm, its belly is fleshy, extending distally, becoming tendinous near the carpus. Still in the proximal third of the forearm, the said muscle divides into four parts, whose tendons go to the metacarpals and phalanges of the II to V toes. Observations in the Hoary fox show a close relationship of concordance with the citations of Miller et al. (1964), and Getty in Sisson and Grossman (2008), in the domestic dog, since an origin is identified in the caudal part of the medial epicondyle and divides into four tendons that insert into the middle phalanx of the four main toes, consistent observations, also, with the citations in wild animals, Vaz et al. (2011), in Short-eared dog and Crab-eating fox; Aversi Ferreira et al. (2005), in Capuchin monkey; Santos, Bertassoli e Rosa (2010) in Crab-eating Raccoon; Lima et al. (2010) in Crab-eating Raccoon; Santos et al. (2010), in Coati; and Pereira et al. (2019), in Maned wolf). In domestic animals, Getty in Sisson and Grossman (2008) states that, in Horses, the Superficial Digital Flexor Muscle is located between the Ulnar Flexor Muscles and the Deep Flexor, and its origin occurs on the Medial Epicondyle of the Humerus and the proximal part of the Superficial Radius Crest, it is divided into two parts: Umeral, fleshy, and Radial, fibrous. The umeral part is multipennate and partially merges with the Deep Digital Flexor. Near the carpus, both parts converge to form a thick tendon that crosses the Carpal Tunnel, along with the tendon of the Deep Digital Flexor Muscle, inserting into the proximal end of the middle phalanx. In Ruminants, the said muscle, near its origin, is partially fused with the Ulnar Carpal Flexor but then divides into two bellies: Superficial and Deep, each ending in an individual tendon in the middle part of the forearm. The tendon of the middle part crosses superficially over the Flexor Retinaculum and joins the tendon of the deep part, which crossed under the Retinaculum,

inserting into the middle part of the phalanx of the III and IV toes, and in Cats, the said muscle originates from the medial epicondyle of the humerus and inserts into the metacarpals and phalanges of the II to V toes. In Humans, Moore, Dalley, and Agur (2019) mention that the Superficial Digital Muscle is one of the Superficial Forearm Flexors that originates with the Common Digital Flexor Muscle, being the largest flexor muscle of the forearm. One of its parts forms an intermediate layer between the superficial and deep flexors. Near the wrist, it gives rise to four tendons that cross the Carpal Tunnel to the toes.

Observations in the Hoary fox indicate a close agreement with Miller et al. (1964) and Getty in Sisson and Grossman (2008) in the domestic dog, which describe that the Ulnar Carpal Flexor Muscle is formed by two parts: Umeral, much larger, and Radial, smaller. Both parts converge into a single tendon that inserts into an accessory carpal bone. It lies on the caudolateral side of the forearm, with the ulnar head located very superficially, partially covering the Umeral head. The Umeral head, the second largest muscle among the forearm flexors, originates from the medial epicondyle of the Humerus and is located between the Deep Flexor and Superficial Flexor muscles, while the Radial head, very thin, originates from the medial surface of the olecranon and inserts into the carpus. Vaz et al. (2011) in Short-eared dog and Crab-eating fox; Aversi Ferreira et al. (2005) in Capuchin monkey; Santos, Bertassoli e Rosa (2010) in Crab-eating Raccoon; Lima et al. (2010) in Crab-eating Raccoon; Santos et al. (2010) in Coati; and Pereira et al. (2019) in Maned wolf; only indicate the muscle in figures. In Humans, on the other hand, Moore, Dalley, and Agur (2019) state that the Ulnar Carpal Flexor Muscle is the most medial of the superficial forearm flexors, originating from the medial epicondyle of the Humerus and inserting into an accessory carpal bone.

In addition to the superficial forearm flexor muscles, the Pronator teres Muscle, Supinator Muscle, and Brachioradial Muscle are located on the anterior aspect of the elbow joint.

In domestic dogs, Miller et al. (1964) and Getty in Sisson and Grossman (2008) state that the Pronator teres Muscle is located in the proximal part of the medial aspect of the forearm. It originates from the medial epicondyle of the humerus, cranially to the origin of the Radial Carpal Flexor, crosses the elbow joint and Antebrachial Fascia, resting under the skin, then crosses the middle radioulnar joint obliquely in a distolateral direction, forming a tendinous band that inserts into the medial edge of the radius. Similarly, authors such as Vaz et al. (2019) in Short-eared dog and Crab-eating fox and Pereira et al. (2019) in Maned wolf mention similar observations. In domestic animals, Getty in Sisson and Grossman (2008) describes the muscle in pigs, stating that it is fusiform, slender, and superficially located on

the palmar aspect of the elbow and proximal part of the forearm, originating from the medial epicondyle of the humerus and Medial Collateral Ligament of the Elbow, inserting into the medial edge of the radius. In cats, the Pronator teres Muscle is larger than in domestic dogs, as indicated in figures. In Humans, Moore, Dalley, and Agur (2019) describe the Pronator teres Muscle as fusiform and the most lateral of the superficial forearm flexors. Its lateral edge forms the boundary of the Ulnar Fossa.

Observations in the Hoary fox show that the Supinator Muscle is a small, fleshy, flattened band that originates from the Medial Epicondyle of the Humerus and the Medial Collateral Ligament of the Elbow, via a short but thick tendon. It rests on the cranial aspect of the radius, following distally, obliquely, and medially to insert into the cranial-lateral surface of the middle part of the radius. These observations align with the descriptions provided by Miller et al. (1964) and Getty in Sisson and Grossman (2008) in domestic dogs, stating that the muscle is a broad muscular band, completely surrounded by a thin fascia, situated on the lateral aspect of the elbow, covered by the Radial Carpal Extensor and Extensor muscles. It originates via a short but strong tendon from the lateral epicondyle of the humerus, extends distally and obliquely in a medial direction, inserting near the medial edge of the radius. In Maned wolves, according to Pereira et al. (2019), the Supinator Muscle originates from the lateral epicondyle of the humerus, following distally and medially to insert into the middle part of the radius. In Humans, Moore, Dalley, and Agur (2019) describe the Supinator Muscle as deeply situated in the ulnar fossa, spiraling obliquely, distally, and medially to insert proximally into the surface of the radius.

In domestic dogs, according to Miller et al. (1964) and Getty in Sisson and Grossman (2008), the Brachioradial Muscle is often long, very slender, and occasionally absent, located in the flexor angle of the elbow. It originates from the proximal part of the Lateral Supraepicondylar Crest of the Humerus, proximally to the Radial Carpal Extensor, extending distally between the Radial Carpal Extensor and the surface of the radius, until its insertion into the periosteum of the middle part of the radius, through a thin aponeurosis. Observations in the Hoary fox partially align with the descriptions of Miller et al. (1964) and Getty in Sisson and Grossman (2008) in domestic dogs, as it is relatively slender but agrees with other aspects. In Humans, Moore, Dalley, and Agur (2019) describe the Brachioradial Muscle as fusiform, superficially located on the anterolateral aspect of the forearm, forming the lateral margin of the Ulnar Fossa. It originates between the forearm extensor muscles, spirals around the radius, and inserts into the anterior surface of the radius in its middle part.

Figure 3. Medial view of the right forearm of the Hoary Fox.
a- Flexor Carpi Ulnaris muscle; b- Superficial Flexor Digitorum muscle; c- Flexor Radialis Carpi muscle; d- Pronator Teres muscle; e- Supinator muscle; f- Extensor Carpi Radialis muscle; (Photo: Matheus Matutino, 2023).

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Stricto Sensu Editora

ANATOMY OF THE THIGH MUSCLES OF THE HOARY FOX

(*Lycalopex vetulus* Lund, 1842)

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ABSTRACT
The Hoary Fox is the only species of Brazilian canid endemic to the Cerrado, the second largest biome in Brazil (LEMOS et al. 2013). We conducted the dissection, analysis, and description of the thigh muscles of the Hoary Fox, prioritizing a comparative approach with domestic canids. The carcasses were donated by the Institute for Wildlife Research (IPEVIS) and the Wildlife Screening Center (CETAS) of Catalão, and/or collected along highways in Goiás and Minas Gerais (Authorization - SISBIO 37072-2). The research has a favorable opinion from CEUA/UFCAT nº 01/22. The experimental protocols are conducted in accordance with the recommendations of the Brazilian College of Animal Experimentation (COBEA). After analyzing the thigh, we identified the following muscles: Tensor Fasciae Latae; Biceps Femoris; Semitendinosus; Quadriceps Femoris; Semimembranosus; Sartorius; Gracilis; Pectineus; and Adductor. The observations of this work are similar to the citations of Miller et al. (1964) in Domestic Dogs.
Keywords: Anatomy, Muscles and Wildlife.

1. INTRODUCTION

The Hoary Fox is a species of Brazilian canid, easily confused with the Crab-eating Fox, especially by laypeople, making it difficult to distinguish between the endemic areas of these two groups. Observational studies indicate that the distribution of the Hoary Fox occurs

throughout the Brazilian Cerrado biome (ROCHA et al., 2008). The distribution area of the Hoary Fox is vast, and the species not only occurs in different types of environments but also faces different types and levels of anthropogenic pressure. According to the IUCN, the species can be locally abundant, but its population is smaller than that of the Crab-eating Fox, for which population estimates in Brazil are also fragile (DALPONTE; COURTENAY, 2008).

Thus, the Hoary Fox, like other species in the Cerrado, suffers from the advance of anthropogenic activities in the biome, with an increase in cultivated areas, leading to a decrease in the diversity of flora, directly impacting the fauna, intensifying competition for food, reducing cooperation, and affecting other important ecological relationships. In addition to the loss of physical spaces and vegetation fields (LEMOS et al., 2013).

In this context, it becomes important to study endemic species to improve the knowledge of their habits, forms of adaptation, diet, and osteomuscular composition. Consequently, the objective of this study is to seek specific knowledge of the anatomy of this species, about which there is little literature, especially regarding the muscular system, including the thigh muscles.

2. MATERIAL AND METHODS

As a descriptive and comparative research, three adult Hoary Fox (*Lycalopex vetulus*) specimens are used, although their exact ages are not defined. These specimens are donated by the Wildlife Research Institute - IPEVIS, the Wildlife Rehabilitation Center - CETAS/Catalão, and/or collected from the sides of highways in Goiás and Minas Gerais (Authorization - SISBIO 37072–2).

This research is conducted at the Comparative Anatomy Laboratory of Wild Animals in the Department of Biological Sciences at the Federal University of Catalão (LACAS-UFCAT).

The carcasses are fixed with a 10% formalin solution and are preserved in this solution both before and after dissection, which is performed using standard techniques in Macroscopic Anatomy studies, with the use of scalpels, scissors, and anatomical forceps. Documentation is carried out through photographic records taken with an iPhone 12 camera, and the terminology adopted is based on the Nomina Anatomica Veterinaria (International Committee on Veterinary Gross Anatomical Nomenclature, 2017).

Given the scarcity of literature on the Anatomy of the Muscular System of canids, our discussion references the work of Miller et al. (1964) “Anatomy of the Dog”, as this is the most comprehensive work on the Anatomy of canids available to us.

3. RESULTS AND DISCUSSION

According to Miller et al. (1964), the thigh muscles in Domestic Dogs can be classified according to their location into **Caudal Thigh Muscles**, comprising: m. Tensor Fasciae Latae; m. Biceps Femoris; m. Caudal Crural Abductor; m. Semimembranosus; m. Semitendinosus. **Cranial Thigh Muscles**: m. Quadriceps Femoris, formed by m. Rectus Femoris; m. Vastus Medialis; m. Vastus Lateralis; and m. Vastus Intermedius. m. Capsularis Coxae and m. Articularis Genus. **Medial Thigh Muscles:** m. Sartorius; m. Gracilis; m. Pectineus; mm. Adductors.

Miller et al. (1964) do not describe the m. Tensor Fasciae Latae but only indicate it in figures. However, in the Hoary Fox, it has an approximately triangular shape, originates from the Anterior Superior Iliac Spine and the Iliac Crest, and spreads in the proximal region of the lateral thigh, proximal to the m. Vastus Lateralis, joining distally to the Fascia Lata.

Figure 1. Lateral view of the right thigh of the Hoary Fox.
1- Cranial part of the Sartorius muscle; 2- Tensor Fasciae Latae muscle; 3- Vastus Lateralis muscle; 4- Biceps Femoris muscle; 5- Semitendinosus muscle.

In Domestic Dogs, the m. Biceps Femoris, according to Miller et al. (1964), is the largest and most lateral of the caudal thigh muscles. It originates from the ischial tuberosity and the distal third of the sacrotuberous ligament, runs distally, and inserts through a thick fascial sheet, which joins the Fascia Lata and the Crural Fascia along the tibia, extending to the calcaneal tendon. Observations in the Hoary Fox reveal that the m. Biceps Femoris closely resembles the descriptions by Miller et al. (1964) in Domestic Dogs, being a bulky and thick muscle with an approximately quadrilateral shape, located in the caudolateral region of the lateral thigh.

The m. Semitendinosus in the Hoary Fox is thinner than the Biceps Femoris and, together with the m. Semimembranosus, forms the caudal contour of the thigh. In all its anatomical characteristics, it resembles those described by Miller et al. (1964) in Domestic Dogs, who state that this muscle is thin and forms the caudal contour of the thigh. It originates from the ischial tuberosity, caudal to the origin of the m. Biceps Femoris, courses medially, and in the popliteal region forms the medial boundary of the popliteal fossa. It inserts on the cranial surface of the tibia, distal to the m. Gracilis (MILLER et al., 1964).

According to Miller et al. (1964), the m. Semimembranosus in dogs is very fleshy, originating from the ischial tuberosity, caudally and medially to the m. Semitendinosus, becoming cranial to the latter muscle as it bends medially to insert on the cranial surface of the tibia. Observations in the Hoary Fox indicate a close resemblance to the descriptions by Miller et al. (1964) in Domestic Dogs. According to observations in the Hoary Fox, the m. Caudal Crural Abductor is not observed in the preparations of this research.

The m. Quadriceps Femoris in Domestic Dogs is a large muscle situated cranially, medially, and laterally on the femur. It consists of four parts: Rectus Femoris; Vastus Medialis; Vastus Lateralis; and Vastus Intermedius, which end in a common tendon that encases the patella and inserts on the tibial tuberosity. The Rectus Femoris is the longest part and the only one originating from the pelvic bone. It is situated cranially on the femur relative to the other parts, originating from a bony tuberosity immediately cranial to the acetabulum. Medially, it is adjacent to the Vastus Medialis, and laterally, to the Vastus Lateralis. Deep between the Rectus Femoris and the femur lies the Vastus Intermedius, the smallest of the four (MILLER et al. 1964). Observations in the Hoary Fox reveal characteristics closely similar to those described in Domestic Dogs, with the Vastus Lateralis being the largest and the only one visible on the lateral thigh.

Figure 2. Lateral view of the right thigh of Hoary Fox.
1- Cranial part of the Sartorius muscle; 2- Vastus Lateralis muscle; 3- Biceps Femoris muscle (sectioned); 4- Semitendinosus muscle; 5- Semimembranosus muscle.

Figure 3. Medial view of the right thigh of the Hoary Fox.
1- Gracilis muscle (reflected); 2- Semimembranosus muscle; 3- Adductor muscle of the thigh; 4- Vastus Medialis muscle; 5- Rectus Femoris muscle.

The Femoral Capsular Muscle and the Knee Articular Muscle, present in Domestic Dogs according to Miller et al. (1964), are not observed in the Hoary Fox.

According to Miller et al. (1964), in Domestic Dogs, among the muscles composing the Medial Thigh Group, the Sartorius muscle consists of two narrow and long parts resembling straps: the Cranial Part and the Caudal Part, which extend from the iliac bone to the medial surface of the knee joint. The two bellies are situated cranially to each other, proximally on the cranial surface of the Rectus Femoris muscle and more caudally cranially and then medially on the Quadriceps Femoris muscle. The Cranial Part inserts together with the Rectus Femoris muscle and the Vastus Medialis, while the Caudal Part inserts, through an aponeurosis, more distally, on the cranial margin of the tibia (Miller et al. 1964). Similarly, in the Hoary Fox, anatomical characteristics and location are consistent with observations in Domestic Dogs.

Figure 4. Medial view of the left thigh of the Hoary fox.
1- Cranial part of the Sartorius muscle; 2- Caudal part of the Sartorius muscle; 3- Pectineus muscle; 4- Adductor muscle of the thigh; 5- Gracilis muscle.

Regarding the Gracilis muscle, in the Hoary Fox, the observations made in this study are similar to the descriptions by Miller et al. (1964) in dogs, stating that this is a flat and wide muscle located on the caudal part of the medial surface of the thigh, originating

aponeurotically from the pubic symphysis tendon and inserting aponeurotically into the Crural Fascia up to the calcaneal tendon.

The pectineus muscle is the smallest and most cranial of the thigh adductor muscles, belonging to the deep medial group of the thigh. It is relatively small and fusiform, originating on the cranial surface of the pubis, and extends distally and medially to insert on the caudomedial surface of the femur, according to Miller et al. (1964). Observations in the Hoary Fox closely agree with the descriptions by Miller et al. (1964) in the Dog, but in some cases, it is absent in the Hoary Fox.

The Adductor muscle of the thigh, in Domestic Dogs according to Miller et al. (1964), as well as in the Hoary Fox, exhibits similar morphological characteristics. It forms the caudal part of the deep medial thigh muscles, with the main part of the muscle, the Adductor Magnus and the Adductor Brevis, located between the Pectineus and the Gracilis muscles, superficially. In both cases, it originates from the pubic symphysis and the symphyseal tendon, inserting distally to the trochanteric fossa.

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ANATOMY OF THE PECTORAL MUSCLES OF THE CRAB-EATING FOX (*Cerdocyon thous* Linnaeus, 1766)

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ABSTRACT

The Crab-eating Fox (*Cerdocyon thous*) is a versatile canid adapted to different environments and plays an important role in the ecology of the ecosystems where it lives. Like other canids, it acts as a regulatory factor in rodent populations and other small animals, contributing to maintaining the ecological balance of the ecosystem. This highlights the importance of understanding the species' biology as a subsidy for the development of protection and conservation programs, as well as veterinary medical procedures. Two carcasses donated by CETAS - Catalão/GO are used in this study. The specimens are fixed and preserved in a 10% aqueous formalin solution. The preparation of the material for analysis uses standard dissection techniques in Macroscopic Anatomy. Documentation is carried out through photographs using an iPhone 11 camera. The description of the pectoral muscles developed in this research emphasizes their morphology, location, origin, and insertion. The discussion of the results is conducted with literature consulted on the Anatomy of Domestic Animals. The terminology follows the Veterinary Anatomical Nomina (2017).

Keywords: Anatomy, Myology and Crab-eating Fox.

1. INTRODUCTION

The Cerrado, the second largest Brazilian biome, covering an approximate area of two million km², exhibits a highly diverse geographical formation that includes open fields, high-altitude fields, cerradão, grassy cerrado, gallery forest, and rocky cerrado. This geographical environment harbors a high biological diversity, including species endemic to the region, in line with its physiognomic heterogeneity (MARINHO FILHO, 1996; OLIVEIRA; MARQUIS, 2002).

Among the fauna of the cerrado are 194 species of mammals belonging to nine orders and 30 families, including the Carnivora (MARINHO FILHO, RODRIGUES; JUAREZ, 2002).

The Crab-eating Fox (*Cerdocyon thous*) is a neotropical canid that primarily inhabits the Brazilian cerrado. It has a generalist diet, which is one of the reasons it adapts well to environments already modified by humans. They can even feed on carrion when necessary. Their body length ranges from 57 to 77 cm, plus a tail varying from 22 to 41 cm. They can weigh from 4.5 to 8.5 kg. The fur is light gray in color, but shades may vary among individuals of the species. They have dark paws and rounded ears. There is no apparent sexual dimorphism. They have predominantly nocturnal and crepuscular habits. They are omnivorous animals, with their diet including various fruits, vertebrates, insects, amphibians, crabs, and other crustaceans, birds, and carrion. Gestation lasts approximately 56 days, and they give birth to two to six pups, weighing between 120 and 160 g. Litters are produced once a year, usually from June to December. The species' conservation status is "least concern" according to both the ICMBio national list and the IUCN. In most countries, populations are considered stable. In Brazil, the species is not considered threatened. The crab-eating fox is hunted by rural residents, accused of predating mainly chickens and lambs. Being able to live close to urban areas, it is a constant victim of roadkill throughout Brazil (ONÇAFARI ASSOCIATION, 2024).

The Crab-eating Fox is still poorly studied, and despite being a canid, it presents small anatomical differences compared to the domestic dog, where Anatomy is well established. On the other hand, it is important to emphasize that animal biology can vary among individuals or even among species of the same family. In this context, the aim of this study is to dissect, analyze, and describe, comparatively with literary data in domestic animals, the pectoral muscles of the Crab-eating Fox, in order to seek more accurate knowledge about its biology and add subsidies for the organization of programs for the preservation and conservation of

wild fauna, as well as for the application of increasingly frequent and necessary veterinary medical procedures for animal health.

Cerdocyon thous is taxonomically described by Linnaeus (1966) as follows: Kingdom: Animalia; Phylum: Chordata; Class: Mammalia; Order: Carnivora; Family: Canidae; Genus: *Cerdocyon*; Species: *Cerdocyon thous* (Linnaeus 1766); Common names: Crab-eating Fox, Little Fox.

2. MATERIAL AND METHODS

This is a descriptive and comparative research study where muscles are dissected, analyzed, and described focusing on shape, location, origin, and insertion, and compared with literary data described in domestic animals. Two adult Crab-eating Fox (*Cerdocyon thous*) carcasses, of unknown age, were donated by CETAS - Catalão/GO. The use of a small number of specimens is related to the descriptive nature of this work, without statistical focus, as these are wild animals found dead or deceased in CETAS units.

Experimental procedures are conducted at the Laboratory of Comparative Anatomy of Wild Animals of the Department of Biological Sciences of the Federal University of Catalão (LACAS-UFCAT).

Experimental protocols are carried out following the recommendations of the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee on Animal Use of the Federal University of Catalão - CEUA/UFCAT no. 01/22.

The specimens are fixed in a 10% aqueous formalin solution and preserved in it. Dissection follows usual patterns in Macroscopic Anatomy.

Documentation is performed with an iPhone 11 mobile phone camera.

The terminology adopted to name the structures is in accordance with the Veterinary Anatomical Nomina (ICGVAN, 2017).

3. RESULTS AND DISCUSSION

Like other members of the Canidae family, the Crab-eating Fox possesses well-developed pectoral muscles responsible for moving the thoracic limbs, crucial for locomotion and movement. They are located in the thoracic region, caudally to the neck and over the

ribs. These muscles are fundamental components of the musculoskeletal system, allowing body movement and functionality. The pectoral muscles are strong and well-developed, enabling the Crab-eating Fox to perform agile movements such as jumps and fast runs, as well as aiding the animal in climbing obstacles, capturing prey, and defending against predators. Additionally, the pectoral muscles play an important role in maintaining posture, ensuring stability and balance during movements.

The comparison of the results of this research with relevant literature is made only with well-established literary data in domestic animals, as consistent citations in wild animals could not be identified. Thus, observations on the Anatomy of the Pectoralis muscle in the Crab-eating Fox reveal the existence of two units or two pectoral muscles: the Superficial Pectoral Muscle and the Deep Pectoral Muscle, similar to the descriptions by Miller et al. (1964) in the domestic dog and Getty *in* Sisson and Grossman (2008) in domestic carnivores.

Superficial Pectoral Muscle - In the Crab-eating Fox, the Superficial Pectoral Muscle is a broad muscle band located on the cranioventral aspect of the thorax, situated between the sternum and the arm. It is smaller and thinner than the Deep Pectoral Muscle, as described by Miller et al. (1964) in the domestic dog, where they affirm that this muscle is the smallest of the two pectorals and is located on the cranioventral aspect of the thorax, between the sternum and the humerus. Furthermore, according to Miller et al. (1964) in the domestic dog, the Superficial Pectoral Muscle runs laterally and distally, penetrating between the Brachial and Brachial Biceps Muscles to insert on the cranial medial aspect of the humerus, in agreement with observations in the Crab-eating Fox.

Miller et al. (1964) also mention that in the Superficial Pectoral Muscle of the domestic dog, three parts are discernible, but they do not describe each part, while Getty in Sisson and Grossman (2008) provides a more accurate description in Equine, Porcine, Ruminant, considering that each part is actually an individual muscle: Descending Superficial Pectoral Muscle; Transverse Superficial Pectoral Muscle, and Ascending Superficial Pectoral Muscle. However, it states that in carnivores, it is more convenient to describe the Superficial Pectoral Muscle as a single muscle divided into different parts. Observations in the Crab-eating Fox are consistent with the citations of Getty in Sisson and Grossman (2008) in domestic carnivores since the three parts in the Crab-eating Fox are not clearly separated, although two parts can be clearly identified: Descending Part and Transverse Part, with no identifiable Ascending Part, as in other domestic animals.

According to Getty in Sisson and Grossman (2008) in Equine, the Descending Pectoral Muscle is short but very thick, exhibiting a rounded aspect, originating from the Manubrium of

the Sternum, coursing caudolaterally to insert into the Deltoid Tuberosity and the Crest of the Greater Tubercle of the Humerus, and due to its large volume, it is superficially convex and flat on the deep surface. Observations in the Crab-eating Fox reveal that the Descending Part of the Superficial Pectoral Muscle is a narrow and thin muscular blade originating from the Manubrium of the Sternum, coursing caudally and laterally over the surface of the Transverse Part, to insert into the cranial medial aspect of the middle part of the Humerus. In Ruminant, the Descending Pectoral Muscle is a thin muscle band located on the surface of the Transverse Pectoral Muscle. Its origin occurs at the midline of the first Sternebra and insertion at the Crest of the Greater Tubercle of the Humerus. Its fibers are arranged obliquely caudolaterally. In Porcine, the same author only mentions that this muscle is thin (GETTY *in* SISSON; GROSSMAN, 2008).

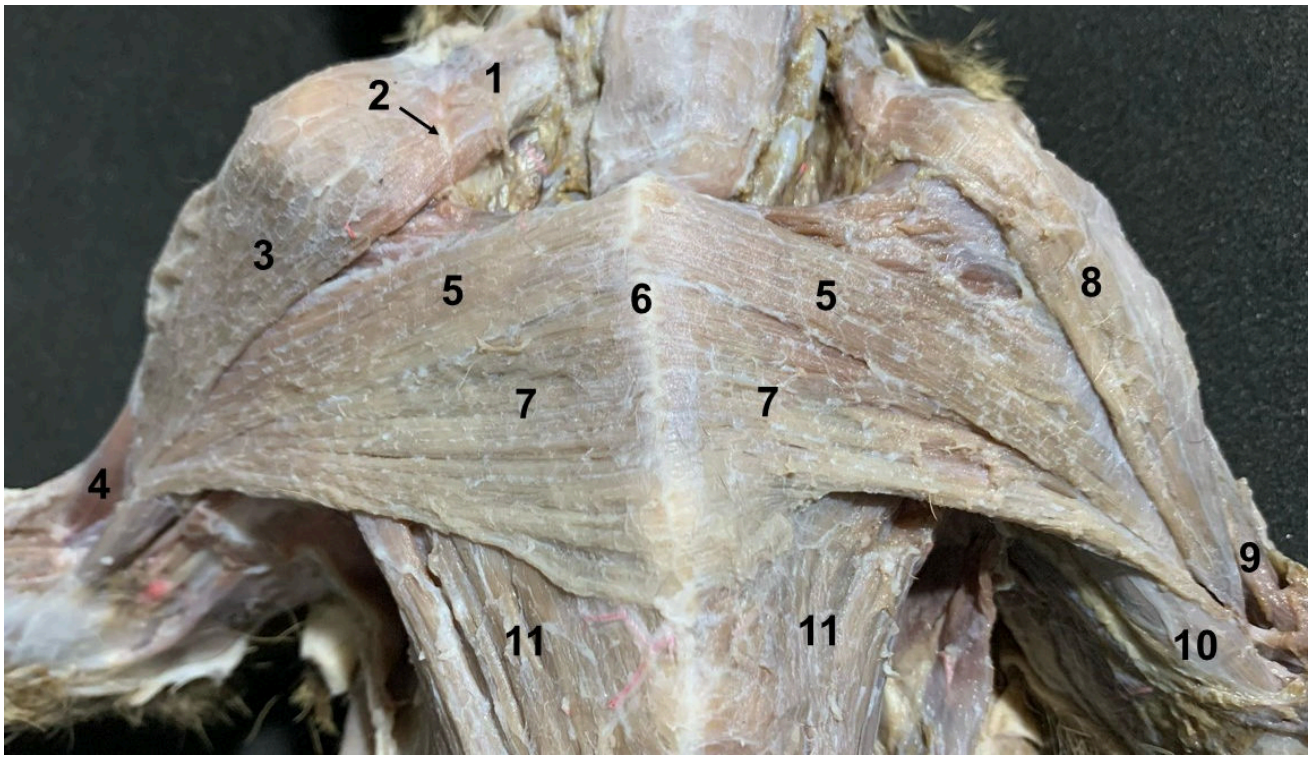

Figure 1. Ventral view of the thoracic region of the Crab-eating Fox.
1- m. cleidocervicalis, 2- tendinous intersection, 3- m. cleidocervicalis right, 4- humerus, 5- descending part of the m. pectoralis superficialis, 6- median raphe of the sternum, 7- transverse part of the m. pectoralis superficialis, 8- m. cleidocervicalis left, 9- m. brachialis, 10- m. biceps brachii, 11- m. pectoralis profundus.

It's possible that the significant differences observed in the volume and position of this muscle between equines and other domestic animals may be related to the strength and endurance functions typical of equines, while the lighter structure seen in the Crab-eating Fox may be adapted for agility and dexterity.

Regarding the Transverse Part of the Superficial Pectoral Muscle, observations in the Crab-eating Fox indicate that it is a wide but thin muscular band originating from the midline of the ventral surface of the first two Sternebrae, the Manubrium of the Sternum, and a long Cranial Process of the Manubrium, in accordance with the descriptions by Getty *in* Sisson and Grossman (2008) in domestic carnivores. However, in Equine, the Transverse Pectoral Muscle is described as a wide and thin muscular band located on the ventral aspect of the thorax, originating from the midline of the Sternum, from the Manubrium to the 6th costoesternal joint, coursing laterally to insert into the cranial aspect of the proximal third of the forearm. In Ruminant, the same author describes the Transverse Pectoral Muscle as thin, originating from the midline of the 2nd - 4th Sternebrae and inserting into the forearm, and in Porcine, the Transverse Pectoral Muscle is divided into two parts (GETTY *in* SISSON; GROSSMAN, 2008).

Regarding the Ascending Part of the Superficial Pectoral Muscle, observations in the Crab-eating Fox show that it is not present, in agreement with the citations by Getty *in* Sisson and Grossman (2008) in domestic carnivores. On the other hand, in Equine and Ruminant, the Ascending Pectoral Muscle is the largest among the pectoral muscles, triangular or fan-shaped, very long and thin near its origin on the abdominal aponeurosis, xiphoid cartilage, and median raphe of the distal part of the sternum, becoming thicker cranially as its fibers converge cranially and laterally to insert into the Greater and Lesser Tubercles of the Humerus, along with the Coracobrachialis Muscle (GETTY *in* SISSON; GROSSMAN, 2008). It's worth noting that in Equine and Ruminants, the pectoral muscles are well divided into individual muscles, as shown by Getty *in* Sisson and Grossman (2008).

In the Crab-eating Fox, it is observed that the Deep Pectoral Muscle is a large muscular blade with an approximately triangular shape, wider at the origin, which occurs along the median raphe of the sternum and the aponeurosis of the Rectus Abdominis Muscle, converging cranially and laterally to insert into the Greater and Lesser Tubercles of the Humerus and the proximal part of their crests, with the cranial part covered by the Superficial Pectoral Muscle, in accordance with the descriptions by Miller et al. (1964) in the domestic dog. According to Getty *in* Sisson and Grossman (2008), in Equine, Ruminant, and Porcine, there is no Deep Pectoral Muscle, as it is functionally replaced by the Ascending Pectoral Muscle, since the author describes each part of the Superficial Pectoral Muscle as an individual muscle. However, in domestic carnivores, the Superficial Pectoral Muscle is described as a single muscle divided into parts, as described by Miller et al. (1964) in the domestic dog and observed in the Crab-eating Fox.

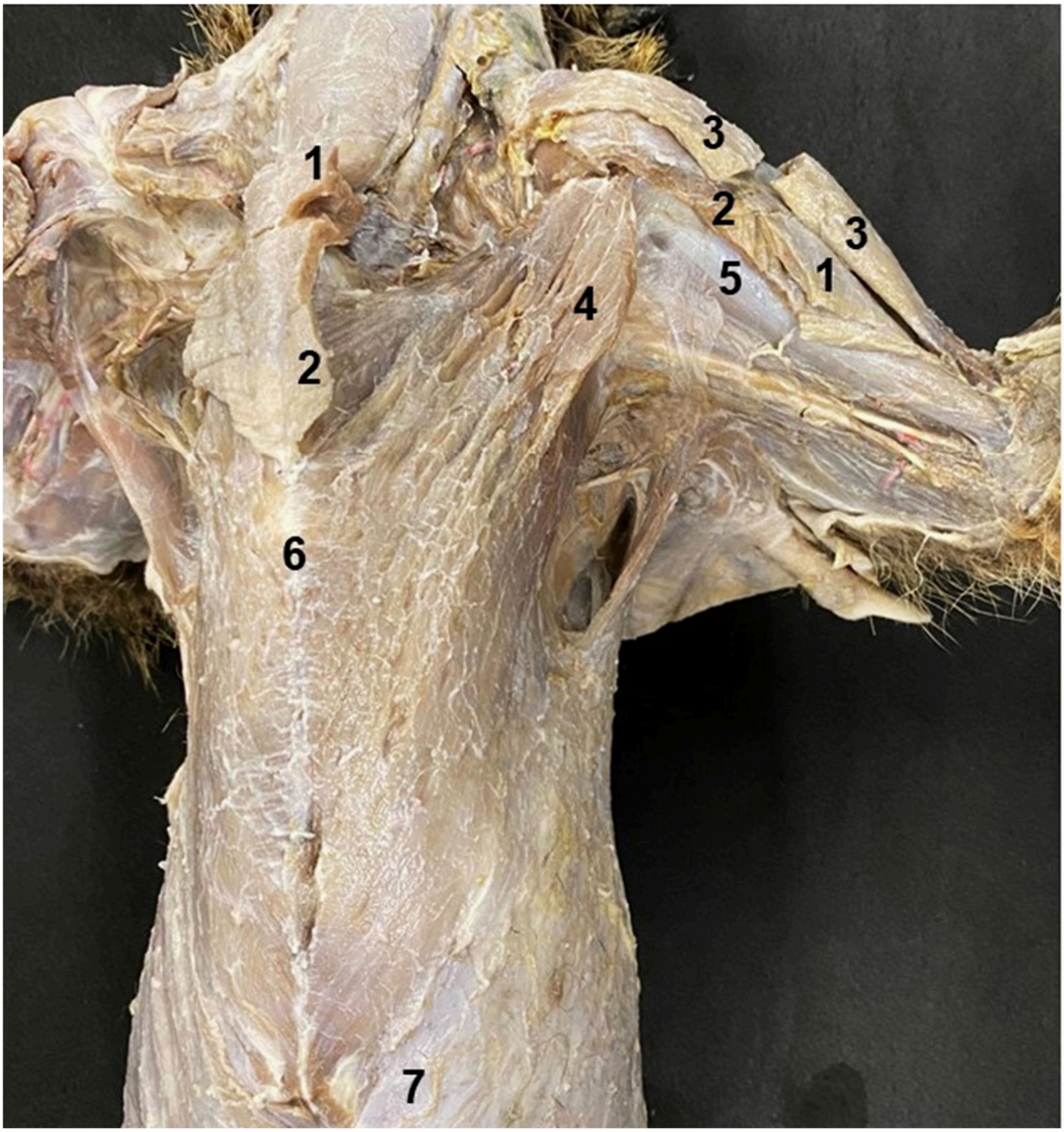

Figure 2. Ventral view of the thoracic region of the left antimere of the Crab-eating Fox.
1- descending part of the m. pectoralis superficialis (sectioned), 2- transverse part of the m. pectoralis superficialis (sectioned), 3- m. cleidocervicalis, 4- m. pectoralis profundus, 5- m. biceps brachii, 6- median raphe of the sternum, 7- aponeurosis of the m. rectus abdominis.

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Stricto Ensu Editora

ANATOMY OF THE LEG MUSCLES OF THE HOARY FOX

(*Lycalopex vetulus* Lund, 1842)

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ABSTRACT

Literary citations in the biology of wild canids are scarce, not presenting the same level of knowledge in Anatomy as other species in the biome. The Hoary Fox (*Lycalopex vetulus* Lund, 1842) is one of the least known canids. It is typical of the cerrado, where most of the faunal components are poorly known, and many are at risk of extinction due to anthropogenic pressure on their habitat. Animal muscle structure is associated with the way of life, where different specializations favor specific types of locomotion and foraging. Comparative Anatomy provides important data inherent to evolution and its adaptations to the numerous modifications introduced in the primary habitat, leading to adaptive requirements associated with feeding and reproduction, functions dependent on the locomotor apparatus. The development of descriptive-comparative anatomical studies contributes to a better understanding of habits and relationships with the environment. Legs constitute important segments for survival, as an essential condition for locomotion activities, searching for food, or in escape situations. This study aims to describe the Anatomy of the leg muscles, comparing the data with literature on domestic carnivores. Two specimens of Hoary Fox, adults, with undefined age, donated by the Wildlife Research Institute - IPEVIS and by the Wildlife Screening Center - CETAS/Catalão, or collected on the margins of highways in Goiás and Minas Gerais (Authorization - SISBIO 37072-2) were used. The procedures for the study took place at the Laboratory of Comparative Anatomy of Wildlife (LACAS-UFCAT). The

results indicate that the muscle structure of the Hoary Fox follows the pattern of domestic carnivores.
Keywords: Anatomy, Muscles and Hoary Fox.

1. INTRODUCTION

Anatomical descriptions of wild animals are scarce, yet describing the Anatomy of wild species contributes to the enrichment of knowledge about these groups, as well as to the understanding of topics related to habitat, development, and conservation and evolutionary programs (LIMA; PEREIRA; PEREIRA, 2010). Literary citations about the biology of neotropical canids are scarce, and the Anatomy of the Hoary Fox does not have the same level of knowledge as that found for other species of the same biome and domestic animals.

The Hoary Fox (*Lycalopex vetulus* Lund, 1842) is one of the seven least known canids in the world (AZEVEDO; LEMOS 2012). It is a typical animal of the Cerrado, and although this is the second largest Brazilian biome, some of its fauna components are at serious risk of extinction or are already on the verge of extinction, as anthropogenic pressure on this biome is increasingly strong, given the characteristics of this biome, favorable for agribusiness and mining exploitation. Among the morphological characteristics of *L. vetulus*, according to the title of the smallest Brazilian canid, the body is slim and small, the limbs are slender, and the tail is long and bushy, characteristics that ensure the adaptation of the animal to move in search of food in its habitat, where the grass vegetation is low (DALPONTE, 1997). The Hoary Fox shows agility and speed, for example, in escape situations, where the animal needs to run zigzag to escape, behavior that requires a lot of skill, as well as a suitable physical build, such as a small and slender body type, reducing body contact with vegetation and facilitating escape (DALPONTE, 2009).

According to Bocchiglieri, Mendonça, and Henriques (2010), the components of the Cerrado fauna can occupy all its geographical patterns, but the Hoary Fox prefers open and high-altitude fields. It is the smallest Brazilian canid and, although considered a carnivore for ingesting small vertebrates, it feeds mainly on insects and even fruits (AZEVEDO; LEMOS, 2012).

Muscular structure is associated with the lifestyle of species; different specializations favor specific types of locomotion in different types of habitats. There are extensive variations in the shape and position of mammalian musculature because a pattern is unviable, but

distinctive characteristics can be identified (BENEDITO, 2017). Anatomical structure expresses the plastic image of the function it performs at a given moment (LOCCHI, 1978), so the structural organization or the shape of anatomical structures can reveal the natural history of a species.

The comparative focus of Anatomy will certainly provide important data inherent to the evolution of species and their adaptations to the numerous modifications introduced in the primary habitat, leading to adaptive requirements, especially regarding feeding and reproduction, which are intimately dependent on the locomotor apparatus. Thus, studying the Anatomy of muscles under the aegis of comparisons is certainly of great value, as body movement or parts thereof intrinsically depend on the muscular system.

The search for food and/or quiet places for reproduction and/or rest requires a perfectly adapted muscular system, especially the parts directly involved in locomotion, which are fundamental in walking, running, or jumping, as Oliveira, Teixeira and Conchalo (2004) advocate, stating that the scope of the locomotor apparatus is based on support and movement, both of which are necessary for the effective function of food apprehension and processing. Thus, a detailed knowledge of the biology of Hoary Fox muscles may provide important collaboration to propose and develop conservation and preservation programs for the Cerrado, its fauna, and flora.

2. MATERIAL AND METHODS

For the present study, two specimens of Hoary Fox, one male and one female, adults, but with undefined age, were used. These specimens were donated by the Wildlife Research Institute - IPEVIS and by the Center for Wild Animal Screening - CETAS Catalão and/or collected along the highways of Goiás and Minas Gerais (Authorization - SISBIO 37072-2) and approved by the CEUA/UFCAT (Ethics Committee on Animal Use of the Federal University of Catalão) under the number 01/22. All experimental protocols were followed in accordance with the recommendations and guidelines of the Brazilian College of Animal Experimentation (COBEA).

The necessary procedures for the study, such as fixation and the use of dissection techniques, took place at the Laboratory of Comparative Anatomy of Wild Animals of the Department of Biological Sciences of the Federal University of Catalão (LACAS-UFCAT). The

carcasses were fixed in a 10% aqueous solution of formalin and preserved submerged in the same medium.

The preparation of the specimens followed usual techniques in Macroscopic Anatomy, using instruments such as anatomical forceps, scalpel, and scissors. The study of leg muscles is developed with emphasis on the shape, location, origin, and insertion of each muscle, and the analysis, description, and documentation are carried out concurrently with the dissection process. Once the anatomical specimens are prepared, the aspects found are discussed comparatively, with emphasis on the identified similarities and differences. Comparison and discussion with domestic canids reference Miller, Christensen, and Evans (1964) "Anatomy of the dog", as this is the most comprehensive work on Anatomy of domestic canids in our environment.

Photographic documentation is carried out using a Samsung Galaxy A32 cell phone camera, and the terminology adopted is based on the Veterinary Anatomical Nomina (International Committee on Veterinary Gross Anatomical Nomenclature, 2017).

3. RESULTS AND DISCUSSION

According to Miller, Christensen, and Evans (1964), in the domestic dog, the leg muscles are located on the cranial, lateral, and caudal surfaces, while the medial surface is essentially free. Observations in the Hoary Fox show results similar to those cited by Miller, Christensen, and Evans (1964). Functionally, the leg muscles in the domestic dog can be classified into the Flexor Group and the Extensor Group, but they are not anatomically separated in the leg as they are in the forearm. On the cranial-lateral aspect of the leg are the Extensors of the Toes and Flexors of the Tarsus muscles, while caudally, there are the Flexors of the Toes and Extensors of the Tarsus muscles. These functional muscle groups are mixed in the leg because the tarsal joint is positioned at an angle opposite to that of the toe joints. The tarsal joint has its flexor surface facing dorsally, while each of the toe joints has an extensor surface facing dorsally; therefore, the muscles supported on the dorsal surface must be Flexors of the Tarsus and Extensors of the Toes. The Hoary Fox, being a canid, has the same limb arrangement; thus, our observations on the legs of the Hoary Fox show characteristics similar to those described by Miller, Christensen, and Evans (1964) in the domestic dog, with the mentioned muscle groups not physically separated but located in

the cranio-latero-posterior region of the leg, as in the domestic dog (MILLER; CHRISTENSEN; EVANS, 1964).

Although the arrangement of leg muscles in the Hoary Fox is similar to that described in the domestic dog, for the purposes of description in this article, a Cranio-lateral Group and a Posterior Group are considered.

Cranio-lateral Muscles of the Leg - The flexors of the Tarsus, which are located on the cranio-lateral side of the leg, are: Cranial Tibial muscle, Long Fibular muscle, Long Extensor of the Toes, Lateral Extensor of the toes, and Long Extensor of the Hallux (MILLER; CHRISTENSEN; EVANS, 1964).

The Cranial Tibial muscle, in the domestic dog, is a large, relatively flat muscle that is superficially located on the cranial surface of the leg. It originates via two short tendons at the medial part of the knee joint margin, as well as at the proximal lateral edge of the tibial crest. Near its origin, the muscle is wider, converging distally into a flat, thin tendon that extends obliquely over the tarsus to insert at the base of the first and second metatarsals (MILLER; CHRISTENSEN; EVANS, 1964). Observations in the Hoary Fox reveal that the Cranial Tibial muscle presents origin, location, and insertion similar to the citations by Miller, Christensen, and Evans (1964) in the domestic dog.

In the Hoary Fox, the Long Digital Extensor muscle is relatively short and fusiform. It is located on the cranio-lateral aspect of the leg, between the Cranial Tibial muscle and the Long Fibular muscle, partially covered by these muscles, in accordance with the descriptions by Miller, Christensen, and Evans (1964), who state that, in the domestic dog, the muscle is fusiform and located between the Cranial Tibial muscle and the Long Fibular muscle, being covered by the Long Fibular muscle proximally and free distally. Like in the Hoary Fox, it originates at the lateral epicondyle of the femur.

The Long Fibular muscle, in the domestic dog, as well as in the Hoary Fox, is the most superficial among the muscles that make up the Fibular Group. It has a relatively short, fusiform belly and a long insertion tendon. Located on the proximal half of the lateral surface of the leg, between the Cranial Tibial muscle and the Long Extensor of the Hallux. It originates at the lateral condyle of the Tibia, the fibular collateral ligament, and the proximal end of the Fibula, inserting into the first, second, and fourth metatarsals (MILLER; CHRISTENSEN; EVANS, 1964).

The Long Extensor of the Hallux muscle, in the domestic dog, is a relatively long, fusiform muscle band, partially covered proximally by the Gastrocnemius muscle and the Long Fibular muscle, as observed in the Hoary Fox as well. It originates from the cranial part

of the fibula, between the proximal third and middle third. The muscle extends obliquely, ending through a thin tendon in the middle part of the first metatarsal (MILLER; CHRISTENSEN; EVANS, 1964). A similar condition is present in the Hoary Fox.

The Short Fibular muscle, in the Hoary Fox, is relatively small, located on the lateral surface of the distal third of the fibula, deep to the tendon of the Long Fibular muscle, following distally and deeply to it until inserting into the first, second, and fourth metatarsals. These observations in the Hoary Fox are similar to the citations by Miller, Christensen, and Evans (1964).

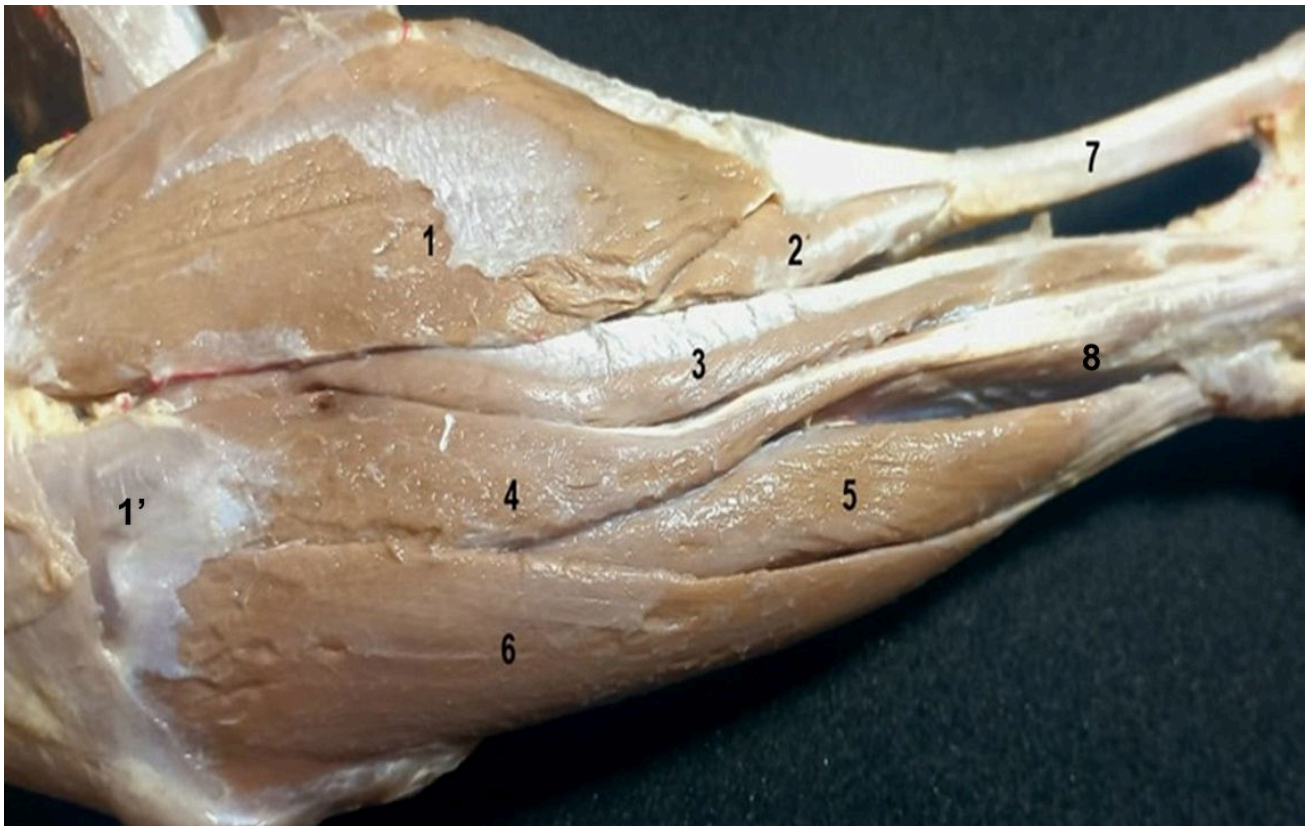

Figure 1. Lateral view of the left leg of the Hoary Fox.
1- Lateral head of the Gastrocnemius muscle; 1'- Popliteus muscle; 2- Superficial Digital Flexor muscle; 3- Long Extensor muscle of the Hallux; 4- Fibularis Longus muscle; 5- Long Digital Extensor muscle; 6- Cranial Tibial muscle; 7- Calcaneal Tendon; 8- Fibularis Brevis muscle.

In the Hoary Fox, the Superficial Digital Flexor muscle is a large muscle interposed between the two heads of the Gastrocnemius muscle, which almost completely covers it in the posterior view, leaving only a small part visible on the posterolateral aspect of the leg, located cranially in the distal part of the Gastrocnemius, situated caudally to the distal end of the Gastrocnemius, almost completely covered by it. Its origin occurs together with the Gastrocnemius muscle, and the insertion tendon runs together with the calcaneal tendon,

enveloped by the aponeurosis of the Biceps Femoris muscle, until inserting into the second, third, fourth, and fifth metatarsals. These observations are consistent with citations in domestic dogs, where only a small distal part appears caudally, in the distal part of the Gastrocnemius. It originates together with the Gastrocnemius on the popliteal surface of the Femur (MILLER; CHRISTENSEN; EVANS, 1964).

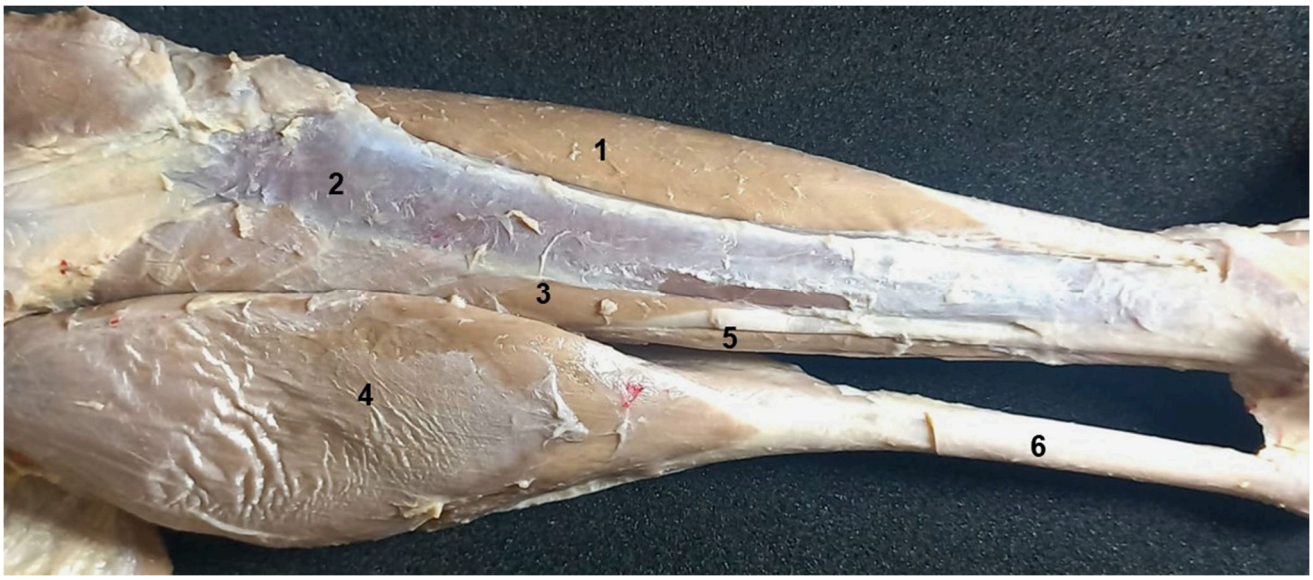

Figure 2. Medial view of the leg of the Hoary Fox.
1- Cranial Tibial muscle; 2- Tibia; 3- Long Digital Flexor muscle; 4- Gastrocnemius muscle; 5- Long Flexor of the Hallux muscle; 6- Calcaneal Tendon.

In the domestic dog, on the caudomedial side of the leg, the following muscles are visible: the Gastrocnemius muscle, the Superficial Digital Flexor muscle, the Long Flexor of the Hallux muscle, the Deep Digital Flexor muscle, and the Popliteal muscle (MILLER; CHRISTENSEN; EVANS, 1964).

In the Hoary Fox, the Gastrocnemius muscle is the largest representative of the leg muscles. It is located superficially on the caudal aspect of the leg and extensively envelops the Superficial Digital Flexor muscle, with which it is closely related. It partially covers the Deep Digital Flexor muscles, Long Flexor of the Hallux, and the Popliteal muscle. These observations are consistent with the citations in the domestic dog by Miller, Christensen, and Evans (1964), who mention that the Gastrocnemius muscle has two heads: the Lateral Head and the Medial Head. The Lateral Head originates, through a large tendon, from the lateral plantar tuberosity of the Femur, while the Medial Head originates from the Medial Plantar Tuberosity. The two heads almost completely envelop the Superficial Digital Flexor muscle, and they merge distally, forming a voluminous flattened muscle that occupies the caudal

aspect of the leg. All these characteristics related to the Gastrocnemius muscle are present in the Hoary Fox.

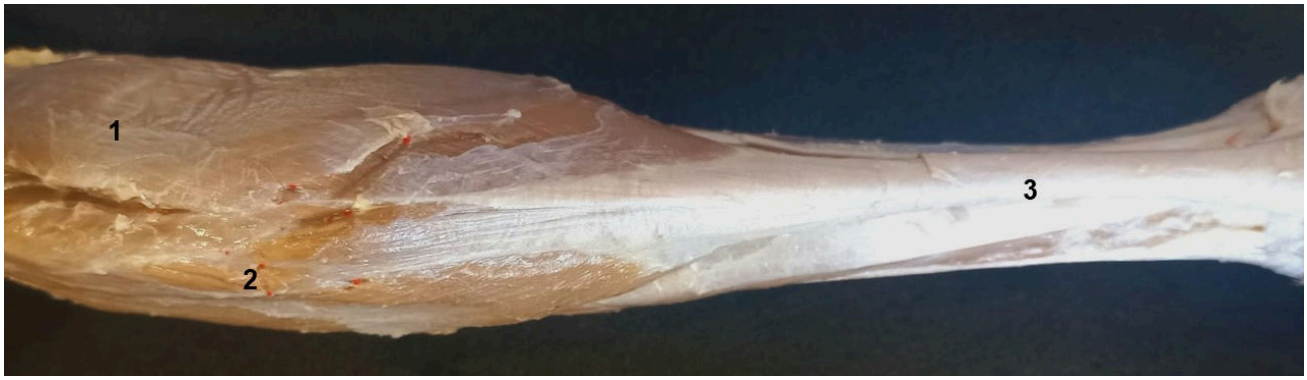

Figure 3. Caudal view of the leg of the Hoary Fox.
1- Lateral head of the Gastrocnemius muscle; 2- Medial head of the Gastrocnemius muscle; 3- Calcaneal Tendon.

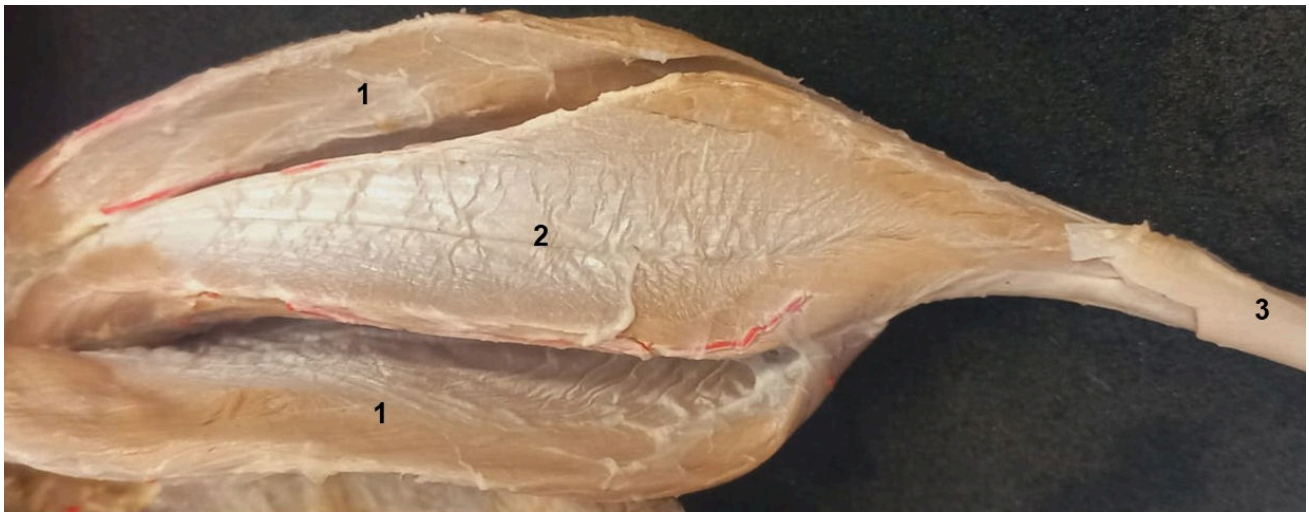

Figure 4. Cranial view of the caudal leg muscles of the Hoary Fox.
1- Gastrocnemius muscle; 2- Superficial Digital Flexor muscle; 3- Calcaneal Tendon.

The calcaneal tendon results from the fusion of the tendons of the Gastrocnemius muscle and the Superficial Digital Flexor muscle, both enveloped by the aponeurosis of the Biceps Femoris muscle, and inserts into the calcaneal tuberosity.

According to Miller, Christensen, and Evans (1964), in the domestic dog, the Popliteal muscle is strong and triangular, located in the popliteal space, partially covered by the Gastrocnemius and Superficial Digital Flexor muscles. It originates from the plantar surface of the lateral condyle of the Femur and inserts into the popliteal line, located on the medial border of the Tibia. Observations in the Hoary Fox show that the Popliteal muscle is wide but thin, exhibiting an irregular quadrilateral shape, yet the origin, insertion, and location are consistent with the citations by Miller, Christensen, and Evans (1964) in the domestic dog.

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Stricto Sensu Editora

MACROSCOPIC ANATOMY OF THE TELENCEPHALON OF A CRAB-EATING FOX (*Cerdocyon thous* Linnaeus, 1758)

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ABSTRACT

Anatomy is the science that studies the morphofunctional organization of bodies, establishing approaches upon which different lines of study and research can be created, including Comparative Animal Anatomy. The aim of this research is to study the macroscopic anatomical organization of the Telencephalon of the Crab-eating fox, a neotropical canid, a component of the Brazilian cerrado fauna. In this study, two adult male specimens were dissected and analyzed, collected dead on the margins of highways in Southeast Goiás. (SISBIO 37072/2). The data obtained are described and compared with those from the well-established literature of the domestic dog. The Telencephalon of the Crab-eating fox is relatively large compared to the animal's physical size, divided by the Longitudinal Fissure into two Hemispheres. The surface of each hemisphere is marked by sulci and gyri, similar to what is described in the Dog; however, the lesser development of the Cortex determines sulci that are more straight and less branched, as well as gyri that are smoother and less tortuous, than those described in the Dog. Although fewer in number, sulci and gyri described in the Dog are present in the Crab-eating fox, occupying similar locations and positions: Rhinal Sulcus; Sylvian Sulcus; Ectosylvian Sulcus; Suprasylvian Sulcus; Sylvian Gyrus, Suprasylvian Gyrus; Ectosylvian Gyrus; Piriform Area, among others. In all macroscopic morphological aspects, the Telencephalon of the Crab-eating fox exhibits less complex forms than those of the domestic Dog, suggesting a more developed Telencephalon in the Dog compared to the Crab-eating fox.

Keywords: Anatomy, Neural System and Crab-eating fox.

1. INTRODUCTION

Anatomy is a unified science, yet there are different approaches under which a line of study or research can be established. Among them, Animal Anatomy studies the way anatomical structures are organized to form the bodies of animals, classified taxonomically. One of the fundamental objectives of Comparative Anatomy is to compare and relate the form and function of anatomical structures to those already described in other species, which is the scope of this proposal in the Crab-eating fox.

The Crab-eating fox (*Cerdocyon thous*) is a little-studied neotropical canid that inhabits the Brazilian cerrado. Despite being a canid, it presents anatomical differences compared to the Domestic dog, where Anatomy is well established.

On the other hand, the Cerrado, the second largest Brazilian biome, occupying an approximate area of two million km², has a highly diversified geographical formation that includes open fields, high-altitude fields, closed woodland (cerradão), open woodland (cerrado campestre), gallery forest, and rocky outcrop savanna (cerrado rupestre). This geographical environment harbors a high biological diversity, including species native to the region and endemic species, in correlation with the physiognomic heterogeneity.

The cerrado fauna comprises 194 mammal species belonging to 30 families and nine orders, including the Carnivora (MARINHO FILHO; RODRIGUES; JUAREZ, 2002). The specimens proposed for analysis in this research belong to the order Carnivora, family Canidae, and species *Cerdocyon thous* (Crab-eating fox).

The telencephalon is the highest part of the brain, considered by some authors as the "summit" of evolution, the site of occurrence of functions referred to as "higher functions" or "superior functions" of the neural system. The irregular surface of the telencephalon is strongly divided by fissures and sulci that delimit areas called Lobes and Gyri. Some of the fissures are deeper and wider, earning them the designation of Fissures, while others are shallow and only referred to as sulci. In humans and many other animals, the Longitudinal Median Fissure, the Lateral Fissure, and some more evident and uniform sulci divide the Telencephalon into right and left cerebral hemispheres, and these into Frontal Lobe, Parietal Lobe, Temporal Lobe, Occipital Lobe, and Insular Lobe.

Studying the Anatomy of the Neural System of species that make up the cerrado fauna seems to us to be of great value, firstly because many of these species are at risk of extinction or on the verge of entering this risk, secondly because the Neural System undoubtedly represents one of the most important aspects of evolution, and thirdly because studies of this

nature in these taxonomic groups are not found in the specialized literature. The integrity or restoration of the integrity of biodiversity and biomes in general depends on knowledge of the biology of their components. Knowing the biology of the members of a biome is fundamental when the interest is in organizing and implementing preservation and protection programs.

The aim of this research is to dissect and describe the Macroscopic Anatomy of the Telencephalon of the Crab-eating fox, enumerating and characterizing the sulci and gyri of the dorsolateral, inferior, and medial surfaces of the hemispheres, confronting the results with the relevant literature on other canids.

2. MATERIAL AND METHODS

This is a descriptive research where the Telencephalon is dissected and its component structures are analyzed and described in as much detail as possible given the available material. The results are compared with relevant descriptions in the Domestic Dog literature, as it is relatively well-established in species of similar groups.

Statistical data are not relevant since this involves descriptive results on a small number of specimens, also because they are animals at risk of extinction or on the verge of entering this risk. Data will be collected from the dissection of three specimens of the Crab-eating fox, which are collected dead due to roadkill on the margins of highways in Goiás and/or Minas Gerais (Authorization SISBIO no. 37072/2).

The experimental procedures are carried out in the Laboratory of Comparative Anatomy of Wild Animals at the Federal University of Catalão (LACAS-UFCAT). The animals are fixed in a 10% aqueous solution of formalin and preserved in it.

The preparation of anatomical specimens is done by macroscopic dissection of the cranial vault and posterior neck region until complete exposure of the cranial vault and vertebral column. With a plaster saw, cuts are made in the cranial vault bones in the median sagittal plane and para-median three centimeters from the first, on each antimer. The careful removal of the bones is done using osteotomes, chisels, gouges, and hammer until total exposure of the telencephalon wrapped in meninges. In the vertebral column, paramedian cuts are made, and the vertebral arch is removed, along with the spinous process, until exposure of the cervical spinal cord. The removal of the study material is done by detachment of the dura mater and sectioning of the optic nerves and olfactory tract, which are not preserved because they were not spared in the process that led the animal to death. Once

outside the carcass, the study material is carefully cleaned, removing the meninges with a watchmaker's forceps and under a 10X magnifying glass. The sulci and gyri, the main objects of analysis, are preserved, described, and photographed with a 7.2-megapixel Sony Cyber Shot camera.

The nomenclature adopted is based on the Veterinary Anatomical Nomenclature (International Committee on Veterinary Gross Anatomical Nomenclature, 2017).

This study was developed with the approval of the Ethics Committee of the Institution (CEUA/UFCAT nº. 01/22). The experimental protocols were carried out following the recommendations of the Brazilian College of Animal Experimentation (COBEA) guidelines.

3. RESULTS

The telencephalon of the Crab-eating fox is relatively small compared to the size of the animal. It represents the most developed part of the brain and, when viewed as a whole, it exhibits a latero-lateral compressed aspect, giving it an elongated piriform outline. The widest part of the telencephalon is oriented caudally, while the narrowest part is strongly compressed and tapered rostrally. It is composed of two parts known as Cerebral Hemispheres, which are separated by a deep longitudinal fissure.

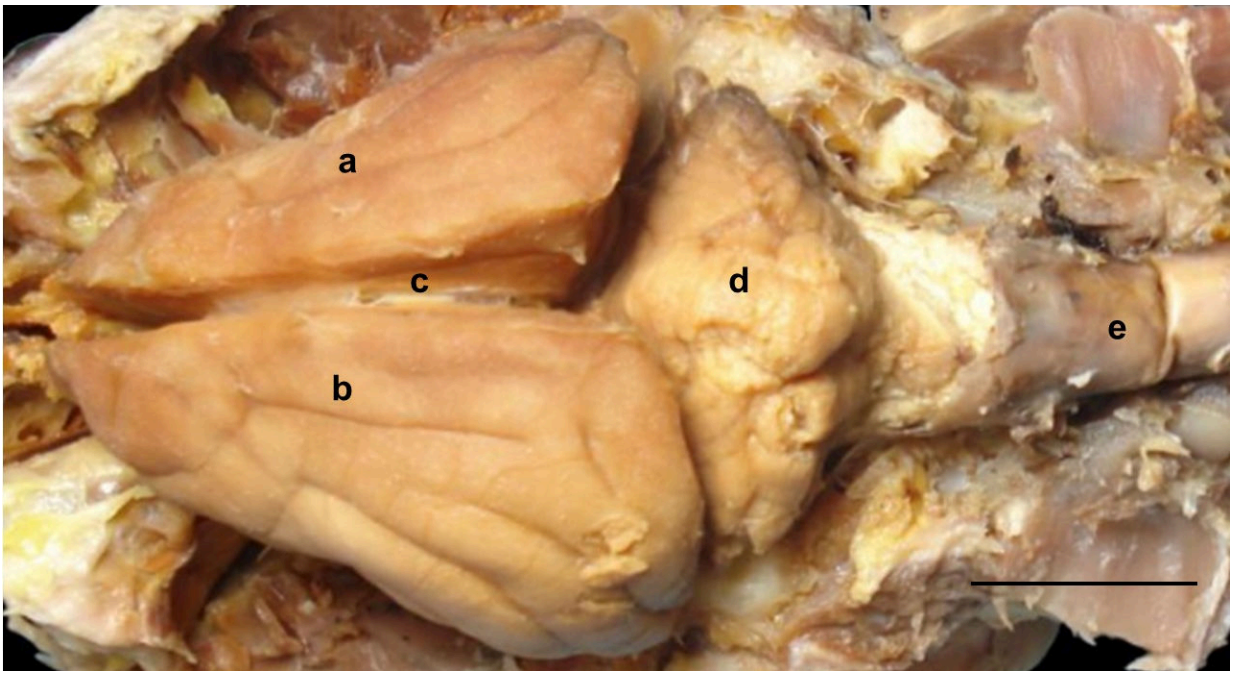

Figure 1. Dorsal view of the Telencephalon of Crab-eating fox.
a - right cerebral hemisphere, b - left cerebral hemisphere, c - longitudinal fissure, d - cerebellum, e - spinal cord. — 20%: 11.00 cm.

Sulci of the Dorsolateral Face - The Dorsolateral Face is the largest of the three and the most clearly visible, with its surface marked by several sulci and gyri, some of which are more distinct while others are somewhat obscured. Although some sulci are deeper and wider, and relatively consistent, such that they can be termed fissures, it is not possible to identify topographic areas that are identified as Lobes, as most sulci and gyri extend throughout or nearly the entire hemisphere.

On this face, a deep and long sulcus extends throughout the hemisphere, known as the Rhinal Sulcus. The Rhinal sulcus is one of the most prominent sulci on the dorsolateral face of the cerebral hemisphere of the Crab-eating fox. It is located in the ventrolateral part of the hemisphere, between the neocortex above, which includes most of the sulci and gyri of the telencephalon, and the olfactory area below, which includes the Olfactory Tract and the Piriform Area. At the junction of the olfactory tract with the piriform area, the Rhinal fissure divides into two branches: an ascending branch that extends caudally and superiorly, which is called the Sylvian Sulcus, and a descending branch that extends posteriorly and inferiorly between the piriform area and the parietal neocortex, called the Posterior Rhinal Sulcus. At the middle part of the Rhinal sulcus, a well-marked sulcus arises, directed cranially-superiorly towards the longitudinal fissure, known as the Pre-Sylvian Sulcus, which, near its cranially-superior end, branches into a cranial and a caudal branch, both positioned parallel to the Longitudinal Fissure, at the same level and in opposite directions (cranio-caudally), constituting a single sulcus called Proreus Sulcus. Encircling the end of the Sylvian sulcus is an inverted U-shaped sulcus, called the Ectosylvian Sulcus. Slightly above the ectosylvian sulcus, exhibiting the same shape but wider, is a well-marked sulcus, the Supra-Sylvian Sulcus. These two U-shaped sulci can be divided into anterior and posterior as described in the Domestic Dog (Anterior Ectosylvian Sulcus, Posterior Ectosylvian Sulcus, Anterior Suprasylvian Sulcus, and Posterior Suprasylvian Sulcus). Postero-superiorly to the Posterior Supra-Sylvian sulcus, there is a sulcus that follows the curvature of it, called the Ectolateral Sulcus. Above the Suprasylvian Sulcus, halfway between it and the Longitudinal Fissure, there is a sulcus that extends almost the entire length of the hemisphere, called the Lateral Sulcus.

Gyri of the Dorsolateral Face - The dorsolateral face of the cerebral hemisphere of the Crab-eating fox presents several gyri or convolutions, some of which extend throughout the entire length of the hemisphere, while others are confined to parts of it. Near the rostral end, in front of the Presylvian Sulcus, there is a large gyrus called the Proreus Gyrus. In the

middle and caudal parts of the hemisphere, there are several gyri arranged relatively orderly in the rostro-caudal direction along the convex surface of the hemisphere.

Encircling the Sylvian Fissure, between it and the Ectosylvian Sulcus, there is a small U-shaped gyrus called the Sylvian Gyrus, which can be divided into two parts. The anterior part is the Anterior Sylvian Gyrus and the posterior part is the Posterior Sylvian Gyrus. Encircling the Ectosylvian Sulcus, between it and the Suprasylvian Sulcus, there is a continuous U-shaped gyrus called the Ectosylvian Gyrus. Above the Suprasylvian Sulcus, extending almost the entire length of the hemisphere, there is a large gyrus called the Supra-Sylvian Gyrus, which is posteriorly subdivided by the Ectolateral Sulcus into two branches called the Anterior Ectolateral Gyrus and the Posterior Ectolateral Gyrus. The undivided rostral part of this gyrus is called the Coronal Gyrus. Occupying almost the entire extent of the hemisphere, bounded by the Longitudinal Fissure above and the Lateral Sulcus below, there is the large Lateral Gyrus.

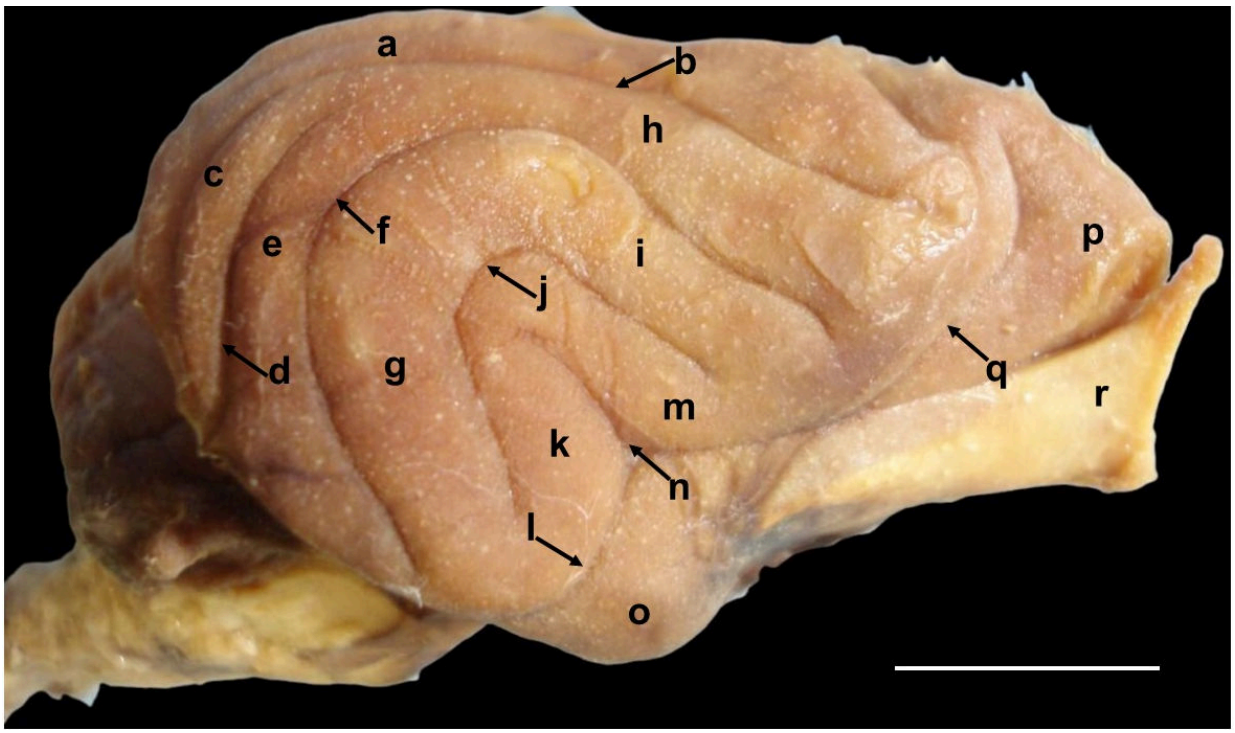

Figure 2. Dorsolateral view of the right hemisphere of the Telencephalon of Crab-eating fox. a- endolateral gyrus, b- lateral sulcus, c- ectolateral gyrus, d- ectolateral sulcus, e- posterior suprasylvian gyrus, f- suprasylvian sulcus, g- ectosylvian gyrus, h- coronal gyrus, i- posterior Rhinal gyrus, j- ectosylvian sulcus, k- posterior silvian gyrus, l- posterior Rhinal sulcus, m- anterior silvian gyrus, n- silvian sulcus, o- piriform area, p- proreus gyrus, q- anterior Rhinal sulcus, r- olfactory tract. — 20%, 11.00 cm.

Sulci and Gyri of the Inferior Face - The inferior face of the cerebral hemisphere of the Crab-eating fox is relatively irregular, but it does not present any well-defined sulcus or gyrus like the dorsolateral face. The most developed telencephalic structure on the inferior

face is the Piriform Area, which is a large pear-shaped cortical area, with its narrow part facing caudally, continuing with the Parahippocampal Gyrus. The broader part of the Piriform Area is oriented rostrally. It receives the Olfactory Tract. Given its appearance, the piriform area might be better termed if considered as a large gyrus of the Telencephalon that is separated from the caudal gyri of the dorsolateral face by the Posterior Rhinal Sulcus. Other structures visible on the inferior face, such as the optic chiasm, tuber cinereum, mammillary body, and cerebral peduncles, do not belong to the telencephalon but rather to other segments of the brain.

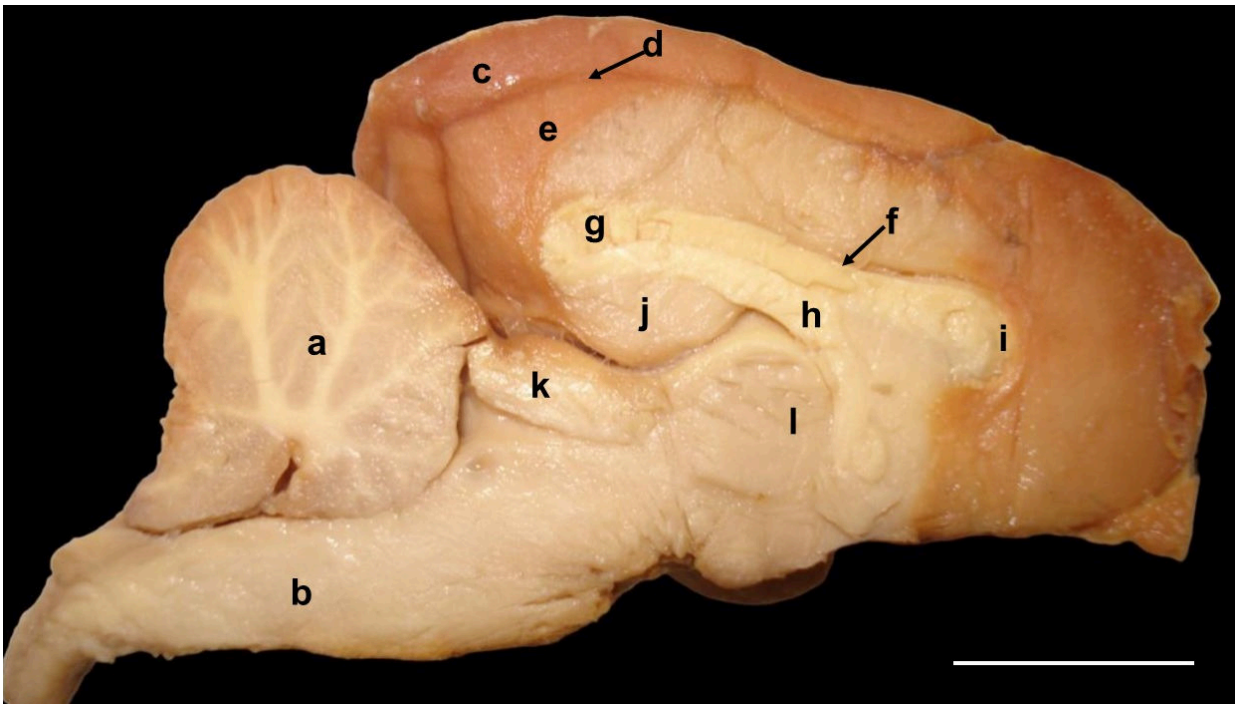

Figure 3. Medial view of the left hemisphere of the Telencephalon of Crab-eating fox.
a- cerebellum, b- brainstem, c- supraesplenial gyrus, d- cingulate sulcus, e- cingulate gyrus, f- corpus callosum sulcus, g- splenium of corpus callosum, h- fornix, i- splenium of corpus callosum, j- parahippocampal gyrus, k- quadrigeminal bodies, l- thalamus. — 20%: 11.00 cm

The cortical area located posterolateral to the cerebral peduncles and in contact with the cranial surface of the cerebellum clearly exhibits the caudal end of the Posterior Rhinal Sulcus, the caudal end of the Cingulate Sulcus, and the Hippocampal Sulcus. The Cingulate Gyrus extends over this area, between the Posterior Rhinal and Cingulate Sulci, as the Parahippocampal Gyrus, until it meets and joins the caudal end of the Piriform Area. The caudal end of the Marginal Gyrus advances over this face until it meets the Posterior Suprasilvian Gyrus.

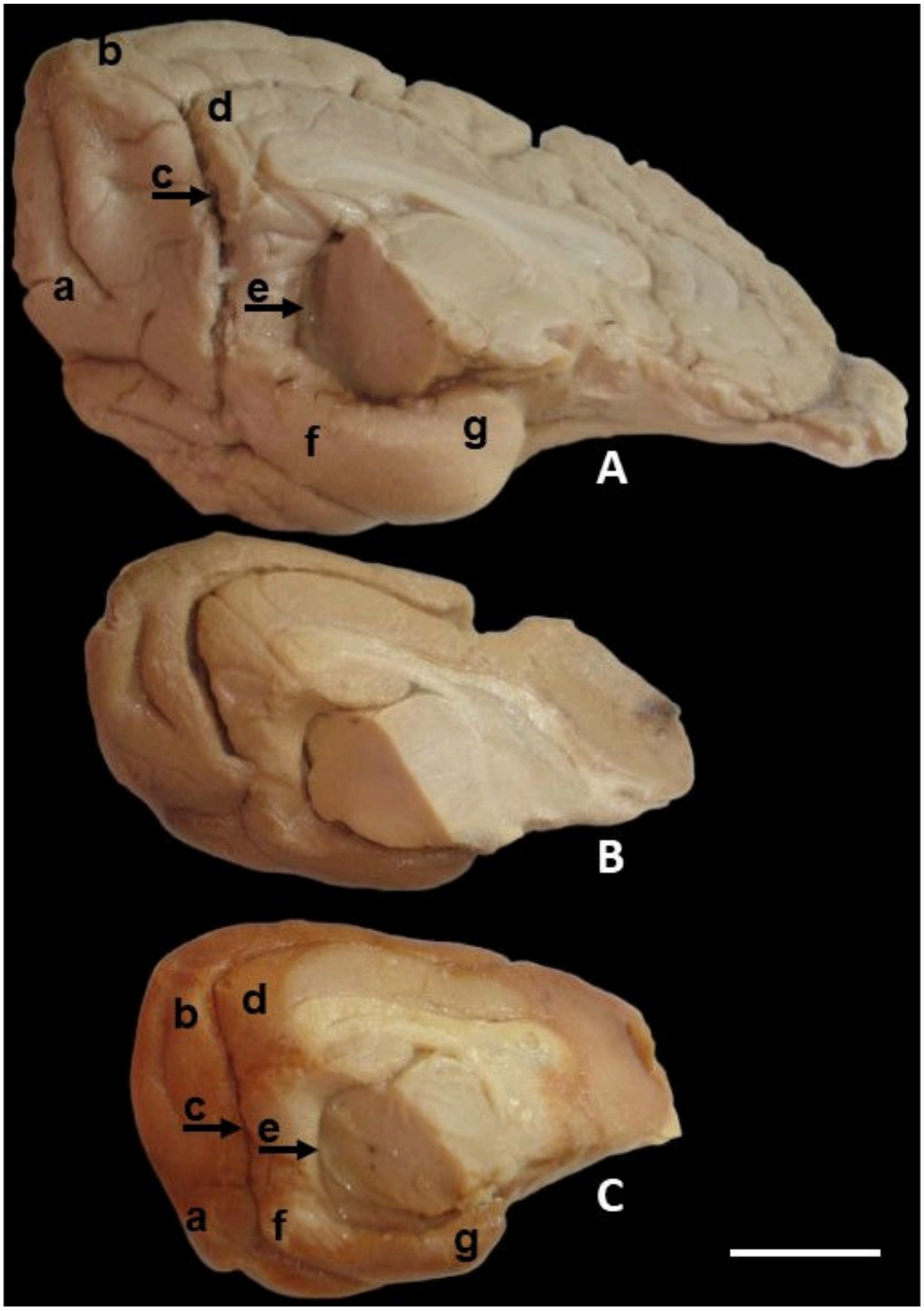

Figure 4. Caudomedial view of the Telencephalon of.
A- Domestic Dog, B- Crab-eating fox (specimen 1), C- Crab-eating fox (specimen 2): a- Postero-lateral gyrus, b- Supraesplenial gyrus, c- Cingulate sulcus, d- Cingulate gyrus, e- Hippocampal sulcus, f- Parahippocampal gyrus, g- Piriform area. — 20%: 11.00 cm.

Sulci and Gyri of the Medial Face - The medial face of the Crab-eating fox Telencephalon is relatively smooth, with few irregularities identifiable. Two sulci are more easily identifiable. The Corpus Callosum Sulcus follows the superior surface of the corpus callosum along its entire length. The Cingulate Sulcus is positioned far from the Corpus Callosum Sulcus, near the upper edge of the hemisphere. Other sulci are very superficial, appearing only as slight grooves, mainly near the rostral end. Most of the medial face of the hemisphere is occupied by the Cingulate Gyrus, which is much larger compared to the domestic dog. The rostral part of the medial face is almost smooth, with only slight sulcus visible. The Corpus Callosum of the Crab-eating fox is relatively small, but its three parts can be identified: the Knee, Body, and Splenium. The Fornix and Septum Pellucidum are well-developed. There seems to be no separation between the right and left thalami.

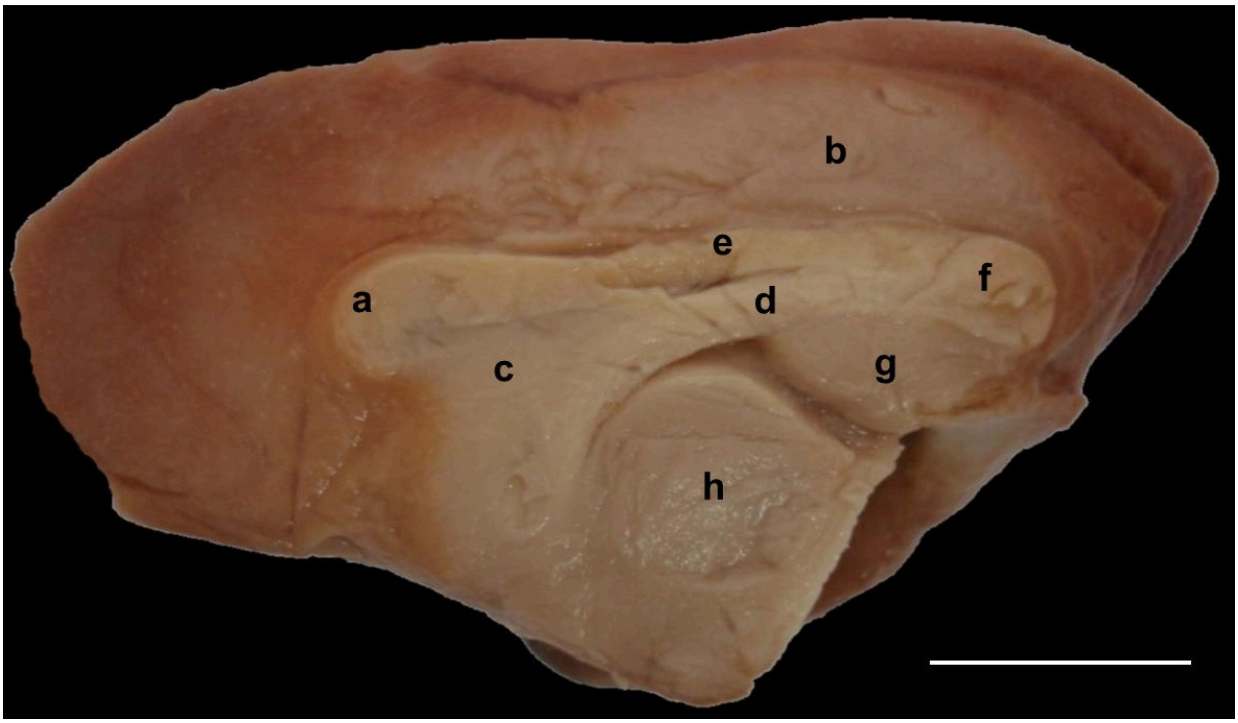

Figure 5. Medial view of the right hemisphere of the Telencephalon of Crab-eating fox.
a- Knee of corpus callosum, b- Cingulate gyrus, c- Septum pellucidum, d- Fornix, e- Body of corpus callosum, f- Splenium of corpus callosum, g- Parahippocampal gyrus, h- Thalamus. — 20%: 11.00 cm.

4. DISCUSSION

The results of this research are compared with the quotations compiled from Miller, Christensen, and Evans (1964) and Getty in Sisson and Grossman (2008), two classics of animal anatomy. No reference is made to other animals because they represent different

groups of canids and therefore any differences and/or similarities present do not find solid grounds for discussion.

According to Miller, Christensen, and Evans (1964), the Telencephalon of *Canis familiaris* (Domestic Dog, referred to hereafter as Dog) is formed by two hemispheres separated at the midline by the Longitudinal Fissure. Observations in the Crab-eating fox (*Cerdocyon thous*) reveal the same configuration of the telencephalon, but the fissure is less deep in this group, since the thickness of the cortex is noticeably smaller.

Getty in Sisson and Grossman (2008) mentions that a striking feature of the cerebral hemispheres of the dog is its strongly compressed extremity or rostral pole, configuring an almost triangular aspect in dorsal vision with the thinner part facing rostrally, and the caudal pole being quite rounded. The specimens of Crab-eating fox analyzed in this research present cerebral hemispheres with configurations described in Dogs, however, exhibiting an even more tapered rostral pole and less convex middle and caudal parts, with the caudal end being beveled, characterizing a very thin edge instead of rounded, which gives the hemisphere a pear-like shape. Perhaps this characteristic is related to the lower development of the cortex in this species.

Miller, Christensen, and Evans (1964) state that the Telencephalon of the dog is subdivided into regions called lobes: Frontal, Parietal, Occipital, and Temporal, although their boundaries are not clear. In the Crab-eating fox, there is no evidence delimiting telencephalic lobes. The gyri located caudally to the Sylvian Sulcus curve rostrally and continue with the Piriform Area, characterizing a region resembling the Temporal lobe of other groups, but no mark is seen to suggest division between the Frontal, Parietal, and Occipital lobes. Therefore, in this research, they will be treated as Frontal, Parietal, and Temporal Regions.

According to Miller, Christensen, and Evans (1964) and Getty in Sisson and Grossman (2008), more than in other species, the sulci and gyri of the Dog's Telencephalon are arranged concentrically around the Sylvian Fissure, however, in the Crab-eating fox, there is an arrangement of sulci and gyri as concentric around the Longitudinal Fissure as it is in the descriptions in dogs, even though the cortex of this canid is less convoluted than that of the dog. The gyri of the cortex of the Crab-eating fox are less circumvolute, and therefore the sulci are less deep and less branched.

For Miller, Christensen, and Evans (1964), the configuration of the telencephalon sulci and gyri is similar within a species, but variations occur between different species and between the hemispheres of a specimen. These citations are confirmed in the Crab-eating fox, among different specimens, as observed in the cingulate sulcus.

Each cerebral hemisphere of the Crab-eating fox has a very narrow and elongated cavity, along the lower surface of the corpus callosum, are the Lateral Ventricles, however, there is no Third Ventricle, clearly delimited as described in Dogs by Miller, Christensen, and Evans (1964).

Still according to Miller, Christensen, and Evans (1964) and Getty in Sisson and Grossman (2008), the sulci and gyri that mark the surface of the Dog's Telencephalon are practically the same, but in the Crab-eating fox, although most of the gyri and sulci are present, they are less demarcated, because the sulci are less deep and less branched, and the gyri less convoluted.

The Rhinal Sulcus is one of the most evident and well-marked sulci of the Dog's Telencephalon (MILLER; CHRISTENSEN; EVANS, 1964 and GETTY in SISSON; GROSSMAN, 2008). In the Crab-eating fox, the observations are in line with the statements of the aforementioned Authors, who also mention that the Rhinal Sulcus is located on the ventrolateral aspect of the cerebral hemisphere, along its entire length, separating the most recent part of the cortex (neopalio or neocortex), located above, from the olfactory cortex (Paleopalio or paleocortex) inferiorly. The rhinal sulcus of the Crab-eating fox is also arranged.

The Rhinal Sulcus in Dogs exhibits, in its middle part, a branching. The upper branch is short, runs posteriorosuperiorly and is called the Fissure or Sylvian Sulcus. The lower branch, on the other hand, is longer, flexes strongly in the caudo-ventral direction and is called the Posterior Rhinal Sulcus, while the part of the same sulci, before its division, is called the Anterior Rhinal Sulcus (MILLER; CHRISTENSEN; EVANS, 1964 and GETTY in SISSON; GROSSMAN, 2008). The observations made in the Crab-eating fox reveal a similar configuration of the cerebral hemisphere.

At the height of the middle part of the Anterior Rhinal Sulcus, a large groove originates, the Pre-Sylvian Sulcus, which is oriented rostrally and superiorly in the direction of the longitudinal fissure. Its rostral end may join an inconstant groove parallel to the longitudinal fissure called the Proreus Sulcus, Miller, Christensen, and Evans (1964) and Getty in Sisson and Grossman (2008). The observations in the Crab-eating fox are in agreement with the aforementioned citation, regarding the Pre-Sylvian and Proreus sulcus, however, the Proreus Sulcus could be described as rostral and caudal branches of the Sylvian sulcus.

According to Miller, Christensen, and Evans (1964) in Dogs, the Sylvian fissure or sulcus is identical to that described by Sisson and Grossman (1953) when they state that it is relatively short and surrounded by three concentric semicircular sulci. The three sulci that are

arranged around the fissure are, in order, the Ectosylvian Sulcus, the Suprasylvian and finally the Ectomarginal. In the Crab-eating fox, the same sulci are present in a similar way, however, they are less deep and have few collateral branches, suggesting a less developed brain than that of the Dog.

The pattern of sulci and gyri of the dorsolateral aspect of the cerebral hemisphere of the Dog, described by Miller, Christensen, and Evans (1964) and Getty in Sisson and Grossman (2008), shows a greater number of variations caused by the presence of more robust and convoluted gyri determining deeper, tortuous and branching sulci, that is, the more accentuated growth of the cortex of the Dog. It required more space, which does not exist, due to the limitations imposed by the cranial bone case, a fact that determines a more accentuated folding of the cortex, culminating in more voluminous and more convoluted gyri and deep, tortuous and more branched sulci.

The concentric arrangement around the Sylvian Fissure of the Ectosylvian, Suprasylvian and Lateral sulci described by Miller, Christensen, and Evans (1964) and Getty in Sisson and Grossman (2008) in dogs are also present in the Crab-eating fox, but they are smoother and more uniform.

The Ectolateral Sulcus, which divides the caudal part of the Suprasylvian Gyrus into Posterior Suprasylvian and Ectolateral Gyrus, is present in the Crab-eating fox in accordance with the citations of Miller, Christensen, and Evans (1964) and Getty in Sisson and Grossman (2008) in Dogs. The middle part of the Lateral Gyrus of Dogs has a small sulcus parallel to the longitudinal fissure called the Ectolateral Sulcus. It divides the Lateral Gyrus into Ectolateral and Lateral (MILLER; CHRISTENSEN; EVANS, 1964, and GETTY in SISSON; GROSSMAN, 2008). This and some other sulci and gyri are not found in the Crab-eating fox, possibly due to the lower development of the cortex in this canid. Thus, Miller, Christensen, and Evans (1964) and Getty in Sisson and Grossman (2008) describe on the rostral half of the dorsolateral surface of the hemisphere of Dogs, a Coronal Sulcus that is a rostral continuation of the Lateral Sulcus, after strong ventral flexion, which is not verified in the Crab-eating fox. The Ansate Sulcus and the Coronal Gyrus are not clearly delimited in the Crab-eating fox as they are described, in dogs, by the aforementioned authors. The Crossed Sulcus and the Anterior and Posterior Sigmoid Gyri are poorly delimited in the Crab-eating fox, but in one specimen, the Crossed Sulcus is strongly marked and continues on the medial aspect of the hemisphere as the Cingulate Sulcus, although the Pre-Cruciate and Post-Cruciate sulci are not present. On the medial aspect of the hemisphere, Miller, Christensen, and Evans (1964), and Getty in Sisson and Grossman (2008) describe the Corpus Callosum as the

largest white commissure between the hemispheres, which forms the roof of the lateral ventricles. Observations in the Crab-eating fox also reveal the presence of a Corpus Callosum, but thinner and poorly delimited. The Knee, the Trunk, and the Splenium, well-defined parts in Dogs, according to Miller, Christensen, and Evans (1964), and Getty in Sisson and Grossman (2008), are not always easily visible in the Crab-eating fox, but the Fornix and the Septum Pellucidum are. The Cingulate Sulcus as well as the Cruciate Sulcus are well-demarcated in one specimen, but in two others it is obscure as shown by Miller, Christensen, and Evans (1964), and Getty in Sisson and Grossman (2008), who similarly indicate other sulci and gyri on the medial surface (Suprasplenial Sulcus, Postsplenial Sulcus, Genual Sulcus, Genual Gyrus, Splenial Gyrus, and Others), which are not identified in the Crab-eating fox.

As described in Dogs, by Miller, Christensen, and Evans (1964), and Getty in Sisson and Grossman (2008), the Cingulate Gyrus proceeds caudally and continues on the inferomedial surface with the Parahippocampal Gyrus. The dimensions of the Parahippocampal Gyrus of the Crab-eating fox are much larger than shown by Miller, Christensen, and Evans (1964), and Getty in Sisson and Grossman (2008) in Dogs, when analyzed from the point of view of the animal's size. The Parahippocampal Gyrus of the Crab-eating fox is delimited dorsally by the caudal part of the Cingulate Sulcus and ventrally by the Parahippocampal Sulcus. It continues rostrally with the Piriform Area and caudally with the Cingulate Gyrus, as described by Miller, Christensen, and Evans (1964), and Getty in Sisson and Grossman (2008) in Dogs.

Sulci and gyri of the dorsolateral surface advance over the inferior caudal aspect of the cerebral hemisphere, as in Dogs, but their nomenclature does not change.

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Stricto Ensu Editora

BRANCHING OF THE AORTIC ARCH IN A LESSER ANTEATER

(*Tamandua tetradactyla* Linnaeus, 1758)

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ABSTRACT

The anatomical study of anteaters has been limited despite their importance as mammals that inhabit Brazil. Populations of these animals are declining due to pressure from civilization, habitat fragmentation, and other threats. The lesser anteater (*Tamandua tetradactyla*) is a medium-sized mammal, with short, dense fur and a wide geographic distribution in South America. It belongs to the Superorder Xenarthra, which includes species with specific anatomical and physiological characteristics. Thus, the present study aimed to describe the branching of the aortic arch in a *T. tetradactyla* and its anatomical relationships. A *T. tetradactyla* carcass donated by the Instituto de Pesquisa da Vida Silvestre – IPEVIS and by the Centro de Triagem de Animais Silvestres – CETAS from Catalão, was dissected. The aortic arch was formed at the level of the third intercostal space and bifurcated into the brachiocephalic trunk and the left subclavian artery. The brachiocephalic trunk trifurcated into the left common carotid, right common carotid, and right subclavian arteries. Regarding anatomical relationships of the aortic arch, it stabilished relationship on the right side with the trachea and cranial vena cava and on the left with the phrenic nerve, cranial lobe of the left lung, and esophagus. The branching of the aortic arch was the same as previously reported by other authors in the same species, but the brachiocephalic trunk of our specimen did not

form a bicarotid trunk. The branches showed divergence in relation to other species of the Superorder Xenarthra, such as armadillos and sloths.
Keywords: Anatomy, Angiology and Artery.

1. INTRODUCTION

The anatomical study of wild animals has been seen with great interest worldwide. However, scientific literature about most groups, including anteaters, are still scarce.

Anteaters are mammals that inhabit the entirety of Brazilian territory, where there are three species: the giant anteater (*Myrmecophaga tridactyla*), the lesser anteater (*Tamandua tetradactyla*), and the silky anteater (*Cyclopes didactylus*). The main characteristic of these species is the total absence of teeth and the presence of a long and conical snout, in which there is a vermiform tongue that helps in capturing food (CUBAS et al, 2007).

Currently, the anteater population has been decreasing significantly due to the increase in urbanization, and consequently the expansion of the highway network and energy matrix. Also, hunting and capture, loss and degradation of habitat, fragmentation of forests, predation by exotic and domestic species, indirect poisoning, and loss in genetic variability. Consequently, the number of animals rescued in fauna screening and rehabilitation centers has increased (MANGUEIRA, 2023).

T. tetradactyla is a medium-sized mammal, belonging to the family Myrmecophagidae, which has a wide geographic distribution and can be found in several Brazilian biomes, as well as several South American countries (SUPERINA; MIRANDA; ABBA, 2010). This species measures between 47 to 77 cm (NOWAK, 1999) and has a body weight of approximately 7 Kg (WETZEL, 1975). Its coat is short and dense, and the color varies according to the region where it is found. It can be golden with a black, gold, ocher, black, or dark brown in some locations (SUPERINA, 2012). They are insectivores, adapted to arboreal strata, with predominantly nocturnal habits, and have a compact body with a long, conical snout, in addition to a prehensile tail measuring between 40.2 and 67.2 cm (CUBAS et al., 2007). There is no evident sexual dimorphism between the sexes of *Tamandua tetradactyla*, with the genitalia found internally in the abdominal cavity (HOSSOTANI; LUNA, 2016; RODRIGUES et al., 2008).

Furthermore, these animals have predominantly solitary behavior, except for parental care. Due to this, when placed in captivity there are minimal social interactions between

individuals to defend food, territory, shelter, or even sexual partners. In the natural environment, the interactions of these animals are mediated through vocalization and postures (DONNARUMA et al., 2020).

The *T. tetradactyla* belongs to the superorder Xenarthra that presents some specific anatomical characteristics, such as additional joints between the lumbar vertebrae, a restricted characteristic of this Superorder, which allows the members of this group to assume an upright posture based on the pelvic limbs and the tail, thus forming a tripod. There are other exclusive morphophysiological aspects observed in this superorder, such as reports of the presence of a paired caudal cava vein, undifferentiated external genitalia, low metabolism, and body temperature that varies between 32.7 ºC and 35.5 ºC (McNAB, 1985; REIS et al., 2006).

The aortic arch is formed before the aorta continues as descendent aorta. The aortic arch forms branches that extend to the head, neck, thoracic limbs, and thoracic cavity. Its branching varies among species of the superorder Xenarthra, such as in the anteaters (PINHEIRO et al., 2012; DOS SANTOS et al., 2020), sloths (ALBUQUERQUE et al., 2018) and armadillos (DOMENICONI et al., 2004; SANTOS et al., 2020). The anatomical knowledge in the branching of the aortic arch is not only used for comparative proposes but also veterinary procedures, such as radiological diagnosis and surgeries in the cranial mediastinum to identify and correct possible vascular anomalies in wild animals (VÉLEZ GARCÍA, 2020). Considering the wide geographic distribution of these mammals, the anatomical particularities and, at the same time, the scarcity of morphological investigations in this species, as well as the importance of the aortic arch for the distribution of oxygenated blood throughout the body, the present study aimed to describe the branching of the aortic arch in a *T. tetradactyla* and its anatomical relationships.

2. MATERIALS AND METHODS

The study was developed using a carcass of a lesser anteater (*Tamandua tetradactyla*), with no defined age, donated to the Laboratory of Comparative Anatomy of Wild Animals at the Federal University of Catalão (LACAS-UFCAT), by the Wildlife Research Institute (IPEVIS) and the Wild Animal Screening Center (CETAS - Catalão-GO), under environmental license SISBIO 37072-2. In the laboratory, the arterial system of the specimen

was accessed through the femoral artery. A repletion was made with latex (Du Latex) tinctured with red liquid pigment (“Xadrez”) for better visualization of the arteries. Immediately after this process, the material was fixed with 10% aqueous formaldehyde solution, through perfusion via femoral artery. In addition, intramuscular injections of the same solution were made, and the specimen was immersed in it to be preserved.

A few days after fixation, the dissection process began using usual techniques in gross anatomy. Scissors, scalpel, and anatomical forceps were used. The skin was initially removed, along with the coat, followed by adipose tissue and fascia. After that, we proceeded to dissect the vascularization of the thoracic limb, followed by removing the pectoral muscles and opening the thoracic cavity, aiming to expose the recommended vessels (STANDRING, 2020). During the dissection process, the material was photographed with a Canon T5i camera (22 MP) equipped with a macro lens of 50 mm to document the results.

The results were described in accordance with the Veterinary Anatomical Nomenclature (International Committee on Veterinary Gross Anatomical Nomenclature, 2017). The research was developed with a favorable opinion from the animal ethics committee of the Federal University of Catalão, Catalão, Goiás, Brazil, CEUA/UFCAT nº 01/22.

3. RESULTS AND DISCUSSIONS

In the *T. tetradactyla* specimen, it was observed that the aortic arch was formed at the level of the left paramedian line, between the third and fourth rib, which corresponds to the third intercostal space. The first branch was the brachiocephalic trunk and the second, the left subclavian artery. Regarding the anatomical relationships of the aortic arch, it was found that the aortic arch is associated on the right side with the trachea and cranial vena cava and, on the left side, with the phrenic nerve, the cranial lobe of the left lung and in its most medial portion with the esophagus (Figure 1).

The brachiocephalic trunk runs towards the median cranial direction, where it is related dorsally to the trachea, and at the level of the first intercostal space it trifurcates into the left common carotid, right common carotid, and right subclavian arteries (Figure 1).

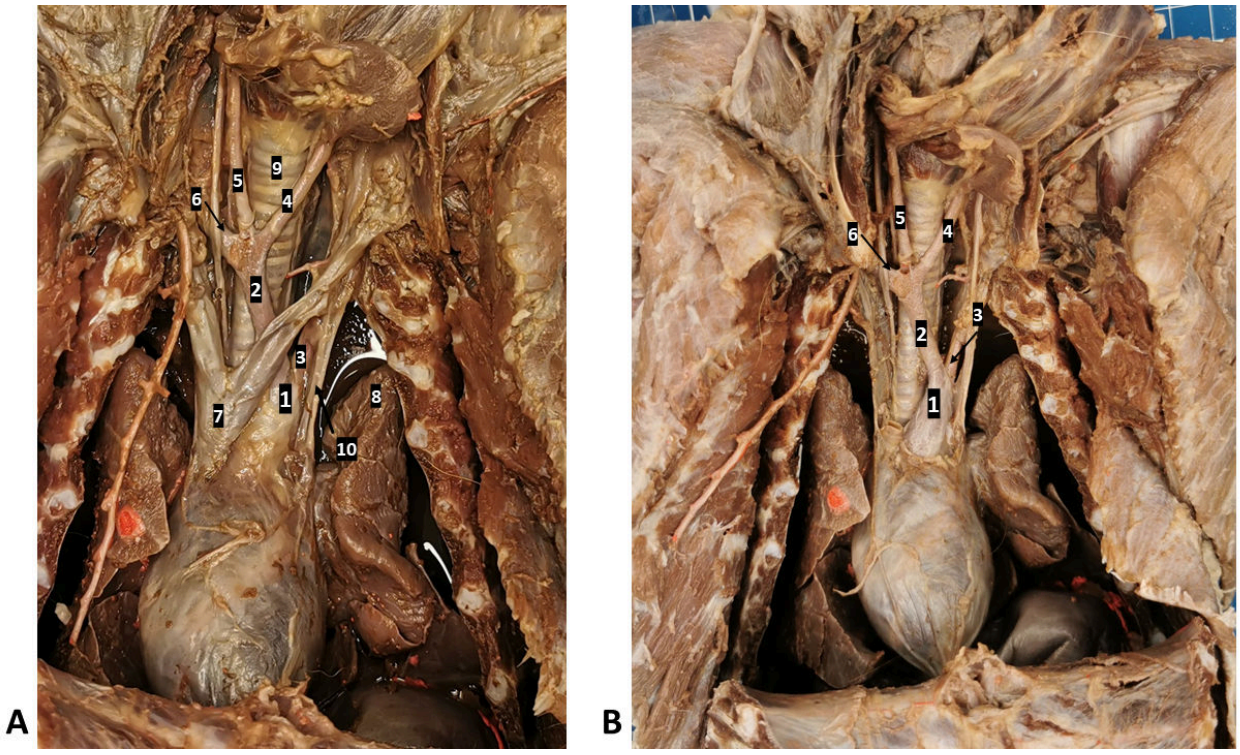

Figure 1. (A) Ventral view of the thoracic cavity of a *Tamandua tetradactyla* with cranial vena cava and (B) without veins;
1- aortic arch; 2- brachiocephalic trunk; 3- left subclavian artery; 4- left common carotid artery; 5- right common carotid artery; 6- right subclavian artery; 7- cranial vena cava; 8-cranial lobe of the left lung; 9- trachea; 10- left phrenic nerve.

The branching of the aortic arch of our *T. tetradactyla* specimen is similar to the reported in the same species (Pinheiro et al., 2012) and *M. tridactyla* (dos Santos et al., 2020). However, Pinheiro et al. (2012), when describing the anatomy of the collateral branches of the aortic arch of three young of *T. tetradactyla*, presents the formation of the brachiocephalic trunk and, from this, originated the right subclavian artery and a bicarotid trunk, from which emerged right and left common carotid arteries. Therefore, it differs from our specimen, in which the brachiocephalic trunk was trifurcated and there was no formation of a bicarotid trunk. Furthermore, it also differs from *M. tridactyla* since in this species a brachiocarotid trunk is ramified, from which the right common carotid and right subclavian arteries originate (dos Santos et al., 2020).

When compared to other species of the superorder Xenarthra, there are differences in the branching of the aortic arch. In armadillos of the species *Dasypus novemcinctus*, it forms two brachiocephalic trunks (right and left) (DOMENICONI et al. 2004). In the giant armadillo (*Priodontes maximus*) and the sloth *Bradypus variegatus*, it forms three branches, the brachiocephalic trunk, left common carotid, and left subclavian arteries (ALBUQUERQUE et.

al, 2018; SANTOS et al., 2020). While in anteaters, there are only two branches from the aortic arch (PINHEIRO et al. 2012; DOS SANTOS et al., 2020; our study).

Thus, the present study corroborates the ramification of the aortic arch into two branches as was previously described by Pinheiro et al. (2012). However, this is the first report of the anatomical relationships of the aortic arch and the trifurcation of the brachiocephalic trunk in *T. tetradactyla*, which also differs from others animals in the Superorder of Xenarthra.

4. CONCLUSIONS

In *T. tetradactyla*, two branches of the aortic arch were identified, the brachiocephalic trunk and the left subclavian artery. In the brachiocephalic trunk there was a trifurcation originating the left common carotid artery, right common carotid artery, and right subclavian artery.

The branching of the aortic arch was the same as previously reported by other authors in the same species, but the brachiocephalic trunk of our specimen did not form a bicarotid trunk. The branches showed divergence in comparison to other species of the Superorder Xenarthra such as armadillos and sloths.

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ANATOMICAL DESCRIPTION OF THE COUGAR'S HEART (*Puma concolor* Linnaeus, 1771)

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ABSTRACT

The heart is a vital organ in the body of vertebrates and the analysis of its morphology can provide information about the physiology and adaptation of a species to the environment. The study of the anatomy of the Cougar's heart (*Puma concolor*), can provide valuable insights for understanding the anatomy of felines in general, and guide conservation measures for the species. The methodology used was the dissection and analysis of two hearts of *Puma concolor*, removed from carcasses donated by the Wildlife Research Institute (IPEVIS) and the Wildlife Screening Center (CETAS) of Catalão (GO). In this study, it was concluded that the hearts of the specimens have an elongated shape, measuring about 8.0 cm in length and 5.5 cm in the transverse direction; The heart are located in the mediastinum of the chest and are enveloped by the pericardium. The Right Ventrolateral and Left Dorsolateral Faces produce Right and Left Cardiac Impressions on the lungs. The results obtained allowed a greater understanding of the cardiac morphology of the species, and it is hoped that they will serve as a subsidy for research on its biology and ecology.

Keywords: Anatomy, heart and *Puma concolor*.

1. INTRODUCTION

The cougar (*Puma concolor*) is a feline native to the Americas, with a broad distribution range from the far south of Alaska to the southernmost tip of South America. In Brazil, it is recorded in all states. It is also known by names such as mountain lion, red tiger, puma, and catamount. The species is solitary, territorial, and exhibits nocturnal and crepuscular behaviors. It has an elongated body, with strong limbs and a long tail. Its coat is uniformly colored, ranging from grayish-brown to reddish-brown, with a lighter chest and a black-tipped tail (PRIST; SILVA; PAPI, 2020). Weight varies according to geographic region, and can exceed 100 kg in some areas. Generally, in tropical regions, its diet consists mainly of medium-sized mammals with an average weight of 18.0 kg, such as peccaries (*Tayassu pecari* and *Pecari tajacu*), deer (*Mazama* spp., *Ozotocerus bezoarticus*, and *Blastocerus dichotomus*), anteaters (*Myrmecophaga tridactyla* and *Tamandua tetradactyla*), and capybaras (*Hydrochoerus hydrochaeris*) (REIS et al., 2011).

In many Brazilian states, the majority of wild felines are classified under some degree of threat, and some species are considered to be critically endangered. The cougar is an important indicator of ecosystem health, as it is an apex predator playing a key role in the population control of its prey and in maintaining ecological balance within its natural habitat (REIS et al., 2011).

Anatomy is the branch of morphology that focuses on the form, structure, topography, and functional interaction of the tissues and organs that make up the body. The most efficient and important method to study and understand anatomy is the dissection of deceased animals (KÖNIG; LIEBICH, 2021). In this context, the study of the heart anatomy of wildlife species is of great significance, as it can provide relevant information about the species' physiology, such as the relationship between diet and cardiac structure, revealing possible adaptations, as well as identifying potential morphological changes related to heart diseases. Therefore, the study of the heart anatomy of *Puma concolor* is essential for the organization and development of preservation and conservation programs for this species, as it can be used to identify risk factors and in the development of effective prevention and treatment strategies for diseases that may affect the cardiovascular health of individuals of this species.

All the component systems of an organism are equally important for its homeostasis. Among them, the circulatory system stands out, with the heart being the central organ (KÖNIG; LIEBICH, 2021), and the respiratory system, for they carry out the transport of

substances between the tissues and the external environment. The respiratory system ensures the introduction of O_2 into the organism and the elimination of CO_2 to the external environment, with the exchanges taking place at the level of the pulmonary alveoli. The transport of these substances between the lungs and tissues, as well as the movement of nutritive substances or metabolites, is primarily the prerogative of the circulatory system (MASSARI; MIGLINO, 2029). Therefore, the investigation of heart anatomy is crucial to expand the knowledge about the structure of the circulatory system in animals. From this perspective, there are still gaps to be filled in understanding the anatomy and physiology of the *Puma concolor*.

Like other wild felines, the adaptation of the cougar (*Puma concolor*) is closely linked to the specific characteristics of its ecological habitat. These adaptations include behavioral, physiological, and morphological aspects that distinguish it from domestic felines, reflecting the unique environmental demands faced by this species (SILVA; MARINHO-FILHO, 2008).

While cougars are considered important predators within their ecosystems, domestic felines do not serve this role. These carnivores are crucial components of ecosystems, controlling the abundance, distribution, and diversity of their prey populations, which makes them key species in the maintenance and restoration of diversity (GHELER-COSTA et al., 2018). The description of the heart anatomy of the cougar (*Puma concolor*) may contribute to the understanding of how this species adapts to different environmental conditions and how its cardiac structure is related to the physiological functions inherent to its feeding and reproductive behavior. In this sense, this study aims to describe the heart anatomy of *Puma concolor*, aiming to provide a basis for potential future investigations on the adaptation and behavior of this species.

2. MATERIAL AND METHODS

This study was conducted at the Laboratory of Comparative Animal and Human Anatomy of the Federal University of Catalão (UFCAT). Two hearts of *Puma concolor* were used, removed from carcasses, both donated by the Wildlife Research Institute (IPEVIS) and the Wild Animal Screening Center (CETAS) of the municipality of Catalão (GO).

Incorporated into the UFCAT Comparative Anatomy research and study collection, the carcasses lack heads and abdominal visceradue to the mutilation conditions that prevent fixation through perfusion. Therefore, the skin was removed, and the carcasses were

submerged in a 10% aqueous formaldehyde solution and kept for fixation. The removal of the hearts was carried out through a sagittal incision in the sternum, sectioning of the diaphragm and subclavian vessels, allowing the removal of the entire cardiopulmonary block. Once the block was isolated, a careful dissection of the basal vessels and bronchi was performed, culminating in the separation of the two systems, isolating the heart and the basal vessels. After a thorough “cleaning” of the vessels in their extrapericardial part, the pericardium was sectioned, removed, and the ‘cleaning’ was completed for the description of the External Anatomy of the heart. Subsequently, incisions were made in the walls of the chambers at the level of their edges to expose and describe the Internal Anatomy. All steps were photographed with an iPhone 11 and Xiaomi Mi 8 cell phone camera.

The experimental protocols were carried out in accordance with the recommendations of the guidelines of the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee on the Use of Animals of the Federal University of Catalão - CEUA/UFCAT No. 01/22.

The terminology adopted to name the structures is in accordance with the International Committee on Veterinary Gross Anatomical Nomenclature (ICVGAN, 2017).

3. RESULTS AND DISCUSSION

The heart of the Puma exhibits an elongated shape, from the “*basis cordis*” to the “*apex cordis*”, with the studied specimen weighing approximately 30kg (anial weight) and measuring approximately 8.0cm in length by 5.5cm transversely at its largest axis. (Fig. 1A).

The heart is located in the Mediastinum, with the larger part (60%) positioned to the left of the median plane. It extends between the 3rd and 6th ribs (7th in the domestic cat and the dog) (KÖNIG; LIEBICH, 2016).

The base of the Puma’s heart is turned cranially and slightly to the left, while the apex is directed caudally and slightly to the left (Fig. 1A). Fully enveloped by the Pericardium, the heart is located in the sagittal plane, between the Left and Right Lungs, occupying a small space called the Thoracic Mediastinum and is almost completely covered by the lungs.

When comparing these results with previous studies, it is observed that the heart of the Puma shares structural characteristics with the hearts of other animals, both domestic and wild. According to Ávila, Machado and Oliveira (2010), similar to the heart of domestic,

wild and laboratory animals, the heart of the paca (*Cuniculus paca*) presents characteristics such as Venous Sinus, Terminal Crest, Fossa Ovalis, Atrioventricular Valves, Papillary Muscles and Tendinous Cords, in addition to smooth Atria and Auricles covered by highly indented Pectinate Muscles.

In mammals, the heart is suspended in the Thoracic Cavity, and the Pericardial Sac is fixed, dorsally, by the root of the large arteries and veins and, ventrally, attached to the Sternum, although its fixation to the Diaphragm varies between species (ÁVILA et al., 2010). A small area next to the apex of the heart is in contact with the Diaphragm, to which it is attached by the Pericardium. Almost the entire area corresponding to the right face is in contact with the ight lung, The same occurs on the left side, where a large area of the left face is in contact with the left lung. In fact, both areas, know as the Right and the Left Face of the heart, due to their positions, would be better named Right Ventrolateral Face (Fig. 1B) and Left Dorsolateral Face (Fig. 1C). The small ventral area, not covered by the lungs, is in contact with the sternum, it's known as the Sterno-costal Face, while the narrow dorsal area is in contact with the vertebral column is the Dorsal Face.

The Right Ventrolateral and Left Dorsolateral faces produce deep marks on the lungs, called Right and Left Cardiac Impressions, of the lungs.

The heart of the Puma is relatively small when compared to the heart of other carnivores, for example, the domestic dog. Hermann (1925) cited by Miller; Christensen; Evans (1964) states that the body weight/heart weight ratio of a domestic dog is approximately 1000g X 8.1g. Although it is not an aspect analyzed in this article, since the carcasses are incomplete, it seems clear that the heart of the Puma is smaller, in relation to body size.

3.1. EXTERNAL ANATOMY OF THE PUMA'S HEART

The analysis of the external anatomy of the Puma's heart revealed that the base of the organ is entirely occupied by the Right and Left Atria, Right and Left Auricles, and basal Vessels; in addition to a large amount of adipose tissue (Fig. 1A). This organization is consistent with the structure found in mammals, where the heart presents four chambers that contract rhythmically, being two atria and two ventricles (DYCE et al., 2010).

The Right and Left Atria are separated from the Right and Left Ventricles by a large and deep transverse groove, the Coronary Sulcus (Fig. 1A-8), which, in its entire extent, is filled with a layer of adipose tissue, the Coronary Sulcus Fat (Fig. 1A-7), which surrounds the

large Coronary Arteries and large Cardiac Veins. The large amount of adipose tissue present throughout the base of the heart prevents the Coronary Sulcus from being clearly visualized. However, it is possible to identify it as described by Dyce (DYCE et al., 2010).

In the right lateral view, a large amount of adipose tissue is identified on the Ascending Part of the Aorta (Fig. 1A-3; Fig. 1B-4). Additionally,, in the right lateral and dorsolateral views, the following are visualized: a lot of fat that fills the Coronary Sulcus (Fig. 1A-7; Fig. 1B-5); the Right Auricle (Fig. 1A-6; Fig. 1B-6); the Cranial Vena Cava (Fig. 1A-1; Fig. 1B-1); the Caudal Vena Cava (Fig. 1A-11 and 1C-11); the Right Atrium (Fig. 1A-5) and the Right Pulmonary Arteries (Fig. 1A-4); the Right Ventricle (Fig. 1A-9, Fig. 1B-8); the Left Ventricle (Fig. 1A-12, Fig.1B-10, Fig. 1C-10).

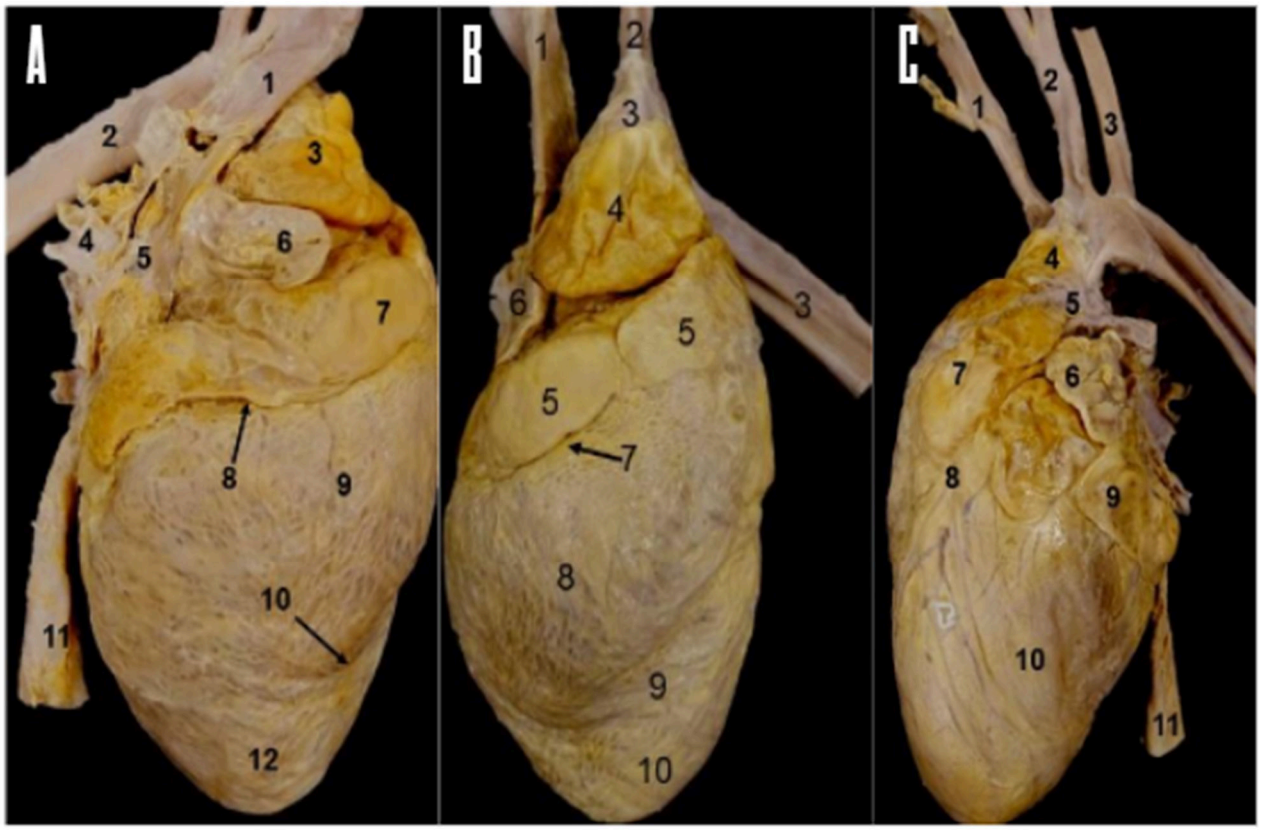

Figure 1. Puma's Heart.
A. Right Lateral View of the Puma's Heart. 1- Cranial Vena Cava; 2- Aorta; 3- Aortic Fat; 4- Right Pulmonary Artery; 5- Right Atrium; 6- Right Auricle; 7- Coronary Sulcus Fat; 8- Coronary Sulcus; 9- Right Ventricle; 10- Ventrolateral Sulcus; 11- Caudal Vena Cava; 12- Left Ventricle. **B. Right Ventrolateral View of the Puma's Heart.** 1- Cranial Vena Cava; 2- Brachiocephalic Trunk; 3- Aorta; 4- Aortic Fat; 5- Coronary Sulcus Fat; 6- Right Auricle; 7- Coronary Sulcus; 8- Right Ventricle; 9- Ventrolateral Sulcus; 10- Left Ventricle. **C. Left Dorsolateral View of the Puma's Heart.** 1- Cranial Vena Cava; 2- Brachiocephalic Trunk; 3- Left Subclavian Artery; 4- Aortic Fat; 5- Pulmonary Trunk; 6- Left Auricle; 7- Arterial Cone; 8- Ventrolateral Interventricular Sulcus; 9- Coronary Sulcus Fat; 10- Left Ventricle; 11- Caudal Vena Cava.

3.2 INTERNAL ANATOMY OF THE ATRIA

3.2.1 Left Atrium

The Left Atrium (Fig. 2A-7) occupies the dorsal part of the Heart Base. Superficially, there is no groove separating the two atria. Most of the atria are covered by adipose tissue and by the basal Vessels, but internally they are separated by the Interventricular Septum. The external surface of the Left Atrium is hidden by a diverticular projection of the Left Atrium, the Left Auricle (Fig. 1C-6), adipose tissue, and vessels. The Left Auricle projects to the right and ventrally and caudally as a pedicled structure, that is, the part that is attached to the atrial wall is narrower than the rest.

The free and wider part of the Left Auricle partially covers the Left Atrium and the base of the Right and Left Ventricles. According to Ávila et al. (2010), the auricular margins of the Crab-eating Raccoon (*Procyon cancrivorus*) are slightly indented, which contrasts with the description of the Left Auricle of the puma, which presents a slightly pleated surface, resembling a “small human ear”.

The Cavity of the Left Atrium (Fig. 2B-6) is relatively small when compared to the volume of the heart. The internal face of its wall is smooth, except for the auricular walls which are covered by numerous muscular folds (crests), together, they are called: Pectinate Muscle of the Left Auricle. The aforementioned muscular crests originate in the atrial wall and head towards the free edge of the auricle, like the teeth of a comb. Apparently, the Pectinate Muscle has the function of strengthening the atrial wall (KARDONG, 2016).

The Left Atrium has five main openings. The largest opening of the Left Atrium is the Left Atrioventricular Foramen that communicates the Atrial Cavity with the Ventricular Cavity. Three smaller openings correspond to the openings of the Pulmonary Veins, being one left and two right. The Lobar Veins of the lungs join before entering the Left Atrium, forming larger trunks. A very small opening can be visualized in the septal wall of the Left Atrium, it is the opening of the Septal Cardiac Vein.

3.2.2. Right Atrium

The Right Atrium (Fig. 2C-5) is visibly larger than the Left Atrium. It occupies the entire dorsocranial region of the Heart Base. The free part of the Right Atrium, the Right Auricle (Fig. 2C-4), is shorter than the left and does not present a pleated surface, not displaying the

aspect of a small ear. It is positioned slightly oblique from right to left and ventrally, partially covering the Ascending Part of the Aorta (Fig. 2C-4).

The cavity of the Right Atrium is visibly larger than that of the Left Atrium, it can be divided into two parts: Venous Chamber (Fig. 2D-8) and the Right Auricular Cavity. The Venous Chamber is the the dorsolateral part. Itis larger and receives the two Vena Cava: Cranial and Caudal, in addition to the Coronary Sinus (Fig. 2D-10) and Minimal Cardiac Veins, originating from the myocardium.

The dorsocranial part represents the Right Auricular Cavity. The majority of the internal face of the Right Atrium is relatively smooth, presenting only small elevations on the wall. On the other hand, the internal face I of the Right Auricle is irregular, presenting numerous muscular crests that originate in the atrial wall and head towards the free edge of the auricle, similar to the teeth of a comb, called the Pectinate Muscle (Fig. 2D-4). In comparison with the observations made by Pereira et al. (2016) in the heart of the paca (*Agouti paca*), the right and left atria are described as smooth, but the auricles are covered by quite indented pectinate muscles.

The Right Atrial Cavity displays four larger openings and several smaller ones. The largest opening of the Right Atrium is the Right Atrioventricular Foramen (Fig. 2C-7) that communicates the Right Atrial Cavity with the Right Ventricular Cavity (Fig. 2C-8). It is located in the caudoventrolateral part of the atrial cavity. Two other openings located in the dorsolateral region represent the openings of the Cranial and Caudal *Vena Cava*. A smaller opening, the *Ostium* of the *Coronary Sinus* (Fig. 2D-10) can be visualized ventrally to the opening of the Caudal *Vena Cava*. Some small openings in the medial wall represent the openings of the Minimal Cardiac Veins, originating from the Myocardium.

In the area between the mouth of the Cranial and Caudal Vena Cava, there is a small elevation, the Intervenous Tubercle. In the dorsal part of the interatrial wall, near the mouth of the Caudal Vena Cava, there is a small depression, the Fossa Ovalis (Fig. 2D-7), a remnant that corresponds to the oval foramen of fetal life (DYCE et al., 2010). The cranial edge of the *Fossa Ovalis* is slightly elevated, forming the *Limbus* of the *Fossa Ovalis*. Emerging next to the *Limbus* of the *Fossa Ovalis* and directing ventrally, in the atrial wall there is a small muscular crest, the Terminal Crest (Fig. 2D-5).

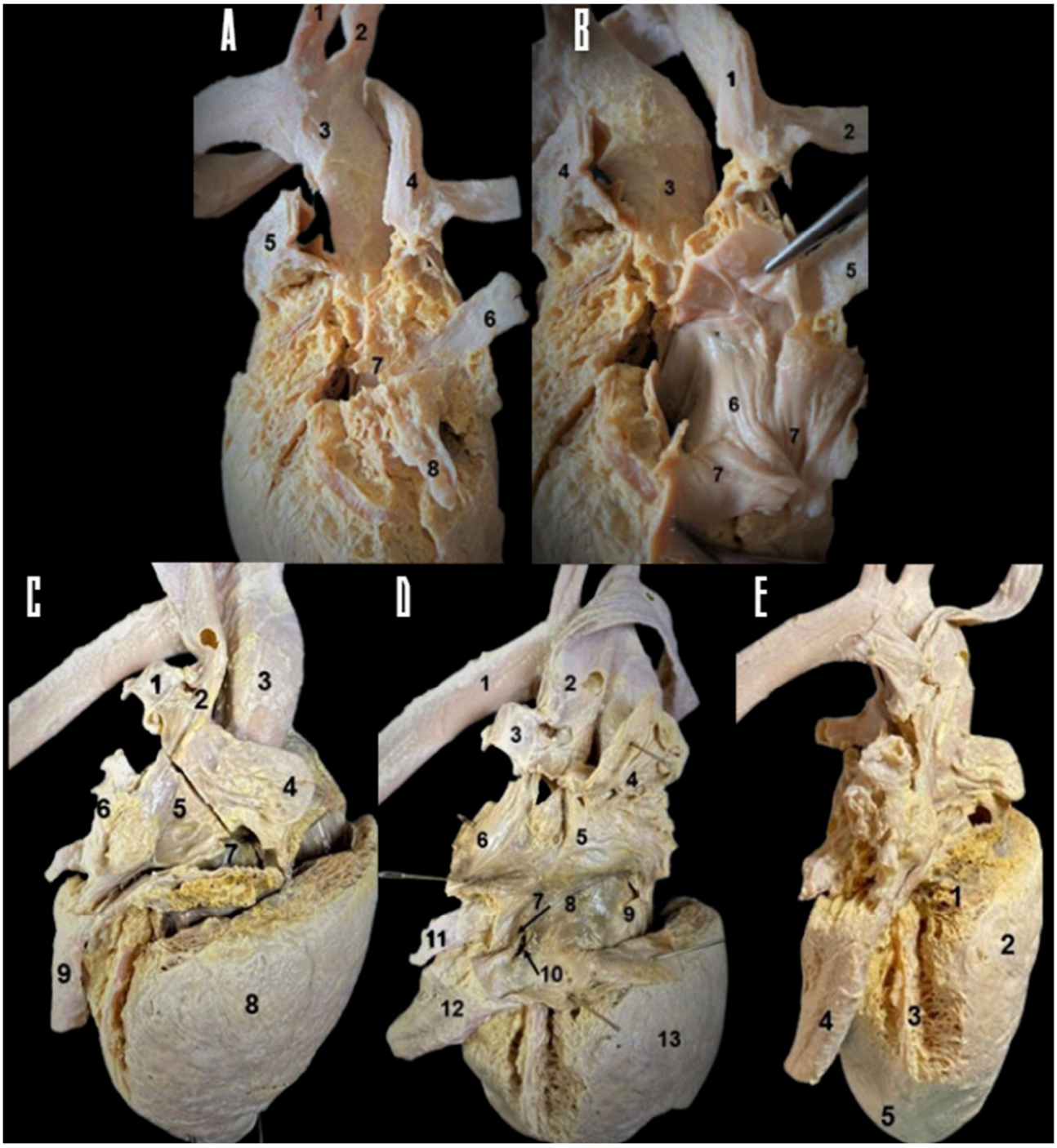

Figure 2. Puma's Heart.

A. Cranial View of the Left Atrium of the Puma's Heart. 1- Brachiocephalic Trunk; 2 Left Subclavian Artery; 3- Aorta; 4- Cranial Vena Cava; 5 Pulmonary Artery; 6- Left Pulmonary Vein; 7 Left Atrium; 8- Right Pulmonary Vein. **B. Cranial View of the Left Atrium of the Puma's Heart.** 1- Cranial Vena Cava; 2- Azygos Vein; 3- Aorta; 4- Pulmonary Artery; 5- Left Pulmonary Vein; 6 Cavity of the Left Atrium; 7- Right Pulmonary Veins; **C. Right lateral view of the Puma's Heart.** 1-Azygos Vein; 2- Cranial Vena Cava; 3-Aorta; 4-Right Auricle; 5-Right Atrium; 6- Right Pulmonary Arteries; 7 Right Atrioventricular Foramen; 8 Right Ventricle; 9. Caudal Vena Cava; **D. Dorsal cranial view of the Puma's Heart.** 1- Aorta; 2-Cranial Vena Cava; 3-Azygos Vein; 4 Pectinate Muscle of the Right Atrium; 5- Terminal Crest; 6- Reflected Atrial Wall; 7- Fossa Ovalis; 8- Venous Chamber; 9-Right Atrioventricular Foramen; 10- Ostium of the Coronary Sinus; 11- Pulmonary Artery; 12-Caudal Vena Cava; 13- Right Ventricle; **E. Dorsal view of the Puma's Heart.** 1- Coronary Sulcus without Fat; 2- Right Ventricle; 3-Dorsal Interventricular Sulcus; 4 Caudal Vena Cava; 5-Left Ventricle.

3.3. ANATOMY OF THE VENTRICLES

The two ventricles together make up the largest part of the heart (Dyce et al., 2010), forming a conical muscle mass, whose base is oriented cranially and discreetly to the right, and the *apex* directed caudally and slightly to the left.

3.3.1. Interventricular Grooves

The Interventricular Grooves are two elongated, yet shallow depressions visible on the surface of the heart, which separate the Right and Left Ventricles from each other. Although the ventricles merge externally, their separate extensions are defined by shallow grooves that descend towards the *apex* (DYCE et al., 2010).

Most of the Right Ventrolateral Face of the heart is formedby the Right Ventricle, which is clearly separated from the Left Ventricle by the Ventrolateral Interventricular Groove (Fig. 1B-9). This groove is very wide and relatively deep, containing a large amount of fat that surrounds the blood vessels of the heart itself. It arises at the Base of the heart, near the Left Auricle (Fig. 1C-6) caudolaterally to the Arterial Cone (Fig. 1C-7), follows caudal and obliquely to the right, between the dorsal edges of the two ventricles, until the middle caudal level of the ventricles, where it curves, more sharply, to the right limiting caudally the Right Ventricle.

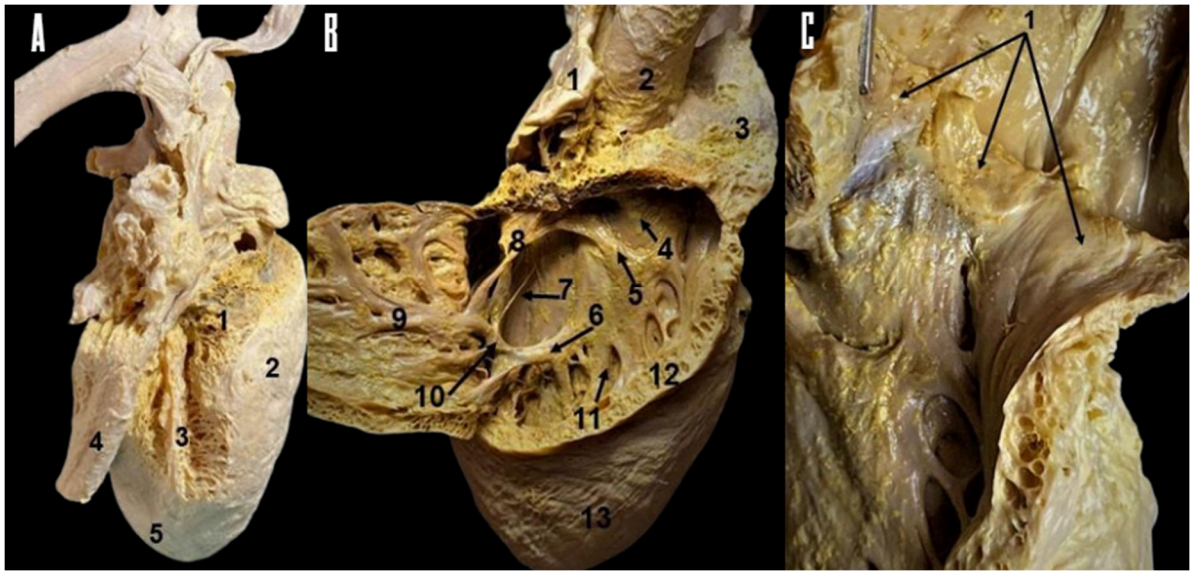

Figure 3. Puma's Heart.
A. Dorsal view of the Puma's Heart. 1- Coronary Sulcus without fat; 2 Right Ventricle; 3- Dorsal Interventricular Sulcus, with the coronary; Caudal Vena Cava; 5 Left Ventricle. **B. View of the Open Right Ventricle of the Puma's Heart.** 1- Right Auricle; 2-Aorta; 3-Pulmonary Trunk; 4 Supraventricular Crest; 5-Septal Papillary Muscle; 6-Septomarginal Trabecula; 7-Tendinous Cord; 8-Lateral Cusp; 9-Muscular belt; 10-Ventrolateral Papillary Muscle; 11-Fleshy Trabeculae; 12-Sectioned Myocardium of the Right Ventricle; 13-Left Ventricle. **C. View of the Open Right Ventricle and Pulmonary Trunk, of the Puma's Heart.** 1- Cusps of the Semilunar Pulmonary Valve.

The Dorsal Interventricular Groove (Fig. 2E-3) is very short and shallow, almost invisible. It is located on the Dorsal Face, in contact with the ventral face of the Caudal Vena Cava. It arises at the Base of the Heart, under the mouth of the Caudal Vena Cava, it goes caudally for a short distance until it disappears into the Myocardium between the two ventricles.

The majority of the heart is made of the Right and Left Ventricles. The Right Ventricle (Fig. 2C-8) is shorter than the Left, composing just over half of the Ventrolateral Face of the heart, with its dorsal limit being the Ventrolateral Interventricular Groove and the ventral limit the Dorsal Interventricular Groove.

In turn, the Left Ventricle (Fig. 1C-10) makes up more than half of the cardiac surface, extending across the entire Dorsolateral Face of the ventricular segment of the heart and almost half of the Ventromedial Face.

Right Ventricular Cavity - The cavity of the Right Ventricle (Fig.3B) is wide, limited medially by the thick Interventricular Septum (Fig. 4B-2), cranially by the Right Atrioventricular Foramen and Tricuspid Valve, while the entire lateral wall that extends from the ventral part of the Ventrolateral Groove to the Dorsal Interventricular Groove is constituted by a thin myocardial wall.

The middle part of the Right Ventricular Cavity is in continuity with the Right Atrial Cavity through the Right Atrioventricular Foramen (Fig. 4A-2) which, in turn, is limited by the Right Atrioventricular Valve or Tricuspid Valve (Fig. 3B-8). The ventromedial end of the right atrial cavity narrows towards the origin of the Pulmonary Trunk (Fig. 3B-3; Fig. 4A-1) forming the Arterial Cone of the Right Ventricle.

The walls of the Right Ventricular Cavity are irregular, especially the Lateral Wall (Fig. 4A-5), due to the presence of numerous muscular projections of the wall itself called *Trabeculae Carneae*, to which the tendinous cords are attached (Fig. 4A-3) (ÀVILA et al., 2010).

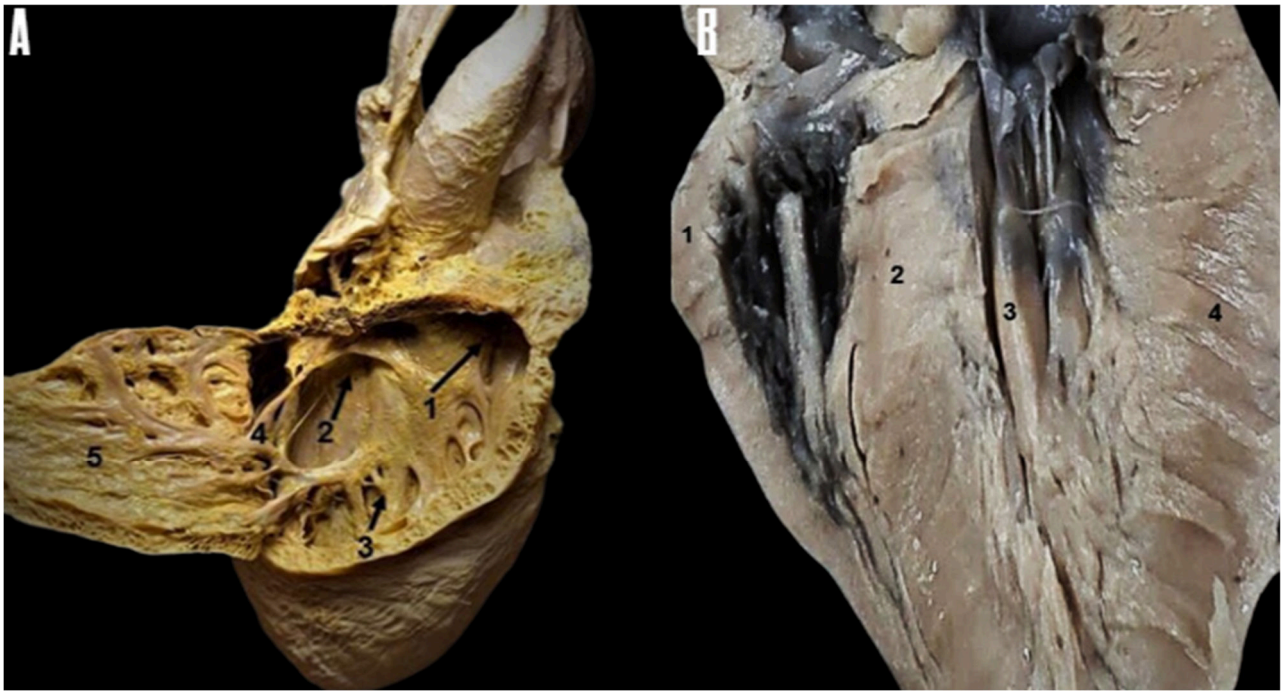

Figure 4. Puma's Heart.
4A. Open Right Ventricle. 1-Pulmonary Trunk; 2-Right Atrioventricular Foramen 3-Fleshy Trabeculae; 4-Ventrolateral Papillary Muscle; 5- Sectioned Ventrolateral Ventricular Wall.
4B. Left Ventricle in Longitudinal Section. 1- Right Ventricular Wall; 2-Interventricular Septum; 3- Papillary Muscle of the Left Ventricle; 4- Wall of the Left Ventricle.

The medial wall of the Right Ventricle is not very flexible, due to the thickness of the Myocardium that forms the Interventricular Septum (Fig. 4B-2). In the cranial-medial part of the Right Ventricular Cavity, near the Pulmonary Trunk Valve, there is a pronounced elevation of the septal muscular wall that originates next to the Arterial Cone, goes obliquely to the right in the direction of the Right Atrioventricular Foramen, it is denominated the Supraventricular Crest (Fig. 3B-4). Caudally to this crest, still from the Interventricular Septum, a wide, but not pronounced muscular fold known as the Septal Papillary Muscle arises, going in the direction of the Atrioventricular Foramen, (Fig. 14-5). It connects at its end through Tendinous Cords (Fig. 3B-7) to the free edge of the Left Cusp of the Right Atrioventricular Valve (Fig. 3B-8). Caudally to the Septal Papillary Muscle, from the septal surface, several muscular crests of different thicknesses arise, they are the *Trabeculae Carneae* of the Right Ventricle (Fig. 3B-11). In comparison with the observations made by Pereira et al. (2016) in the heart of the Crab-eating Raccoon (*Procyon cancrivorus*), the same internal muscular projections are also described. The aforementioned *trabeculae* appear as elevations of the surface of the Interventricular Septum and go laterally until they reach the lateral wall of the Right Ventricle, forming true muscular beams (bridges), whose function, apparently, is to strengthen the lateral wall that has very thin myocardium, thus preventing excessive dilation of the atrium.

The Septomarginal Trabecula islocated approximately in the middle part of the ventricle, emergeson the surface of the interventricular septum, crosses the light of the ventricle, fixing itself in the middle part of the lateral wall, it is the (Fig. 3B-6). According to the description of Pereira et al. (2016), in the Right Ventricle, a long muscular band was noticed, which extended from the interventricular septum to the wall of the said ventricle, similarly to the Septomarginal Trabecula of domestic dogs and cats (GETTY, 1986) and wild animals, such as the paca (ÁVILA et al, 2010) and coati (SOUZA et al., 2016). As it approaches the wall, the Septomarginal Trabecula divides into several branches, which are fixed in the papillary muscles of the lateral wall, or continue in the wall as muscular belts, apparently to strengthen the thin myocardial wall. Several branches of the Septomarginal Trabecula join the Trabeculae Carneae of the atrial wall, to form muscular projections in the ventricular light, they are the Ventrolateral Papillary Muscles (Fig. 3B-10), whose ends connect to the Tendinous Cords of the Ventral Cusp, of the Right Atrioventricular Valve.

Near the dorsolateral end of the ventricular cavity, several smaller Septomarginal Trabeculae connect the Interventricular Septum to the lateral wall of the Ventricle. Several small trabeculae join together to form small papillary muscles, at whose ends tendinous cords are attached, which in turn are fixed to the free edge of the ventral cusp. Thin tendinous cords connect the papillary muscles to each other. Just as very thin tendinous cords connect papillary muscles to the delicate septal cusp of the Right Atrioventricular Valve (Fig. 3B-7).

The end of the Arterial Cone cavity continues out of the heart through the Pulmonary Trunk, with the limit between both being the Pulmonary Trunk Valve, composed of three cusps: Lateral, Dorsal and Medial (Fig. 3C-1).”

3.3.2. Anatomy of the Left Ventricle

The Left Ventricle (Fig. 1C-10) makes up most of the heart’s ventricular mass (about 2/3). The Left Ventricle is formed by a very thick myocardial wall, which gives it a firm and compact texture to endure external pressure, when compared to the Right Ventricle which has thin walls. Studies in *Procyon cancrivorus* also demonstrated that the wall of the left ventricle is thicker when compared to the wall of the right ventricle, also showing similarity with domestic animals (PEREIRA et al., 2016).

The Left Ventricle composes the dorsolateral left part of the ventricular mass, from the Ventrolateral Sulcus to the Dorsal Sulcus, also forming the caudal half on the right side, caudally to the Right Ventricle. Therefore, the entire ventricular mass of the caudal half of the

ventricular mass is formed by the Left Ventricle, including the *"apex cordis".* The ventricular mass is entirely covered by the Epicardium, which is intimately adhered to the surface of the Myocardium. Subepicardial fat is practically non-existent throughout the extent of the ventricular mass. The Myocardium of the Left Ventricle is thick and more compact than the right Myocardium, which is thin and exhibits a spongy aspect.

Left Ventricular Cavity - The cavity of the Left Ventricle (Fig.5C) is less extensive than that of the Right Ventricle, however, it is more globular and relatively conical, whose apex is in topographic agreement with the apex of the heart. This shape of the cavity makes its volume similar or slightly smaller. The myocardial walls of the Left Ventricle are extremely thick, apparently, about three to four times the thickness of the wall of the Right Ventricle (Fig. 4B-1, 4), similar to what occurs in other carnivores like the dog (TRUEX; WARSHAW, 1942 apud MILLER; CHRISTENSEN; EVANS, 1964). This great thickness of the left ventricular wall is responsible for the resistance to high pressure.

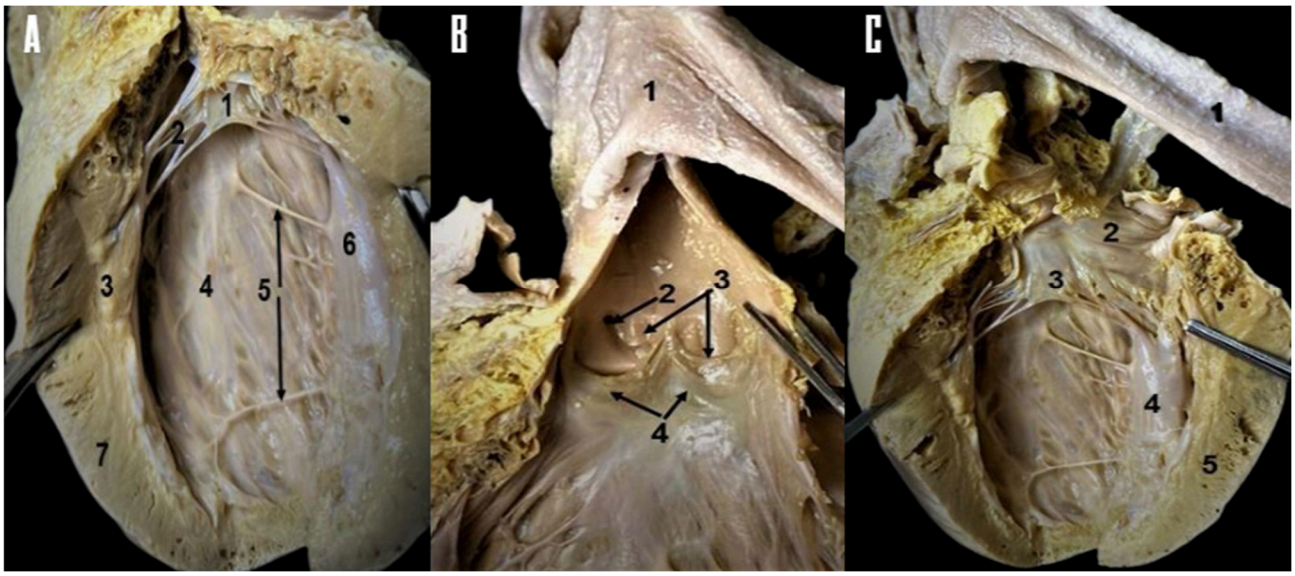

Figure 5. Puma's Heart.
5A. Dorsolateral View of the Open Left Ventricle of the Puma. 1 Ventral Cusp of the Left Atrioventricular Valve; 2- Tendinous Cords; 3- Ventrolateral Papillary Muscle; 4- Interventricular Septum 5- False Tendinous Cords; 6- Ventromedial Papillary Muscle; 7- Myocardium of the Left Ventricle. **5B. Dorsolateral View of the Open Ventricle of the Puma.** 1 Aorta; 2- Ostium of the Left Coronary Artery; 3- Aortic Sinus; 4- Cusps of the Semilunar Aortic Valve. **5C. Dorsolateral View of the Open Left Ventricle of the Puma.** 1-Aorta; 2-Left Atrioventricular Foramen; 3- Cusps of the Tricuspid Valve; 4- Dorsolateral Papillary Muscle; 5- Sectioned Myocardium of the Left Ventricle.

The inner face of the left ventricular walls is less irregular than that of the right ventricle, due to the absence of some fleshy trabeculae and has only two large Papillary Muscles, one Dorsolateral and the other Ventromedial (Fig. 4A-5) The other trabecular formations are slightly pronounced and are oriented approximately parallel to the Papillary Muscles. Each

Papillary Muscle branches out at its end and attaches to several tendinous cords, which in turn attach to the free end of the cusps of the Left Atrioventricular Valve (Fig. 5C-3). Many False Tendinous Cords (Fig. 5A-5) freely cross the ventricular lumen, connecting the wall to the Papillary muscles or different regions of the wall to each other.

The cranial continuation of the left ventricular cavity connects to the Left Atrium through the Left Atrioventricular Foramen, which is surrounded by the Left Atrioventricular Valve, formed by two cusps, one dorsolateral and the other ventromedial whose free edges are fixed to the respective papillary muscles, through Tendinous Cords.

Both papillary muscles of the Left Ventricle are fixed to both cusps, with the Dorsolateral Papillary Muscle (Fig. 5C-4) fixed to the edge of the dorsolateral half of each cusp, while the Ventromedial Muscle (Fig. 5A-6) is fixed to the corresponding half of each cusp.

3.3.3. Cardiac Valves

The valves are divided into Tricuspid or Right Atrioventricular, Bicuspid or Left Atrioventricular (also known as mitral), Pulmonary Valve, and Aortic Valve (SANTOS, 2014).

Right Atrioventricular Valve - The Right Atrioventricular Valve is irregular, consisting of three cusps of different sizes and shapes and serrated edges that connect to the Tendinous Cords and to the base fixed in the Right Fibrous Ring. The three are partially continuous with each other, being identified only by the greater width of the blade at certain points.

Left Atrioventricular Valve - It is similar to its right homonym, however, it is thicker, in line with the larger Papillary Muscles and hefty Tendinous Cords due to the higher blood pressure existing on the left side of the heart, about four times (DUKES, 1955 apud MILLER; CHRISTENSEN; EVANS, 1964).

Aortic and Pulmonary Valves - Both are tricuspid(Right, Left and Dorsal) and Semilunar, whose free edge is facing the vessel and the opposite part, larger fixed in the Aortic Fibrous Ring and in the Pulmonary.

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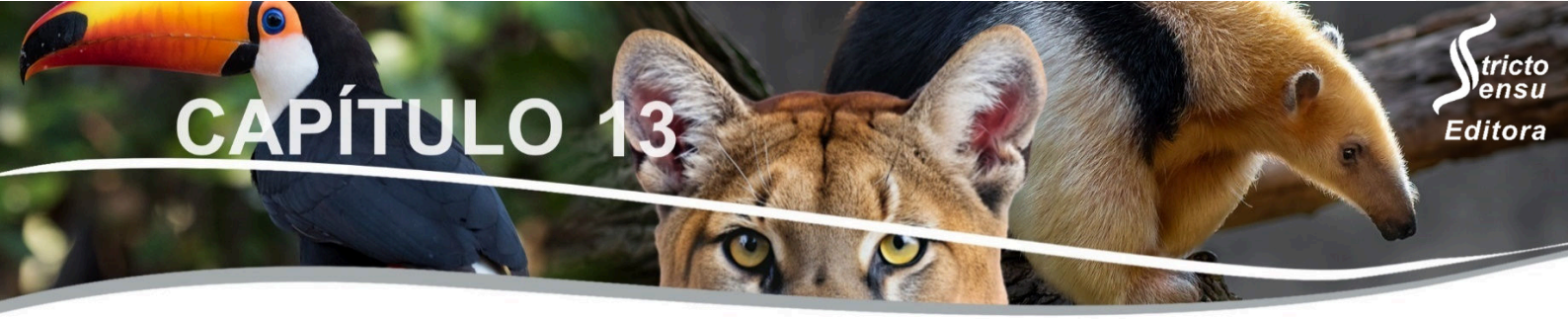

DESCRIPTIVE AND COMPARATIVE ANATOMY OF THE SUPERFICIAL GLUTEAL AND FEMORAL MUSCLES IN PUMA (*Puma concolor* Linnaeus, 1771) AND OCELOT (*Leopardus pardalis* Linnaeus, 1758)

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ABSTRACT

Anatomical investigations on puma (*Puma concolor*) and ocelot (*Leopardus pardalis*) are rare, even though the importance of these species for ecosystems is evident. The muscular anatomy contributes to improve biological knowledge about evolutionary adaptations, such as could have occurred in the family Felidae. Thus, the present study aimed to describe and compare the anatomy of the superficial muscles of the gluteal and femoral regions in ocelot and puma. We used one adult female of each species without a defined age. Both had been donated to the Laboratory of Comparative Anatomy of Wild Animals at the Federal University of Catalão (LACAS-UFCAT) by the Wild Animal Screening Center (CETAS - Catalão-GO) under authorization SISBIO 37072-2. The specimens were fixed in a formaldehyde 10% via femoral artery and kept in the same solution for about three weeks. Gross dissections of the superficial muscles were performed and documented with a photographic camera. The results were discussed with that of other felids. The origin and insertion of the superficial muscles of the gluteal and femoral regions had similarities with that described in puma by other authors and the domestic cat (*Felis catus*). The gluteofemoral muscle was present in both species, such as occurs in the domestic cat. The sartorius muscle was divided into two parts in ocelot differing from puma and domestic cat. Thus, the most superficial gluteal and femoral muscles of puma and ocelot conserve the anatomical arrangement in felids.

Keywords: Insertion, origin and pelvic limb.

1. INTRODUCTION

The ocelot (*Leopardus pardalis*) and puma (*Puma concolor*) are animals belonging to the order Carnivora and family Felidae (NYAKATURA; BININDA-EMONDS, 2012). Both are important felids for conserving the ecological balance of their respective ecosystems as they play the role of predators at the top of the food chain. Furthermore, the presence of these animals serves as a possible preservation bioindicator, considering the necessity of a food source and suitable habitat for their survival (NIELSEN et al., 2015; PAVIOLO et al., 2015; JÚNIOR; CÓSER; STRADIOTTI, 2015).

The ocelot has an appearance marked by well-defined black spots on its fur, varying greatly from gray, yellow and ocher-brown, with the abdomen tending to be lighter. Moreover, it is considered a medium-sized feline due to its slender body, with elongated head and paws and a short tail with black rings. Its weight is approximately on average 15 kg, and can measure between 95 cm and 1.40 cm in length having into account the tail (MACHADO et al., 2005; CUBAS, 2007). It is distributed geographically in Central and South America, occupying diverse habitats. However, despite its wide distribution, the ocelot has significant conservation challenges due to habitat loss, illegal hunting, and road kill (PAVIOLO et al., 2015; MENDONÇA, 2014).

The puma, also known as the "cougar" or "mountain lion", is the second felid largest in the Americas after (*Panthera onca*). The coat has striking characteristics of the species, presenting a uniform brownish-yellow to reddish color throughout almost the entire body, except on the abdomen and medial region of the limbs, which are lighter, while the dorsum is darker (JÚNIOR; CÓSER; STRADIOTTI, 2015). Due to its high adaptability and wide dietary diversity, the puma can be found in a vast area that ranges from the southern United States of America to South America. It occupies habitats ranging from swamps, tropical forests, subtropical zones, and high altitudes. However, like the ocelot, the puma also has challenges due to habitat fragmentation, and conflicts with humans (NIELSEN et al., 2015; AZEVEDO, 2013; JÚNIOR; CÓSER; STRADIOTTI, 2015).

In recent decades, studies that address the morphology of the anatomical aspects of wild animals have increased. Nevertheless, the need for more research on the comparative and descriptive anatomy of these animals is still evident (CUBAS; SILVA; CATÃO-DIAS, 2007). The comparative anatomy of the pelvic limb muscles has been performed in puma (CARO-MUNIZAGA; CONCHA-ALBORNOZ, 2014) and domestic cat (*Felis catus*).

The anatomy of these felines is important for understanding their adaptive characteristics and the development of effective conservation strategies in surgical procedures and well-being care (OLIVEIRA; TEIXEIRA; CONCHALO, 2004). However, anatomical studies in detail about the muscles of these species are scarce, with a significant gap in information, especially concerning the muscles of the pelvic limb. Therefore, this study aims to describe the superficial gluteal and femoral muscles of the ocelot and puma.

2. MATERIALS AND METHODS

We used one specimen of a female ocelot (*Leopardus pardalis*) and one specimen of a female puma (*Puma concolor*), both adults, but without a defined age. Both were victims of roadkill accidents and donated to the Laboratory of Comparative Anatomy of Wild Animals at the Federal University of Catalão (LACAS-UFCAT), by the Wild Animal Screening Center (CETAS - Catalão-GO) under authorization SISBIO 37072-2. They were collected on the highway between Goiás and Minas Gerais near Catalão-GO.

The specimens were fixed with formaldehyde 10% via femoral artery and kept in the same solution for approximately three weeks. To improve the identification of vessels, repletion with colored latex was performed via femoral artery and vein, using red and blue, respectively. The gross dissections were performed according to the dissection guide of the domestic dog (EVANS; DE LAHUNTA, 2017).

The dissection were documented with a 7.2 MP Sony Cyber Shot camera, and the anatomical description follows the Veterinary Anatomical Nomenclature (International Committee on Veterinary Gross Anatomical Nomenclature, 2017). The experimental protocols are carried out according to the guidelines of the Brazilian College of Animal Experimentation and approved by the Ethics Committee on the Use of Animals of the Federal University of Catalão - CEUA/UFCAT nº 01/22.

3. RESULTS AND DISCUSSION

The gluteus superficialis muscle originates from the gluteal fascia, lateral sacral crest, and transverse process of the first caudal vertebra. It inserts distal to the greater trochanter of femur. It is caudal and superficial to the gluteus medius muscle, cranial to the

gluteofemoralis muscle and its distal end was found covered by the gluteofemoralis muscle, mainly in the puma (Figure 1). In contrast, Caro-munizaga e Concha-Albornoz (2014) reported this muscle covered by the biceps femoris muscle.

The gluteus medius muscle originates from the gluteal surface of the ilium and the gluteal fascia, and inserts onto the greater trochanter of the femur, such as that described in the domestic cat (CLAIR, 1982). König e Liebich (2004) describe it originated from the iliac crest and gluteal lines. The tensor fasciae latae muscle partially covers the distal end of the muscle (Figures 1 and 2).

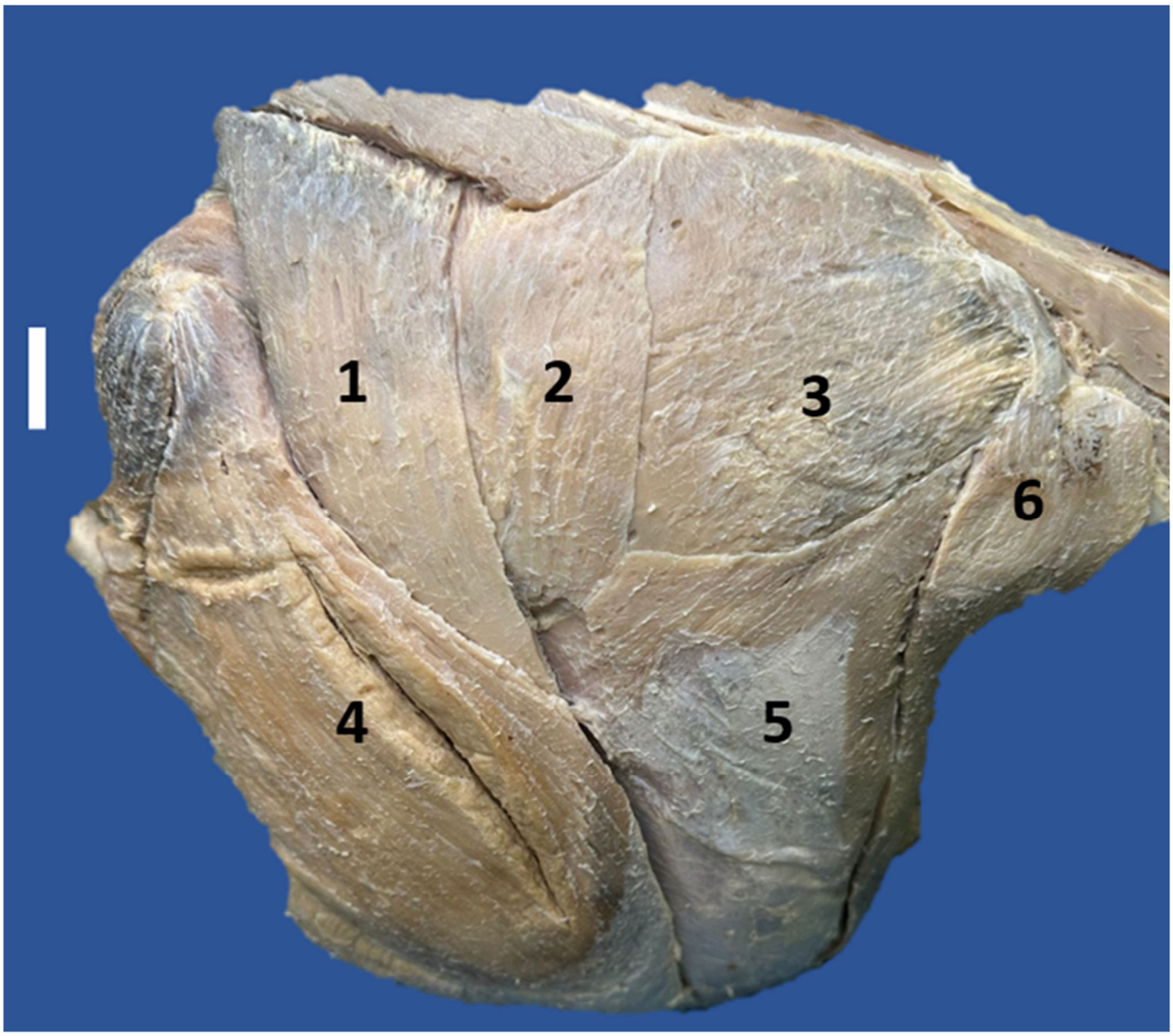

Figure 1. Lateral view of the right thigh of puma (*Puma concolor*).
1- gluteofemoralis muscle; 2- gluteus superficialis muscle; 3- gluteus medius muscle; 4- biceps femoris muscle; 5- tensor fasciae latae muscle; 6- sartorius muscle.

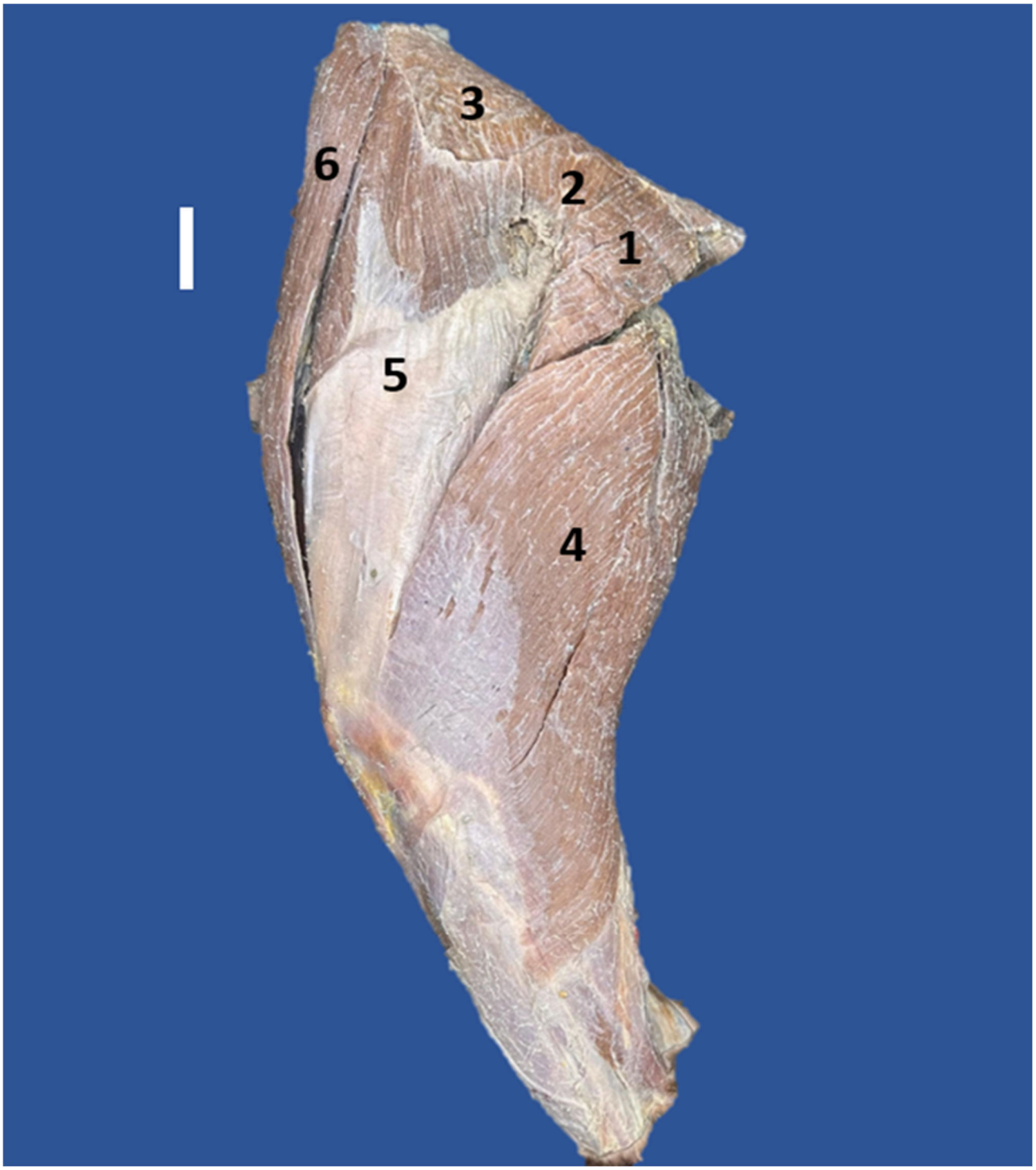

Figure 2. Later view of the left pelvic limb of ocelot (*Leopardus pardalis*).
1- gluteofemoralis muscle; 2- gluteus superficialis muscle; 3- gluteus medius muscle; 4- biceps femoris muscle; 5- tensor fasciae latae muscle; 6-sartorius muscle.

The gluteofemoralis muscle originates from the second and third caudal vertebrae in the ocelot, while in the puma from the second to third caudal vertebrae, such as that described by other authors in puma (CARO-MUNIZAGA; CONCHA-ALBORNOZ, 2014). The muscle is

located between the biceps femoris and gluteus superficialis muscles gluteus (Figures 1 and 2). The insertion was not possible to determine due to the bad conservation of the specimen.

The biceps femoris muscle is the largest and most prominent of the pelvic limb muscles. The division of two parts in the muscle is evident, and there is a clear separation from the first proximal third. It was observed in the puma that the arrangement of the fibers in the two proximal thirds is in a caudo-cranial orientation and in the final third in a cranio-caudal orientation (Figures 1 and 2). Moreover, resistant fascia begins coincidentally with the change in fiber directions. In the ocelot, in turn, the fiber orientation pattern is similar, but the caudo-cranial arrangement limits the caudal head. Besides, the fascia predominantly covers the cranial part. It originates from the ischiatic tuberosity, and inserts via the *fascia latae* into the knee fascia and the crural fascia that attach to the patella, knee ligaments and tibia. It is important to note that in both animals there is participation to the formation of the common calcaneal tendon.

The semitendinosus muscle is long and robust that is found at the caudal femoral region. It originates from the ventral surface of the ischiatic tuberosity in intimate relationship with biceps femoris and semimembranosus. It inserts into the cranial border and proximal third of the medial surface of the tibia, and forms a tendon towards the tuberosity of the calcaneus contributing for the formation of the common calcaneal tendon. Thus, having a diagonal orientation in the latero-medial direction that accompanies the biceps and resumes the direction of its insertion at the height of the popliteal space, as described by König e Liebich (2004) in the domestic cat. An important observation is that the direction of the fibers is evidently observed in the ocelot (Figure 3), while in the puma the condition of the pieces made it difficult to examine the orientation (Figure 4).

The semimembranosus muscle projects in the caudal and medial regions of the thigh. It is related to the semitendinosus and gracilis muscles. A greater width is noticeable compared to the semitendinosus, as it covers part of the caudal and medial region of the thigh (Figures 3 and 4). Caro-munizaga e Concha-Albornoz (2014) describe the origin from the ischiatic tuberosity and ischiatic symphysis. Regarding the insertion, there is the division of the bellies, with the cranial portion inserted into the medial epicondyle of the femur and the caudal portion inserted into the medial condyle of the tibia, such as was described in puma and domestic cat (CARO-MUNIZAGA; CONCHA-ALBORNOZ, 2014; LIEBICH; MAIERL; KÖNIG, 2020).

Figure 3. Caudal view of the left thigh of ocelot (*Leopardus pardalis*).
1- Gracilis muscle; 2- Semimembranous muscle; 3- Semitendinosus muscle.

Figure 4. Caudal view of the right thigh of puma (*Puma concolor*).
1- Semitendinosus muscle; 2- Semimembranous muscle; 3- Gracilis muscle

The *sartorius* muscle is long and flat with two parts, cranial and caudal, the cranial part forms the cranial contour of the thigh and can also be observed from the lateral view of the thigh. In the lateral region of the thigh, the cranial part limits the *tensor fasciae latae* and *gluteus medius* muscles, which are located caudally. In the medial region of the thigh, the caudal belly limits the quadriceps femoris, located medially and the pectineus, located caudally. As described by Caro-munizaga e Concha-Albornoz (2014), in the puma a clear division between the bellies is not observed, however, in the ocelot it is possible to see this division. It originates from the cranioventral iliac spine and thoracolumbar fascia, runs through the medial region covering the quadriceps femoris muscles. It inserts onto the femoral and knee fascia (cranial belly) and inserts next to the aponeurosis of the *gracilis* muscle to the cranial margin of the tibia (Figures 1 and 2).

The *gracilis* muscle is an extensive, blade-shaped muscle located superficially, occupying a large part of the medial surface of the thigh, covering part of the deep muscles of the medial region of the thigh, such as the adductors (Figures 5 and 6). It originates through an aponeurosis on the ventral surface of the pelvic symphysis, such as that described in the domestic cat by König e Liebich (2016). The insertion is via an aponeurosis into the fascia of the leg, and cranial margin of the tibia. From the caudal edge of the aponeurosis, a tendinous band emerges to contribute to the formation of the common calcaneal tendon and ends into the calcaneal tuberosity.

The *tensor fasciae latae* muscle has a triangular shape and is located superficially between the iliac tuberosity and the knee. It originates from the lateral portion of the ilium and insertion tendon of the gluteus medius. The muscle is closely related to the sartorius and gluteus medius muscles. The fascia lata partially covers the quadriceps femoris muscle and continues with a wide aponeurosis located between the quadriceps femoris and biceps femoris muscles. Therefore, the insertion corresponds to the aponeurosis in the patellar ligament. It was evident that in the puma, in which there was a muscular band without fascia covering it, which was not observed in the ocelot (Figures 3 and 4).

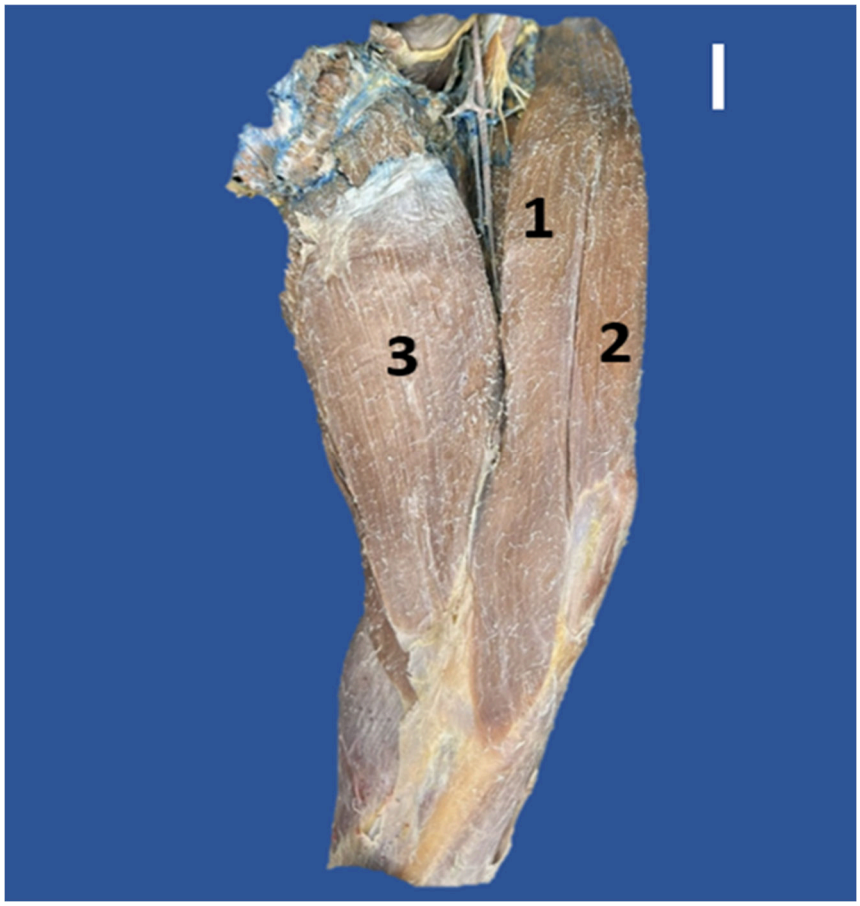

Figure 5. Medial view of the left pelvic limb of ocelot (*Leopardus pardalis*).
1- Caudal part of sartorius muscle; 2- Cranial part of sartorius muscle; 3- Gracilis muscle.

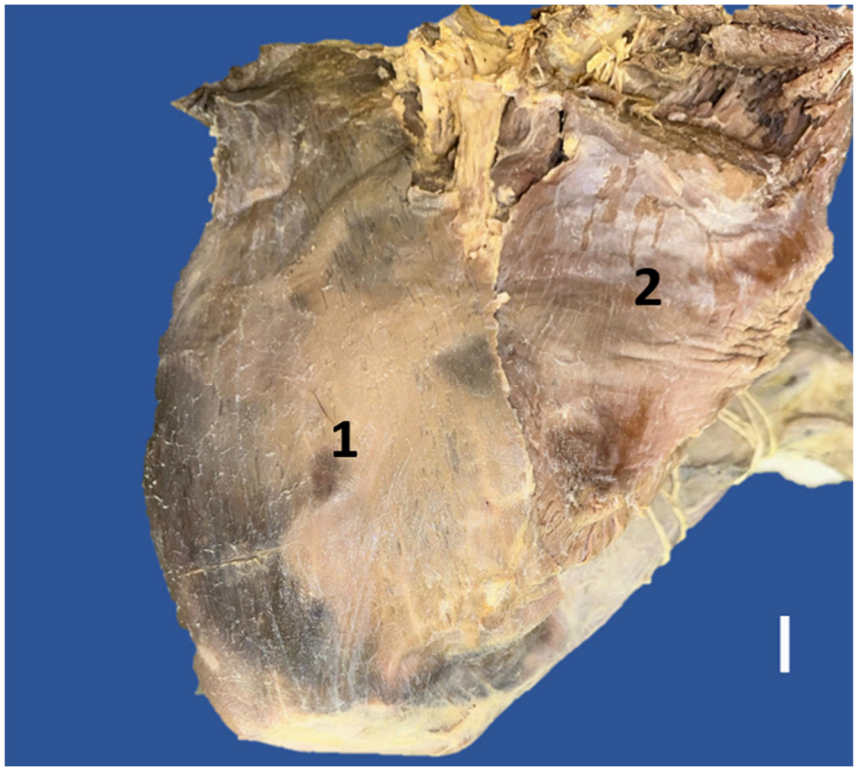

Figure 6. Medial view of the right thigh of puma (*Puma concolor*).
1- Sartorius muscle; 2- Gracilis muscle.

4. CONCLUSIONS

Given the situation in which the specimens were found, some muscle groups were worn out. Therefore, despite attempts to preserve and dissect the pieces, some characteristics could not be described. The importance of studying comparative anatomy between wild and domestic animals is evident. In this study, it was clear that the findings confirmed the similarity between the analyzed species, given their taxonomic proximity. Furthermore, when comparing the two species with the domestic cat, we noticed similarities. The origin and insertion of the superficial gluteal and femoral muscles between the two species are similar in almost all cases, conserving the arrangement of felids. In addition, this study contributes to the understanding of species that have not yet been completely described, and therefore, helps with possible weaknesses that impact the well-being of these species.

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Stricto Sensu Editora

ANATOMICAL DESCRIPTION OF THE ABDOMINAL VASCULARIZATION OF THE OCELOT (*Leopardus pardalis* Linnaeus, 1758)

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ABSTRACT

Anatomy is an important tool in understanding the morphology of wild species, especially those threatened, such as felines like the Ocelot (*Leopardus pardalis*). In this context, studying anatomy allows us not only to get to know these animals better, but also to propose better strategies for treating and promoting their health. The objective of this work was to study the descriptive anatomy of the abdominal aorta and its branches and the inferior vena cava with a focus on the Ocelot. The study was developed from the dissection of two specimens of Ocelot (*Leopardus pardalis*), donated by the Wildlife Research Institute – IPEVIS and the Wild Animal Screening Center -CETAS of Catalão. In our study, we obtained a good exposure of both arteries and veins in the Ocelot. The results found were in line with other partial studies addressing this animal and other wild felines, as well as the anatomy of domestic cats, in addition to being possible to report an important anatomical variation in the venous vascularization of the right kidney. The results obtained corroborate the great importance in terms of knowledge and preservation of the species, making it possible to expand the horizon for a therapeutic approach to species that are already threatened.

Keywords: *Leopardus pardalis*, vorta artery and vena cava.

1. INTRODUCTION

The study of animal anatomy is an important tool both in understanding and developing strategies for the preservation of threatened species. Comparative anatomy allows for the comparison and correlation of the form and function of anatomical structures across different taxonomic groups. In this context, the proposal to study the anatomy of wild animals comparatively aims to discuss possible anatomical similarities among different groups, thereby facilitating a better understanding of evolutionary relationships (OLIVEIRA; TEIXEIRA; CONCHALO, 2004).

Within the scope of wild felid species present in the Americas, it is noteworthy that jaguars, pumas, and ocelots face similar challenges to their survival (WULTSHC et al., 2016). In the case of the ocelot, a medium-sized spotted cat ranging from the southern United States to northern Argentina, there are records of its presence in a wide variety of habitats, playing an essential role in the dynamics of the ecosystems it inhabits (ROCHA et al., 2016; MACHADO et al., 2020). Current literature analysis reveals a scarcity of studies related to these felids, with an even greater gap concerning ocelots. There is an evident lack of knowledge in morphological and anatomical studies that need to be addressed (MACHADO et al., 2020, WANG et al., 2019).

Considering the lack of studies on these animals, the descriptive anatomy of wild animals has been of great interest worldwide. As research progresses, new findings emerge about the vulnerability of various species, primarily due to anthropogenic actions on their habitats.

Regarding cardiovascular structures, it is known that the cardiovascular system is responsible for supplying and maintaining sufficient, continuous, and variable blood flow to various tissues of the body. This system includes the heart and its blood vessels. Arteries, capillaries, and veins form a continuous system lined with uninterrupted endothelium (GARCIA FILHO et al., 2012). The cardiovascular system exhibits significant variations among species, reflecting evolutionary adaptations to habitat demands and ensuring internal and external homeostasis for each species.

Such information can impact the veterinary management of these species, supporting improved conservation strategies and approaches. Therefore, this study aimed to describe the abdominal vascular arrangements of the ocelot (*Leopardus pardalis*) and compare them with information available in the literature on this and other wild and domestic felids.

2. MATERIALS AND METHODS

This study was conducted at the Laboratory of Comparative Anatomy of Wild Animals at the Federal University of Catalão (LACAS-UFCAT). The study was conducted on the carcasses of two specimens of ocelot (*Leopardus pardalis*), donated to the Laboratory of Comparative Anatomy of Wild Animals at the Federal University of Catalão (LACAS-UFCAT) by the Wildlife Research Institute – IPEVIS and the Wildlife Triage Center – CETAS of Catalão, both collected from the roadside in Goiás or Minas Gerais (Authorization - SISBIO 37072 – 2). To enhance vessel identification, Latex “Arte Cola”, colored red for arteries and blue for veins, was injected. Formaldehyde solution at 10% concentration was used for fixation via perfusion, through the jugular vein, supplemented by intramuscular and intracavitary injections as needed. The carcasses were submerged in the same formaldehyde solution for preservation.

The specimens were meticulously dissected using standard macroscopic anatomical techniques, employing scalpels, scissors, and other instruments. Dissection began with the opening of the thoracic and abdominal cavities to expose the targeted vessels. Membranes and other tissues covering and potentially obstructing the view of the targeted structures were removed during dissection.

After dissecting and removing surrounding tissues, anatomical specimens were photographed using a Cyber-Shot camera with a 7.2 megapixel. The results were described following the Veterinary Anatomical Nomenclature (International Committee on Veterinary Gross Anatomical Nomenclature, 2017).

3. RESULTS

In the abdominal vasculature, our study utilized an ocelot (*Leopardus pardalis*) specimen for dissection. This allowed for a detailed examination of both arterial vascularization and venous drainage. Regarding arterial flow, following from the abdominal aorta (Figure 1), the first branch is the celiac trunk, followed below by the larger superior mesenteric artery, which supplies the majority of the gastrointestinal tract. Notably, the artery courses below the level of the inferior vena cava, and the branch to the superior mesenteric artery crosses over the inferior vena cava above the kidneys.

Figure 1. Arterial flow.
a – Aorta, b – Celiac artery, c – Cranial mesenteric artery, d – Vena cava, e – Right renal artery, f – Left renal artery g e h – right renal veins (anatomical variation), i – Left renal vein, j – Left gonadal vein, m – r.a. Right ureter, n – r.a. Left ureter, K – r.v. Right ureter, L – r.v. Left ureter, 1– Rignt Kidney, 2 – Left Kidney, 3 – Right ureter, 4– Left ureter.

Continuing its course, just below, the abdominal aorta branches into the right and left renal arteries (Figure 1). Regarding venous drainage, the inferior vena cava runs right of the abdominal aorta, with the phrenic-abdominal vein observed at a higher level draining into the inferior vena cava. Shortly after, the right and left renal veins follow into the vena cava. An anatomical variation was noted in the right renal vein (Figure 2), where three branches were observed originating from the renal hilum, two of which were larger and one smaller. In the left renal vein, it is also evident that the gonadal vein joins it at a 90-degree angle.

Following the vascularization of the abdominal aorta artery (figure 3), below its branching to the renal arteries, it runs laterally to the inferior vena cava, on the right side and divides on its anterior surface, firstly into the right and left gonadal branches and then into the inferior mesenteric artery. Regarding venous drainage, at the level of the gonadal arteries, the right gonadal vein flows into the inferior vena cava. Laterally, the circumflex iliac arteries are observed leaving the abdominal aorta artery and the circumflex iliac veins flowing into the inferior vena cava.

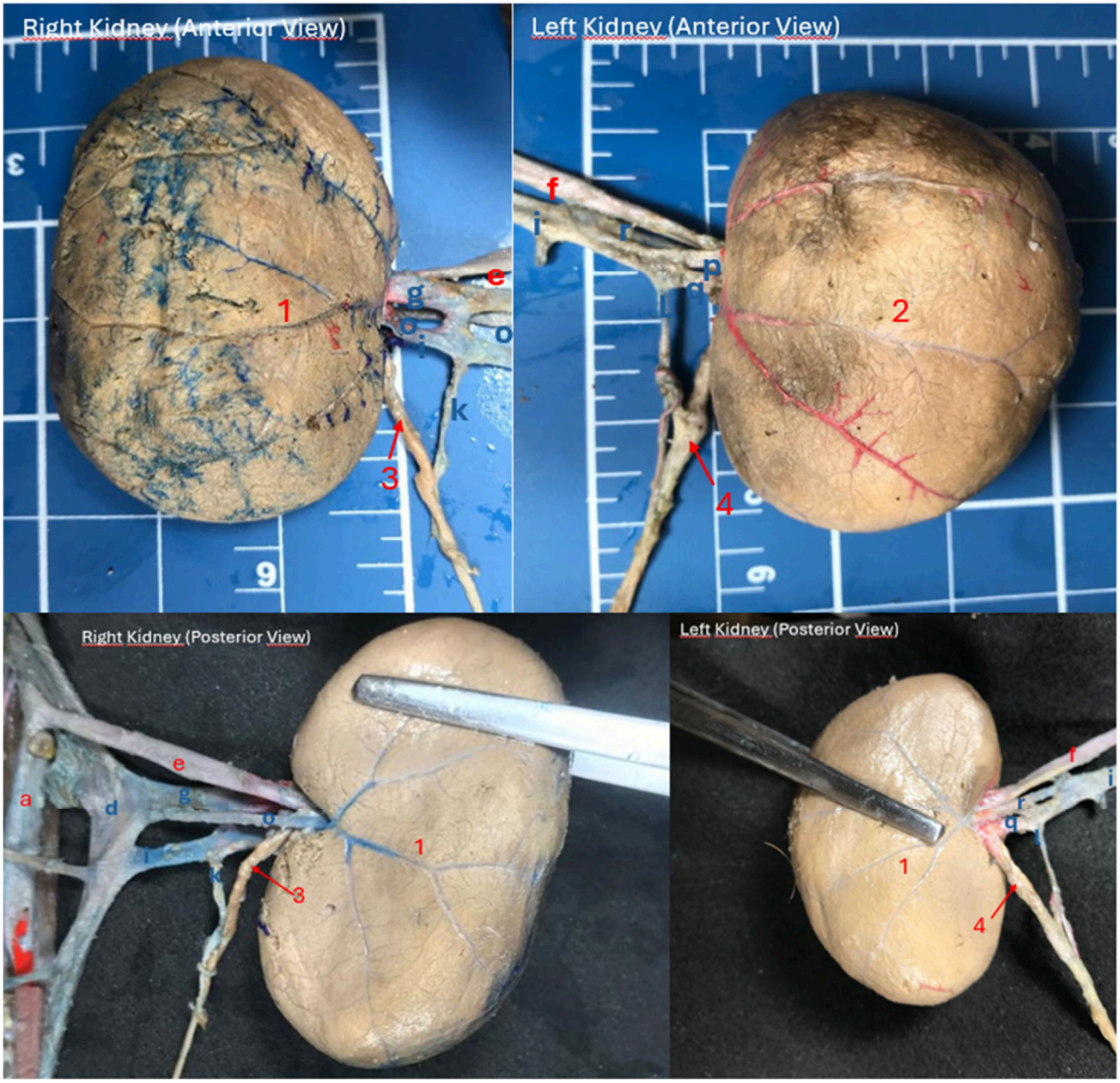

Figure 2. Veia renal.

1 - Right kidney g - Cranial renal vein, i - Caudal renal vein, o - Dorsal vein, 3 r.v ureter, d - Inferior vena cava, a - Aorta artery, e – Renal artery. 2 - Left kidney: i - Renal vein, p - Cranial renal vein, q - Cadal renal vein, r - Dorsal renal vein, L - r.v. ureter.

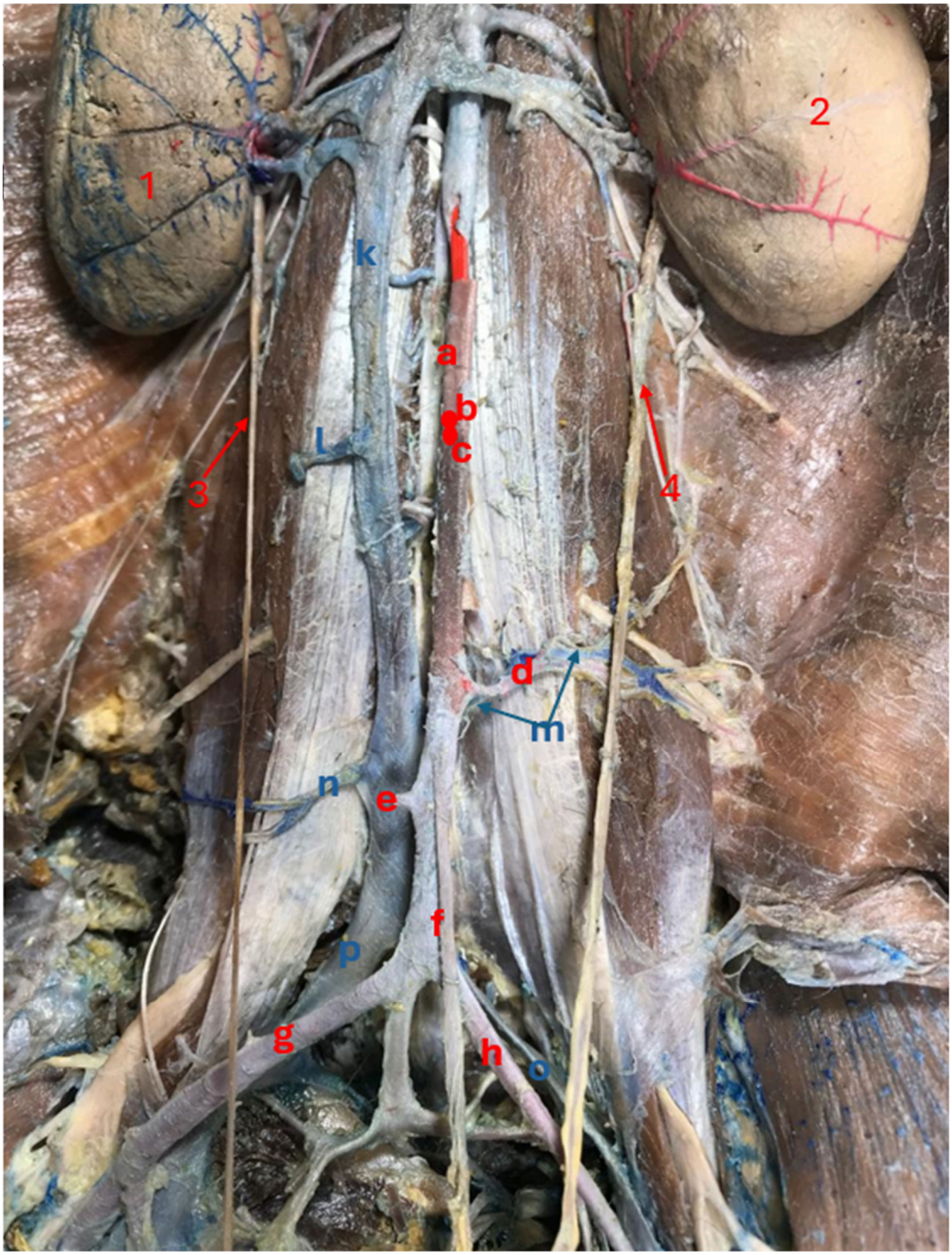

Figure 3. Arterial vascularization.

a – Abdominal aorta, b e c – Gonadal artery ostia, d – Left circumflex artery, e – Right circumflex artery, f – Caudal mesenteric artery, g. Right external iliac artery, h – a. Left external iliac artery, i – a. Right internal iliac artery, j – Left internal iliac artery. Venous drainage: k – Caudal vena cava, l – Right gonadal vein, m – Left circumflex iliac vein, n – Right circumflex iliac vein o – Left common iliac vein, p – Right common iliac vein, 1 – Right Kidney, 2 – Left kidney, 3 – Right ureter, 4 – Left ureter.

Finally, at the pelvic level (Figure 3) the abdominal aorta artery is evident, leaving anteriorly to the inferior vena cava to divide into the right and left external iliac arteries and, starting from a branch running between them, divides into the internal iliac arteries right and left. Regarding venous drainage, it is observed that the inferior vena cava runs posteriorly to the abdominal aorta, where it receives tributaries from the right and left common iliac veins.

4. DISCUSSION

The mammalian aorta is an elastic artery in which the tunica media is formed by four main components: elastin, collagen, smooth muscle cells and a non-fibrous matrix. Living tissues can adapt to mechanical demands, so that specific structures throughout the aorta vary to respond to the physiological wave of blood tension of each species (GARCIA FILHO et al., 2012).

It must be considered that, due to the scarcity of studies on wild felines, the anatomy of domestic animals such as dogs and cats is generally used as a reference. Regarding the anatomy of the ocelot's abdominal aorta, the results were similar to the study by Pinheiro et al., (2014), which described the collateral branches of the abdominal aorta in the ocelot, with the abdominal aorta emerging into the abdominal cavity after crossing the aortic hiatus between the pillars of the diaphragm, and emitting ventrally and sequentially, the celiac artery and the cranial mesenteric artery, highlighting, also, an abdominal arterial vascularization quite similar to that described in domestic felines.

In another study evaluating domestic cats, a pattern similar to that of the ocelot was found, with a sacral vascularization branching towards the internal iliac artery distally to the branching point for the external iliac artery, with no variation between the sexes in this regard and, however, presenting a wide variation in terms of length of the arterial segment between the internal and external iliac branches, without association with its width, or the size of the animal (GONZALES et al., 2014). In association with the anatomy of the sacral branches of domestic cats (*Felis catus*), Geraldo et al., (2013) also found the same pattern as that of the ocelot while. On the other hand, in the study of the domestic dog, an inverted pattern in relation to available to these vessels was found, being that in felines the aorta crosses above the inferior vena cava before branching, while in dogs it passes beneath the vena cava (MOMPEÓ et al., 2020).

Regarding venous circulation, there were not many studies found in the literature addressing wild felines, and it is even scarcer when we talk about anatomical variations, as seen in the one found in our study, with triple venous drainage in the right kidney. In the study carried out by Abidu-Figueiredo et all, 2018, two cases were reported with double venous drainage in the right kidney in shorthair cats in Brazil (*Felis Catus*), associated with cats with a circumcaval ureter, which is also a rare variation in these felines. In another study, carried out by Korim, Kuricová and Erbelová (2023), an interesting change was also reported in domestic cats, such that an abnormal formation of the central venous circulation was found with two separate, symmetrical, right and left caudal vena cava, that joined between L2 and L3, under the caudate process of the liver. Such findings demonstrate the vascular potential of felines to present anatomical variations.

The study of animal anatomy may be relevant in related cases of endangered felines that require a more invasive approach to treatment aimed at preserving the species, as well as being of fundamental importance for knowledge about the animal's body structure, given the scarcity of studies in this context intended for these animals (DIAZ et al., 2021; MACHADO et al., 2020). A better knowledge of these structures enables a better approach to less traumatic and more targeted progressive therapies, in addition to having greater potential to improve the quality of life of these animals. Currently, macroscopic anatomical descriptions of the canine and feline abdominal and thoracic vasculature have been extensively detailed in the veterinary literature, however, this fact is not reflected when the subject is the study of wild felines (MOMPEÓ et al., 2020).

Therefore, it is pertinent to emphasize the importance of the anatomical and morphological description of the blood vascularization of felines, in order to fill gaps that often hinder surgical management or comprehend the clinical and pathological aspects of these animals. Therefore, the importance of further studies on these species in question must also be emphasized, to generate contributions to the maintenance of the biodiversity and species preservation.

5. CONCLUSION

Given the numerous threats to wildlife, a deeper understanding of the biology and anatomy of these felids is essential. In our study of ocelot specimens, we have observed that this species exhibits anatomical differences from domestic animals such as dogs, and

anatomical similarities to cats, which are more commonly documented in the literature. Moreover, there may be anatomical variations within the species, as identified in the dissection of the renal vasculature of the ocelot. Therefore, we highlight the need for further investment in studies on these animals to facilitate new veterinary approaches for their treatment and preservation and enhance our anatomical and pathological knowledge of them.

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Stricto Sensu Editora

PREPARATION OF DIDACTIC MATERIAL OF THE SKELETAL SYSTEM BY MACERATION

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ABSTRACT

The skeleton, composed of bones and adjacent connective tissue, provides essential information about the species studied, such as support and locomotion. Intended for didactic and formative use, osteotechniques are anatomical techniques to properly prepare a skeleton. This chapter presents different methodologies, which can be combined or not, to obtain high-quality material of the skeleton through maceration. In brief, the maceration process consists of removing excess parts of soft tissue from a given specimen for a complete and detailed visualization of its skeletal structure. For mechanical (physical) maceration, this process is conducted with the aid of surgical material to manually remove all soft tissues attached to the skeleton of the study specimen. Biological maceration is performed with the help of living beings that consume the soft tissues and reveal the skeleton, the most common of which are beetles of the genus *Dermestes*. Finally, chemical maceration is usually done after physical and/or biological maceration using multiple possible chemical reagents, such as hot water and hydrogen peroxide; and some less conventional alternatives such as bromelain and papain. Although it is possible to use a single type of maceration to obtain the final skeleton, initial mechanical maceration and chemical or biological maceration are generally used to complete the process. The final specimens are high-quality skeletons rich in detail and excellent for teaching and learning this important component of the body.

Keywords: Anatomy teaching, Osteotechnique and Anatomical techniques.

1. INTRODUCTION

The skeleton forms the framework of the body and is important to protect crucial viscera and to supply structure for muscular and articular fixation. The bones can indicate, through their morphology, a direct indication of the soft tissues of the living individual, and even though these disappear postmortem, the skeleton remains (WHITE; BLACK; FOLKENS,

2011). Thus, such a structure can provide a lot of information regarding the specimen to whom it belonged. Therefore, knowing the skeleton is essential for a complete understanding of a species.

As an object of anatomical investigation, the skeleton, composed of bones and adjacent connective tissue, provides valuable information about the investigated species, such as posture, locomotion, and support (HILDEBRAND; GOSLOW, 2006; SILVEIRA; TEIXEIRA; OLIVEIRA, 2008). To be successfully used in a formative and didactic form, the skeleton must be adequately prepared. Osteotechniques are a set of different anatomic techniques for this aim and combine disarticulation, maceration, whitening, and assembling of the skeleton (AIMI et al., 2022).

There are diverse factors relating to the bones that must be considered for the correct preparation of skeletons. These include the decision between disarticulating the body parts completely or preserving cartilage, connective tissue, and joints, depending on the end goal. Thus, the didactic objective of the desired pieces must be completely clear before technique selection (SILVEIRA; TEIXEIRA; OLIVEIRA, 2008). The present chapter exposes different maceration techniques, which can be either combined or used alone, to obtain final skeletons that are suitable for study, teaching, and research material.

2. MATERIALS AND METHODS

The maceration demands as the main component, the skeleton of the desired specimen, preferably fresh and not formalized, either as a whole individual or as a carcass. In addition, individual protection items are also mandatory, such as gloves, lab coats, and protective masks and glasses. The specific material demanded for each technique depends on the maceration technique and will be informed in each section, along with the description of the osteotechnique. In brief, for mechanical maceration scalpels and blades, surgical scissors and clamps are used; for biological maceration, the biological agents that consume soft tissues are mandatory, and are usually insects; and for chemical maceration, diverse chemical reagents that aid soft tissue removal and detachment are used.

2.1. OSTEOTECHNIQUES: MACERATION

The dissection and removal of humid, soft parts of the piece can be detached from the skeletal structure by three main maceration methods: mechanical, biological, and chemical maceration. To obtain macerated pieces, it is ideal that there is no previous contact with formaldehyde. Fixation completely precludes the conduction of biological maceration, due to direct harm to the biological agents, and can negatively impact chemical maceration. For mechanical maceration, the previous fixation is indifferent. Hereafter, each of the three maceration methods will be discussed in detail to highlight their applications.

2.2. MECHANICAL MACERATION

Maceration consists of removing excessive skin, muscle, membranes, nerves, and viscera from the body to generate a complete and detailed visualization of its bone structure (SILVA et al., 2023). For mechanical maceration, this process is conducted with surgical material, and a manual and thorough removal of all soft tissues is conducted.

The first step is the inspection of the body in the search for possible trauma and fractures, followed by biometry and weighing. Mechanical processes are the ones for fat, muscle, and cartilage removal by instruments such as gloves, anatomical clamps, blades and scalps, scissors, and, if necessary, knives (SILVA et al., 2023). Initially, the skin and superficial muscles are cut and the viscera in the cavities are removed. Then, all soft tissues covering the bones are gradually removed, and as dissection progresses, it is possible to disjoint each bone from the skeleton. Depending on the aim, it is possible to maintain parts of the skeleton articulated, such as the upper/thoracic cingulate bones, lower/pelvic cingulate bones, rib cage, and so on (MARTINS et al., 2021).

In between the maceration sessions, depending on the total size of the skeleton and considering it can demand more or less total dissection time, it is possible to store the bones and the carcass in pots filled with water. This approach aims at softening the muscles and ligaments and making the manual cleaning process easier. When manual cleaning cannot be completed, it is also possible to freeze the carcass, and the freezing process facilitates the loosening of tissue layers and fat after defrosting (LOPES et al., 2019; SANTOS et al., 2022). Mechanical maceration is commonly the first treatment that anatomical pieces are submitted to prepare a skeleton. Although it is possible to obtain a skeleton using only mechanical

maceration, in most cases other techniques will be combined to achieve the result (SILVEIRA; TEIXEIRA; OLIVEIRA, 2008).

According to Silveira et al. (2008), this technique presents low cost and the total time to prepare the pieces is usually short, and it can be done in less than one month. The resulting skeleton is durable and requires no maintenance. Furthermore, as it does not involve chemical processes, it is a technique with a low risk of toxicity. Manual maceration can be more sustainable in terms of energy and resource consumption, as it does not require the use of energy-consuming electrical or mechanical equipment. This can be advantageous in areas where resources are limited or expensive. It also demands less investment in specialized equipment, making the technique an accessible option for laboratories and institutions with limited budgets and resources. Another advantage relates to precision control since operators have absolute control over the mechanical maceration during the whole procedure, which enables the conduction of any necessary adjustments depending on the specific necessities of each specimen. Precision control is particularly important and useful in situations where delicacy is necessary, such as in the preservation of fragile and rare specimens. In addition, all manual procedures can be easily adapted to deal with different types of materials or conditions of the specimen.

This way, operators can manually adjust the intensity, duration, and other process parameters as necessary, providing work flexibility. In some cases, manual maceration can be gentler and more delicate, reducing the risk of damage to specimens. This parameter is especially important when dealing with valuable samples that require special care. Manual maceration allows operators to interact more closely with the material, which can make it easier to identify problems or anomalies during the process. Additionally, this helps ensure a more complete and thorough cleaning of the parts.

Aside from all the advantages, there are also some limitations to mechanical maceration. A major factor is the limitation regarding the complete cleaning of the pieces. In some cases, even after the process, some organic residues may still persist, resulting in Strong, adverse odors. In addition, the pieces may present a degree of oiliness and a darkened color due to fat tissue and residual pigments in the bones. Such effects can be attributed to the incomplete removal of soft tissues during the mechanical process, and a way to overcome this inconvenience would be the combination of complementary methods of cleaning the hard tissues, thus achieving more satisfactory results.

2.3. BIOLOGICAL MACERATION

Biological maceration can be conducted using living beings that prey on soft tissues and demonstrate the hard tissues, and thus the skeleton. In this type of maceration, bones, ligaments, cartilage, and connective tissue can be preserved while the remaining muscles are removed. Most researchers using biological maceration employ insects, and the most commonly used are beetles (Coleoptera), from the family Dermestidae, usually within the genus *Dermestes*.

Beetles constitute the second group of insects of highest forensic interest in Brazil (RODRIGUES et al., 2012). Other insects, such as fly larvae, can also be employed (SIQUEIRA; DA SILVA; SOUZA, 2015). For biological maceration, a constant verification of the process is mandatory, considering that in case no more soft tissues are available, the insects will consume fewer hard tissues, such as ligaments. Biological maceration is of high importance in the case of smaller skeletons and to clean locals of difficult access for physical maceration, such as the cranium (RODRIGUES et al., 2012).

The most employed beetles for maceration are dermestes, vastly geographically distributed, and in nature prey on residual soft tissues in the carcasses of dead animals. It is possible to collect individuals from nature and then proceed to start a colony in the laboratory, in adequately prepared vessels. A well-trained biologist or related professional can Harvest carcasses and identify the species. Maintenance and verification of the well-being of the colony must be conducted constantly, preferably daily, to confirm the availability of food. At times when there is no available skeleton to be prepared, it is possible to offer the insects animal material, in small quantities, and avoid viscera, which can putrefy before are consumed and attract other necrophagous animals (SIQUEIRA; DA SILVA; SOUZA, 2015).

Dermestes are holometabolous insects, meaning that they present four different main stages of differentiation during their life cycle. Those are egg, larva, and adult. Larvae prey on corpses and form two to five-mm holes in the muscle tissue of the carcass and create an irregular network (MAGNI et al., 2015). An example of immature individuals and adult beetles is shown in Figure 1. During this process, the mandibles of these larvae may carve grooves into the cartilage and, on some occasions, into the edges of bones (ZANETTI; VISCIARELLI; CENTENO, 2014), reinforcing the need for constant verification of biological maceration in the laboratory.

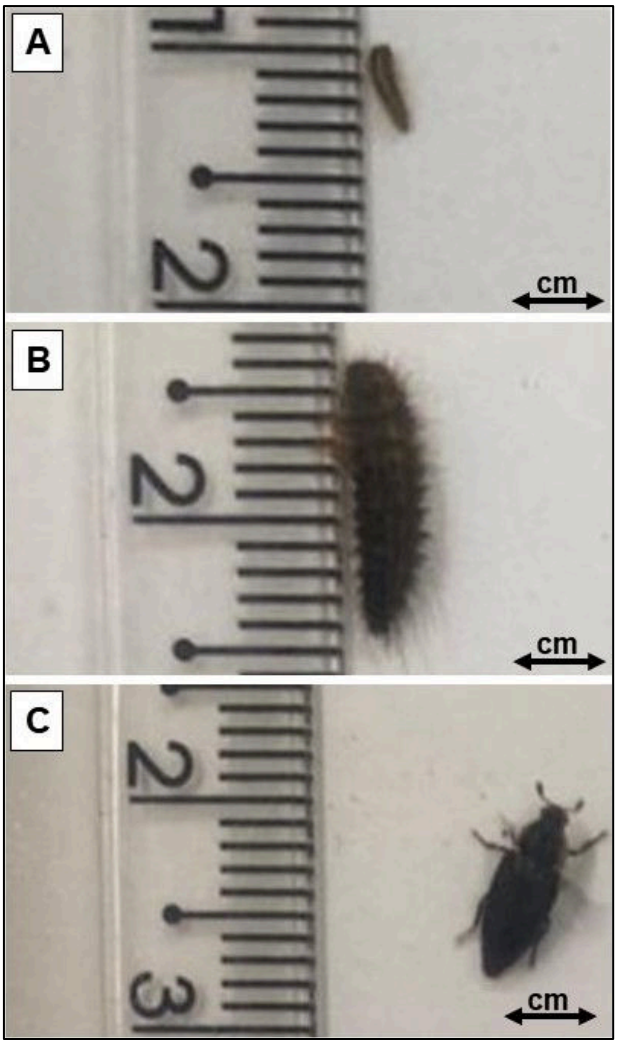

Figure 1. Life cycle of dermestes (*Dermestes maculatus* DeGeer, 1774).
A) Immature individual, in initial larval stage; B) Immature individual, in terminal larval stage; C) Mature individual, in adult stage. Personal collection.

This genus of beetles prefers to feed on fresh meat. Still, because of competition between fly larvae, which can rapidly take over the fresh carcass, the beetles may feed once the fly larvae can no longer survive, meaning that the carcass is already dry. The colony can be fed with fresh or dry material (HINSHAW, 2023). However, dermestes do not like to feed on internal organs, skin, or feathers (SANGER-CIARLEGLIO et al., 2020). As long as there is food available, multiple generations can persist (GATTA et al., 2021).

In the laboratory environment, dermestes are of high value for maceration, particularly in delicate and fragile skeletons, difficult to clean through physical maceration, and when the skeleton and bone structure are intact (SIQUEIRA; DA SILVA; SOUZA, 2015). The demanding time to clean the skeleton depends on the total size of the specimen and the number of animals – in particular, larvae – in the colony (FRANCO et al., 2001). Adult

dermestes also prey on the carcass and help clean, but they consume less material than immature individuals.

As essential care to obtain a high-quality skeleton using dermestes is the need to offer clean anatomical pieces in the vessels containing the insects. The removal of tissues must be manually conducted, with the removal of viscera, skin, and most of the muscular tissue, as long as dissection does not harm the skeleton. The clean carcass must be then placed on cotton, lining the entire container (SIQUEIRA; DA SILVA; SOUZA, 2015).

According to Gomes and Mendes-Oliveira (2015), the vessel used to assemble the dermestary can be made of wood, glass, or plastic. The vessel must be covered with a lid, but allow air flow, so that the lid must contain an opening covered with a thin nylon screen (about 1 mm). The lid avoids the exit of dermestes and prevents undesired animals from entering the dermestary. The suggested size of the vessel is 30 L, and this size holds a reasonable colony and allows easy removal of the carcass for cleaning and moving.

It is important to add materials to serve as a substrate and a sort of nesting material for dermestes (GOMES; MENDES-OLIVEIRA, 2015). The utilization of hydrophilic cotton with around four centimeters high so that the animals can make tunnels and protect themselves during incubation periods. The nesting material also serves as a shelter against a higher incidence of light. Cotton also helps maintain temperature and humidity parameters stable and suitable. The need for water in the colony must be suppressed by spraying water using a sponge inside the vessel (PAHL, 2020).

To know the demanding conditions in detail to maintain the colony healthy, to guarantee a sustainable system. For colonies maintained in closed environments, the temperature must be set between 21 and 26ºC and humidity at 50% to guarantee that the individuals remain healthy (SANGER-CIARLEGLIO et al., 2020). In locations Where the climate is colder and drier, it is necessary to use a heating system with indirect light and artificial humidity. To guarantee the reproduction of an environment that resembles the natural environment of these beetles, it is necessary to avoid clarity, maintain the vessel in a dark place, and use luminosity only at the time of colony manipulation (GOMES; MENDES-OLIVEIRA, 2015). An example of dermestary is shown in Figure 2; in our facility, we have been maintaining the colony for two years – and have already derived over ten colonies from the original one.

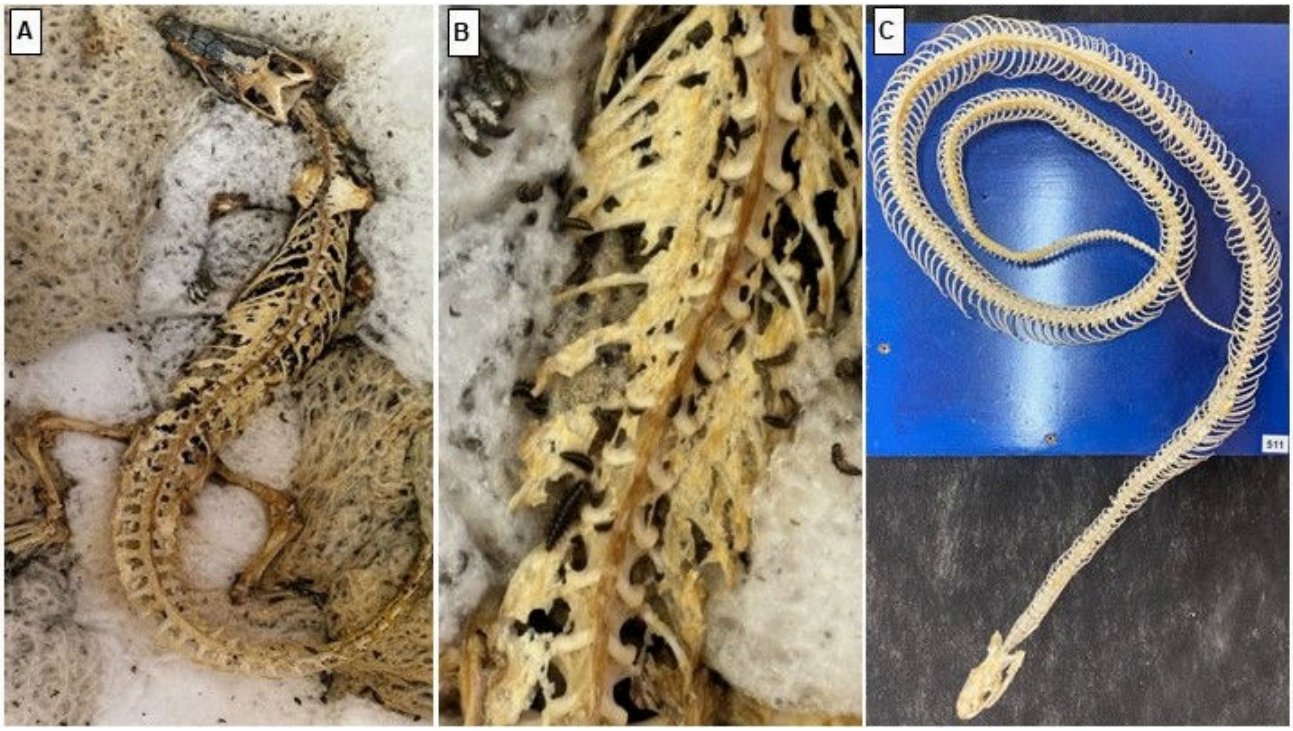

Figure 2. Preparation of skeletons by biological maceration using dermestes.
A) Lizard (Tupinambis sp.) carcass being prepared by maceration using dermestes; B) Closer amplification of the biological process; C) Corn snake (*Pantherophis guttatus*) skeleton prepared using biological maceration with dermestes.

The cleansing of the dermestary must be conducted whenever the substrate is filled with waste; however, it is not indicated to clean the vessel too often since the often manipulation can negatively affect the colony. In case there is overpopulation, it is recommended to divide the colony or to euthanize excessive individuals. It is important to pay attention to the length of time for the pieces to remain under the action of the dermestes, as there is a risk that cartilaginous parts and very delicate bones will be lost due to the lack of food. A daily verification of the process is indicated, and the skeleton should be removed from the vessel as soon as it is clean enough (GOMES; MENDES-OLIVEIRA, 2015).

In the laboratory environment and under controlled conditions for cleansing and preparation of skeletons, dermestes have been used in museums for decades (AURICCHIO, 2002). On the other hand, if not maintained under control, dermestes can be harmful to the collection, having the potential to become powerful degradation biological agents (OLIVEIRA, 2010). In the laboratory, the carcass is dehydrated using ethanol 70% for 48 to 72 hours, allowed to air dry or dry in a forced air greenhouse, and then placed in the vessel with the colony. To remove the larva from the skeleton, ethanol 50% or ammonium hydroxide can be used; other options are hot water, deep freezing, or sodium perborate (PAHL, 2020).

Cleaning skeletons with the help of dermestids is extremely effective, in addition to being viable and practical, even compared to chemical maceration techniques. In chemical maceration, it is necessary to verify the best solutions and concentrations to achieve the desired result and avoid damage to the bone tissue (SILVEIRA; TEIXEIRA; OLIVEIRA, 2008). In addition, the final skeleton after the action of dermestes tends to be completely cleaned and can be kept fully articulated, facilitating the assembly of the skeleton. In addition, there is no fetid odor exhaled. The total time necessary to obtain a clean skeleton is shorter in comparison with other types of osteotechniques. The cost is also low, considering that the animals can be harvested from nature, and are very low cost to maintain in the laboratory.

The first disadvantage is the difficulty in managing the dermestes appropriately. In our case, we acquired some individuals from an already well-established colony with a fellow researcher, who was very helpful in guiding basic care for maintaining a healthy colony. Key factors to be considered to sustain the colony are: checking cotton to completely line the grow box; constantly checking for enough food for the colony; checking the number of individuals - and possible creation of new colonies, in new boxes, if necessary, or even euthanasia (in a freezer), to dispose of supernumerary individuals (SIQUEIRA; DA SILVA; SOUZA, 2015). Moreover, dermestes are not cold-resistant. In locations Where the average climate presents lower temperature, it is necessary to artificially regulate environmental temperature (GOMES; OLIVEIRA, 2015).

The biological maceration using fly larvae is mostly conducted outdoors, in the shadow, allowing flies to lay eggs in the carcass. The decomposition process must be followed daily, or on alternate days. As an advantage, the cost is almost null, since the carcass can be allocated in nature, and the total time to achieve the result is usually short (SIQUEIRA; DA SILVA; SOUZA, 2015). It is ideal to maintain the carcass outdoors because it facilitates the maintenance of humidity. In addition, this maceration liberates an aversive odor exhaled, and it is less inconvenient and less disturbing if it is not indoors. This putrid odor is a severe disadvantage and is caused due to decomposition. Also, the resulting skeleton presents a darkened color. After the action of the insects – either using beetles or flies –, bones are collected and cleaned using acetone and ethanol (SIQUEIRA; DA SILVA; SOUZA, 2015). And considering the disadvantages of using fly larvae, the best strategy to conduct biological maceration is the use of dermestes.

2.4. CHEMICAL MACERATION

Chemical maceration is usually used after physical and/or biological maceration. Its main function is to extract the remaining cadaveric leavings which are still attached to the skeleton. In addition, some chemical products used in chemical maceration can lighten the bones, such as hydrogen peroxide (LOPES, et al., 2019; MARTINS et al., 2021; LIMA et al., 2021). Multiple chemical reagents can be employed to chemically detach soft tissues from hard tissues. The most used will be presented: hot water and hydrogen peroxide, as well as some alternative methods, such as the use of bromelain and papain.

2.4.1. Hot water – heat

Maceration using hot water is considered a type of chemical maceration because heat acts on water to facilitate the removal of tissue residues that were not completely removed through mechanical and biological maceration. Water, when heated, helps detachment of soft tissues from the bone through protein lysis and melting of remaining fat tissue still present in the bone. The literature mentions different techniques that employ hot water for the bone-stripping process. In this chapter, we will focus on three methods: water heated at 100ºC for 36 hours; use of a pressure cooker for 40 minutes; and microwave for five minutes.

Water heated at 100ºC is commonly used in a clay pot with tap water and one tablespoon detergent. The desired material is placed in the pot along with the mixture for 24 hours. After this period, the bones are removed, and the solution is renewed. The skeleton then is maintained immersed for another 12 hours. After this process, the bones are collected and washed with running water (COUSE; CONNOR, 2015).

For the pressure cooker, which has the advantage of taking much less time, the material is placed in the cooker with water and is kept under pressure for 40 minutes. The use of this technique is much simpler. After this period, the heating source is turned off and the cooker remains closed for a further 30 minutes for the resting pause (MARTINS et al., 2021). Once the pause has elapsed, the bones are collected and rinsed.

Microwave maceration requires 1000-watt equipment, a bowl, water, and latex gloves. The bone is submerged in the bowl containing water and placed in the microwave. The microwave is operated at maximum power for five minutes, being monitored every ten seconds to check for changes in the soft tissues. After the procedure, the skeleton is washed with running water to rinse the bones (COUSE; CONNOR, 2015).

The main advantages of using hot water maceration are the low cost of the technique and the fact that it is non-toxic, considering that only water and a heat source are needed. As disadvantages, some precautions must be taken when using water heating; for example, in the 24-hour technique, it is necessary to conduct the method with supervision/monitoring of the procedure at all times since the fire can be put out, or there can be a gas leak. In the pressure cooker technique, there is a risk of mechanical failures, such as in the pressure cooker itself, and the possibility of explosions. However, even though there are risks, they are very low when appropriate precautions are taken.

2.4.2. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) can be used in the maceration process to oxidate remaining soft tissues, causing these to detach from the bones. In addition, because of its whitening properties, hydrogen peroxide can be used to even out the tone of the bones (LIMA et al., 2021; NADEEM et al., 2023). To be used for maceration and whitening, a solution of hydrogen peroxide 130 volumes diluted to 30% is used.

The bones are immersed in the solution inside a plastic vessel hermetically sealed during the procedure. The immersion time depends on the size of the bone, as well as its resistance. Small and fragile pieces, such as carpal and tarsal bones and shoulder blades, can remain in the solution for 24 hours; bigger and more resistant bones, such as femur and tibia, can remain for 72 hours. After this period, pieces are washed under running water and allowed to dry at room temperature (LACERDA et al., 2023).

The advantages of using hydrogen peroxide for maceration include good tissue removal, short preparation time, and ease of whitening the bones. In addition, it is possible to conduct assisted boiling, allowing the removal of the pieces at various stages of maceration (NADEEM et al., 2023; SILVEIRA; TEIXEIRA; OLIVEIRA, 2008). Nevertheless, the method also presents some disadvantages. They include the elevated cost of the product and some associated risks related to human exposure through ocular, inhalation, and dermal routes. The ocular exposition may trigger eye irritation and lead to pain; in more severe cases, it can cause eye ulceration and corneal perforation. Inhalation of the solution can lead to severe pulmonary irritation. Dermal contact can lead to local irritation and temporary discoloration of hair and skin. Any contact with high concentrations of hydrogen peroxide can produce severe burns and the production of blisters (DIVISÃO DE TOXICOLOGIA HUMANA E SAÚDE AMBIENTAL, 2020).

2.4.3. Alternative methods – pineapple and papaya

Chemical maceration has been mostly employed using hot water or hydrogen peroxide. However, it is also possible to employ alternative, natural methods. Literature has shown that the use of pineapple (*Ananas comosus*) and papaya (*Carica papaya*) results in good residual tissue removal and cleansing of the bones. These two-fruit present remarkable characteristics for maceration: pineapple contains bromelain (72 mg/ml in the pulp and 98 mg/ml in the peel) and papaya contains papain (11 mg/g in the latex, from the peel of unripe fruit). These molecules are extremely helpful for maceration because they present proteolytic properties (MAHECHA et al., 2011; SOUZA et al., 2014; VEIRA et al., 2020).

The maceration using pineapple is usually conducted after mechanical maceration. The anatomical pieces are allocated in a plastic vessel, and the pineapple solution (pineapple juice: one kg pineapple to four L water) is poured on top of the skeleton until complete immersion. The pieces are kept inside the solution for 48 hours; then, they are washed using a brush and running water, and exposed to sunlight to dry (CORDEIRO, 2020).

Chemical maceration using papaya juice consists of covering the skeletal material, also after physical maceration, with a solution containing unripe papaya juice in a proportion of 1:1, meaning that one litter juice is diluted in one litter water. After covering all pieces, the material is allowed to rest inside the vessel with the solution from 48 to 96 hours, so that the enzymes can act. Then, the material must be removed from the solution, washed under running water, and allowed to dry in the sun (HORN; MATTE, 2015).

The advantages of using pineapple and/or papaya maceration are the low cost of the technique and the complete absence of toxicity. Thus, the materials can be placed in the laboratory for preparation with no need for specific care while the enzymes are acting on the remaining soft tissues. As a disadvantage, the removal of remaining tissues is not as effective as other maceration techniques, including hot water and hydrogen peroxide (SILVEIRA; TEIXEIRA; OLIVEIRA, 2008). The pros and cons of using these approaches must be considered and evaluated for each working condition.

3. RESULTS AND DISCUSSION

Although it is possible to use one maceration technique alone, it is more common to combine several types of techniques to obtain the final specimen. Disregarding the punctual case of retrieving carcass from nature, already in the advanced decomposition stage and where most of the soft tissues were naturally degraded and only hard tissues remain, generally the mechanical (or physical) maceration is the first approach employed, to manually remove skin, muscles, and viscera.

Once the skeleton is already on display and it is possible to observe the bones and Other hard tissues, the bone cleaning can be conducted either by chemical reactions – chemical maceration – or by biological agents – biological maceration. The definition of biological or Chemical maceration to finish the procedure depends on the infrastructure of the laboratory and the access to the necessary materials for each method. As observed in Table 1, each maceration technique demands certain materials and presents pros and cons; regardless, the resulting skeleton is usually comparable among the techniques.

Table 1. Main characteristics of the different maceration techniques and comparison.

Type Of Maceration	Necessary Materials	Advantages	Disadvantages
Mechanical	Surgical material (scalpel, tweezers, scissors, and so on)	Low cost; Usually short preparation time; Low toxicity; Precision control; No need for specialized material.	Difficulty in achieving a complete removal of soft tissues; Foul odor; Darkened color; Possibility of user injury to the experimenter.
Biological	Beetles (Coleoptera; Dermestidae; genus *Dermestes*); Fly larvae	Easy and practical; No foul odor (Dermestes); Extremely low cost; Complete removal of soft tissues; The skeleton is preserved fully articulated.	Difficulty in adjusting the proper management of Dermestes (non-resistant to cold Weather); Aversive odor and darkened color (fly larvae) If not closely followed, can lead to connective tissue damage.
Chemical	Hot water (heat)	Low cost; Low toxicity.	Demands care related to water heating.
	Hydrogen peroxide	Effective soft tissue removal; Low preparation time; Bone whitening.	Elevated cost; Imposes risk to the experimenter (exposure to chemicals through ocular, inhalation, and dermal routes).
	Pineapple and papaya	Low cost; Low toxicity; No need for special care.	The removal of soft tissues is not as effective.

4. CONCLUSION

There are multiple possibilities to obtain a skeleton through osteotechniques. Among them, maceration is vastly employed and results in superior quality material. Although it is possible to use one single type of maceration, it is common to employ combined techniques to achieve the final specimen, such as an initial mechanical maceration, followed either by biological or Chemical maceration to conclude the process. Regardless of the employed methodology, maceration presents low cost and is of great relevance for anatomical teaching, learning, and research, as an efficient approach to obtaining high-quality skeletal material.

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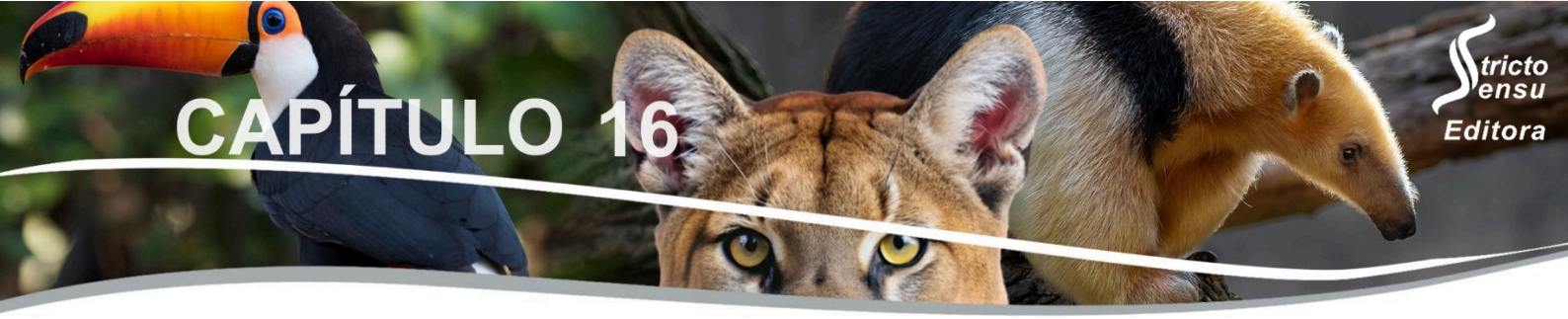

CONTRIBUTION TO THE ANATOMICAL STUDY OF THE CORTICAL REGION OF THE BOAR BRAIN (*Sus scrofa scrofa* Linnaeus, 1758)

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ABSTRACT
Comparative Neuroanatomy is a science focused on the morphological aspects of the organs that make up the nervous system. It enables research on humans, domestic and wild animals, with a descriptive focus on comparing anatomical characteristics of species with taxonomic proximity, providing support for other basic and applied sciences. Thus, the aim of this study is to investigate and describe the neuroanatomical aspects of the cerebral cortex of wild boar (*Sus scrofa scrofa* Linnaeus, 1758) and compare them with descriptions already published regarding the domestic swine (*Sus scrofa domesticus* Erxleben, 1777). To this end, the following procedures were carried out: fixation in formaldehyde and removal of the brains from the cranial cavities of 20 specimens of wild boar, 10 males and 10 females, belonging to the collection of cadaveric parts of the Wild Animal Research Laboratory (LAPAS) of the Faculty of Veterinary Medicine at the Federal University of Uberlândia; carrying out neuro techniques to prepare the material; observation, written and photographic records of the cortical region and its respective parts; description and comparison of findings with similar data present in the literature about the domestic species. Similar characteristics were found in the cerebral cortex of wild boars when compared to domestic swines, with discrete and specific variations in the size and shape of some components. The neopallium region was the one that varied the most with regard to the gyri and sulci visualized. It is expected that the work can contribute to the improvement of the related scientific collection, so that neurosciences benefit from the results found and the information produced serves as support for neurobiological research.
Keywords: Neuroanatomy, Cerebral cortex and Feral swine.

1. INTRODUCTION

To establish comparisons between the anatomical aspects of different animals, most often after the end of their development, Comparative Anatomy is the branch of Morphology that establishes the criteria for comparison. Considered, by nature, the substitute for comparative embryology in method and objective, it has been, mistakenly, named Comparative Anatomy. Comparative is what can be compared or that allows comparison, on the other hand, compared is something that has already been confronted (DI DIO, 2002).

One of the focuses of Comparative Anatomy is the study of the Nervous System, Neuroanatomy, which is at the basis of Neuroscience and has a more morphological approach (LENT, 2010). For a better understanding, this system was divided by neuroanatomists into the Central Nervous System (CNS), composed of the encephalon and spinal cord; and Peripheral Nervous System (PNS), represented by nerves, ganglia and their nerve endings (MACHADO; HAERTEL, 2022).

The encephalon, in turn, is macroscopically divided into three parts, which include the cerebrum, the cerebellum and the brainstem (FRANDSON; WILKE; FAILS, 2011). These parts are strictly adapted to the cranial cavity both in area and shape (DELLMANN; McCLURE, 2008a); together, they constitute the main center of the organs of sense and locomotion. When compared, among vertebrate animals, the brain presents regions that are more or less developed depending on the habits and capabilities of the animals (FARIAS, 2016).

Just like the encephalon itself, the brainstem is also divided into three parts, the medulla oblongata, the pons and the midbrain, which together contain the tracts and nuclei that will integrate the brain with the cerebellum, the spinal cord and the rest of the body (RADANOVIC; KATO-NARITA, 2016).

The medulla oblongata is the part that undergoes the least modifications throughout the evolution of the Nervous System, with no significant differences being observed in this region when comparing domestic animals with humans (FARIAS, 2016). It is located between the pons and the spinal cord, which it establishes communications with, being responsible for regulating vegetative functions. From it emerge the last four pairs of cranial nerves, IX, X, XI and XII (RADANOVIC; KATO-NARITA, 2016).

The pons is located ventral to the cerebellum, between the medulla oblongata and the midbrain (MARTINEZ; ALLODI; UZIEL, 2015); involved with several motor and sensory

functions, four pairs of cranial nerves also emerge from it, V, VI, VII and VIII (RADANOVIC; KATO-NARITA, 2016).

The mesencephalon, located between the pons and the diencephalon, has two regions of greatest relevance: the cerebral peduncles, characterized by large bundles of nerve fibers that connect the cerebral hemispheres to the brainstem and, consequently, to the cerebellum and spinal cord; and the colliculi (quadrigeminal bodies), two small elevations that coordinate ocular reflexes (right and left rostral colliculi) and two others that relay auditory impulses (right and left caudal colliculi) (FRANDSON; WILKE; FAILS, 2011). In lower vertebrates, the quadruple bodies constitute the main center for processing optical and auditory stimuli, unlike higher vertebrates that have this function located in the brain (FARIAS, 2016). Two pairs of cranial nerves emerge from the midbrain, III and IV (RADANOVIC; KATO-NARITA, 2016).

The cerebellum, located dorsal to the pons, is divided into two lateral hemispheres (right and left) and a central region called the vermis. Its surface is equipped with a series of sheets called leaves, composed of white matter internally and gray substance externally (cerebellar cortex), similar to what occurs in the brain. Functionally, the cerebellum is associated with the regulation of body movements through the coordination of muscular activity. Between it and the brain stem is the CSF cavity called the fourth ventricle (FRANDSON; WILKE; FAILS, 2011). As the spinal cord makes connections with spinal nerves and the first two pairs of cranial nerves connect to the brain, it can be concluded that the cerebellum is the only organ that is not related to nerves (MACHADO; HAERTEL, 2022).

The brain, the largest and most important part of the brain, is divided into two distinct areas, the telencephalon and the diencephalon: the first comprised the two cerebral hemispheres, which include the cerebral cortex (focus of this study), the basal ganglia, the others subcortical nuclei, the medullary white center of the brain and the rhinencephalon. The largest CSF cavities, the lateral ventricles, are also located in the telencephalon (FRANDSON; WILKE; FAILS, 2011); the second area is a globose mass covered entirely by the cerebral hemispheres (MARTINEZ; ALLODI; UZIEL, 2015), it is composed of four bilateral and regular regions, arranged on each side of the third ventricle (CSF cavity), called the thalamus, hypothalamus, epithalamus and subthalamus (FARIAS, 2016). It is assumed that the four dilations, normally associated with the thalamus, the medial and lateral geniculate bodies, together constitute a fifth part of the diencephalon, the metathalamus. As already mentioned, the first two pairs of cranial nerves, I and II, connect, respectively, with anatomical structures belonging to the telencephalon (olfactory bulb) and the diencephalon (optic chiasm) (MACHADO; HAERTEL, 2022).

The CNS is entirely covered by the meninges, which protect and support both the brain and the spinal cord. Fish have only one continuous layer called the primitive meninges; in amphibians there are two layers, one more internal, called the pia mater, and another more external, the dura mater, with the cerebrospinal fluid (CSF) present between them (in the subdural space); In mammals, an intermediate layer appears, with a spider web appearance, the arachnoid mater. The cerebrospinal fluid, in this case, is located between the pia mater and the arachnoid (in the subarachnoid space). The brain dura mater is attached to the skull, unlike that found around the spinal cord, which has a space between it and the vertebra (extradural space), filled with adipose tissue (FARIAS, 2016). The cerebral dura mater of mammals has two sheets, between which they form the venous sinuses for draining the brain. It also has three internal folds that separate each of them distinct structures: the sickle of the brain, located between the cerebral hemispheres; the tensor cerebellum, present between the cerebrum and the cerebellum; and the diaphragm sella, which separates the pituitary gland from the ventral surface of the diencephalon (DELLMANN; McCLURE, 2008a). A fourth fold is allowed, between the cerebellar hemispheres, the falx cerebellum. However, its incidence is reported in humans and some wild mammals, but absent in domestic mammals (NOMINA ANATOMICA VETERINARIA, 2017).

As mentioned, in each part of the brain, there are chambers that make up a ventricular (CSF) system. The two lateral ventricles (telencephalon) are connected, through the interventricular foramina, to the third ventricle (diencephalon), which in turn is connected to the fourth ventricle (cerebellum and brainstem) by the midbrain aqueduct. These cavities house the choroid plexuses, responsible for the formation of cerebrospinal fluid that leaves the ventricular system, from openings that connect the fourth ventricle to the subarachnoid space (FRANDSON; WILKE; FAILS, 2011).

The CNS is made up of billions of neurons, also known as nerve or neural cells, which have a high degree of specialization; by neuroglia, sometimes called glial cells; through blood vessels; and by a small amount of connective tissue (GARDNER; GRAY; O' RAHILLY, 1988). This system has a similar structure in all vertebrate animals (KIERNAN, 2003).

During development, most neurons migrate to the surface of the telencephalon, constituting the cerebral cortex. In animals evolutionarily positioned below mammals, the cortical surface is smooth (lisencephalons), without gyri and grooves; Mammals, on the other hand, have gyri and grooves in the brain (gyrencephalons), with some exceptions and characteristics varying according to the species. The cerebral hemispheres of mammals are interconnected by three commissures (a set of fibers that connect opposite sides of the same

organ in the CNS), the anterior and hippocampal (“fornix” in humans), which connect ancient sectors (archipallium); and the corpus callosum, which connects the most recent areas (neopallium) (FARIAS, 2016).

The anatomical study of the brain in Neuroanatomy, from Cláudio Galen to the present day, has undergone major transformations. The dissection of human and animal specimens resurfaced at the end of the 15th century, after an interval of almost 1800 years, having been consolidated in the 17th century by the English doctor and professor Thomas Willis, considered the pioneer in the structural and detailed investigation of the brain (ROONEY, 2018).

As for the brain of domestic swines (*Sus scrofa domesticus* Erxleben, 1777), there are few variations to what has been exposed so far, in a generic way, for mammals. It is necessary to highlight the following characteristics: the bulb is relatively wider when compared to other species; the caudal colliculi of the midbrain are excessively well developed and larger than the rostral colliculi; the cerebellum is very wide, with large lateral hemispheres, which go beyond the lateral limits of the medulla oblongata; Finally, in contrast to what is described for other species, the lateral fissure of the hemisphere, in domestic swines, is not the most notable, but similar to other depressions on the brain surface (DELLMANN; McCLURE, 2008b).

Wild boars (*Sus scrofa scrofa* Linnaeus, 1758), which constitute the focus of this work (figure 1), are considered exotic animals in Brazil, as they originate from Europe and were introduced into the national biome by European colonizers, becoming wild in several locations. They are omnivorous and have, on their skull, a prominent occipital crest, formed from the supraoccipital and parietal bones (TIEPOLO; TOMAS, 2006).

The anatomical aspects of the boar's cerebral cortex have not yet been described, a fact similar to what occurs with several wild animals that lack morphological studies, especially those species with potential for commercial exploitation, such as the wild boar (MENEZES et al., 2001).

There is a tendency for the cortical anatomical characteristics of wild boars to be similar to those found in domestic swines, considering that the first group is considered a direct ancestor of the second. Both are considered the same species, as crossing between them generates fertile offspring (CÂMARA FILHO et al., 2004). For example, the presence of a large olfactory bulb, a special characteristic of the CNS of all suidae (OLIVEIRA, 2003), must also be evident in the wild boar. However, based on what was mentioned by Câmara Filho et al. (2004), that swines have undergone variations over thousands of years, caused by

selection and crossing processes, divergences in brain anatomy must be revealed between the two groups.

Analogy deals with the pairing of different anatomical structures based on functional similarity criteria, homology deals with comparison based on embryological similarity. Both constitute the foundation of morphological research, providing support for other basic and applied sciences. Comparative Neuroanatomy has already enabled great advances in knowledge, because some species have started to be used as experimental models in practical classes and preliminary scientific studies, including the improvement of techniques and procedures; all based on the neuroanatomical characteristics of the animals.

Domestic swines are increasingly used in the field of neuroscience (SAULEAU et al., 2009), as they have many anatomical and physiological similarities with humans, being considered the domestic animal with the greatest potential for use in training and medical research (SWITZER, 2008). A fact confirmed in 2021 with the advent of the first genetically modified swine kidney transplant for humans. Procedure carried out without immediate compromise of the recipient's immune system (BBC NEWS, 2021).

The wild boar, in turn, is a wild species with few reports of its anatomy (OLIVEIRA, 2003). Considered exotic, as it does not originally belong to the Brazilian fauna, it has no natural predators and is seen as a threat to the environment and the country's economy. Both the wild boar and its hybrid, the domestic swine, known as “javaporco”, are constantly released for hunting by the Brazilian Institute of the Environment and Natural Resources (IBAMA), which makes the use of specimens in teaching and research activities viable. (SUSTAINABLE PLANET, 2013).

Thus, the objective is to investigate, describe and publish the external neuroanatomical aspects of the cerebral cortex of the wild boar (*Sus scrofa scrofa*) and compare them with the descriptions already published regarding the domestic swine (*Sus scrofa domesticus*).

It is expected to find different characteristics between the brains of wild boars and domestic swines, because although they are from the same species, with only the subspecies varying (Table 1), significant morphological differences are visible between them (SANTOS, 2016).

Table 1. Systematic and taxonomic classification of Domestic (D.) swines and Wild boar:

Taxonomy	Infraclass	Order	Suborder	Family	Gender	Species	Subspecies
D. swines	*Eutheria*	*Artiodactyla*	*Suina*	*Suidae*	*Sus*	*scrofa*	*domesticus*
Wild boar	*Eutheria*	*Artiodactyla*	*Suina*	*Suidae*	*Sus*	*scrofa*	*scrofa*

Source: Domestic swine (GETTY, 2008); wild boar (MORAES; SANTOS, 2014).

2. MATERIAL AND METHODS

We used 20 specimens of wild boar, 10 males and 10 females, adults of different ages and free from injuries, belonging to the collection of cadaveric parts of the Wild Animal Research Laboratory (LAPAS) of the Faculty of Veterinary Medicine of the Federal University of Uberlândia (UFU), Uberlândia - Minas Gerais. The material was sent to the Anatomy Laboratory of Faculdade Morgana Potrich (FAMP), in Mineiros (Goiás), where the brains were removed from the cranial cavities and then dissection procedures were carried out, removing the meninges and blood vessels to highlight the cerebral cortex and written and photographic records of the observed structures.

In this research, a descriptive and comparative observational study was conducted, according to the classification of types of research proposed by Hübner (2002), limited to the recording, written and photographic, of the neuroanatomical characteristics observed in the cortical region of the wild boar brain (*Sus scrofa scrofa*), comparing them with those described for the domestic swine (*Sus scrofa domesticus*), due to the taxonomic proximity of the species. Considering the descriptive and comparative approach of this research, statistical analysis was not necessary.

The animals, initially stored in a freezer, were thawed and washed under running water. The femoral arteries were cannulated and injected with 10% formaldehyde solution for fixation. Next, formaldehyde was injected into the cavities and the specimens were submerged in the same solution for at least 72 hours. After fixing the material, the brains were removed from the cranial cavities according to the techniques recommended by Fazan and Fazan (2020) and adapted for the animals in this research. The pieces remained immersed in 10% formaldehyde for at least 15 days to complete fixation; Finally, the dissection procedures, written and photographic records took place.

For the dissection and disclosure of the cerebral cortex, the techniques recommended by Mizers and Gardner (1988) who dissected human primates (*Homo sapiens*) and Evans and De Lahunta (1994) who worked with dogs were adapted for the wild boar (*Sus scrofa scrofa*). (*Canis familiaris*); The photographic recording of the material was carried out using a Sony Alpha A6600 24.2MP digital camera. Finally, for the description and comparison of the data found, with emphasis on the similarities and differences identified between the species, the Nomina Anatomica Veterinaria (2017) was adopted to standardize the anatomical terminology used.

As it is cadaveric material belonging to the collection of the Wild Animal Research Laboratory (LAPAS) of the Faculty of Veterinary Medicine of the Federal University of Uberlândia (UFU), already released for use in teaching, research and extension activities, without carrying out of animal sacrifice, the execution of this study was exempt from analysis by the Ethics Committee on the Use of Animals (CEUA), as it is in compliance with the Brazilian Guideline for the Care and Use of Animals for Scientific and Didactic Purposes (DBCA) , published in Brasília/DF - 2013 by the National Council for Animal Experimentation Control (CONCEA) of the Ministry of Science, Technology and Innovation. At the end of data collection, the material was reintegrated into the LAPAS collection of pieces and used for other projects, such as practical classes in undergraduate and postgraduate courses, as well as training that addresses the morphology of the wild boar.

3. RESULTS AND DISCUSSION

For some authors, the diencephalon is recognized as a region of the brain stem, together with the midbrain, the pons and the medulla oblongata, which makes the telencephalon a synonym for the brain, composed of a superficial part, called the cerebral cortex, where most of the neuron cell bodies are concentrated; and another deep or subcortical, characterized by several other anatomical structures, such as the basal ganglia, for example (ROWEN; WILKE; FAILS, 2011).

In line with what was described by Rowen, Wilke and Fails (2011) for farm animals, including the domestic swine (*Sus scrofa domesticus*), the telencephalon of 100% of the wild boar specimens (*Sus scrofa scrofa*) dissected in this study was presented divided, by a very evident longitudinal fissure in the median plane, into two asymmetrical contralateral hemispheres, right and left, with the cerebral cortex similar to that found in domestic mammals in general, that is, formed by various convex folds called gyri and separated by grooves and specific fissures, according to the depth of each depression (Figure 1a).

Similar to what König and Liebich (2016) and Dellmann and Mcclure (2008a) described for domestic animals, where they also considered the domestic swine, the medial faces of each cerebral hemisphere of the wild boar were connected, in corresponding regions, by the main contingent of fibers inter-hemispheric commissural structures, the corpus callosum, varying the size and shape of this anatomical structure. In the medial and lateral view, it is also possible to accurately visualize the depression that separates the brain from the cerebellum, called the transverse cerebral fissure (figure 1b).

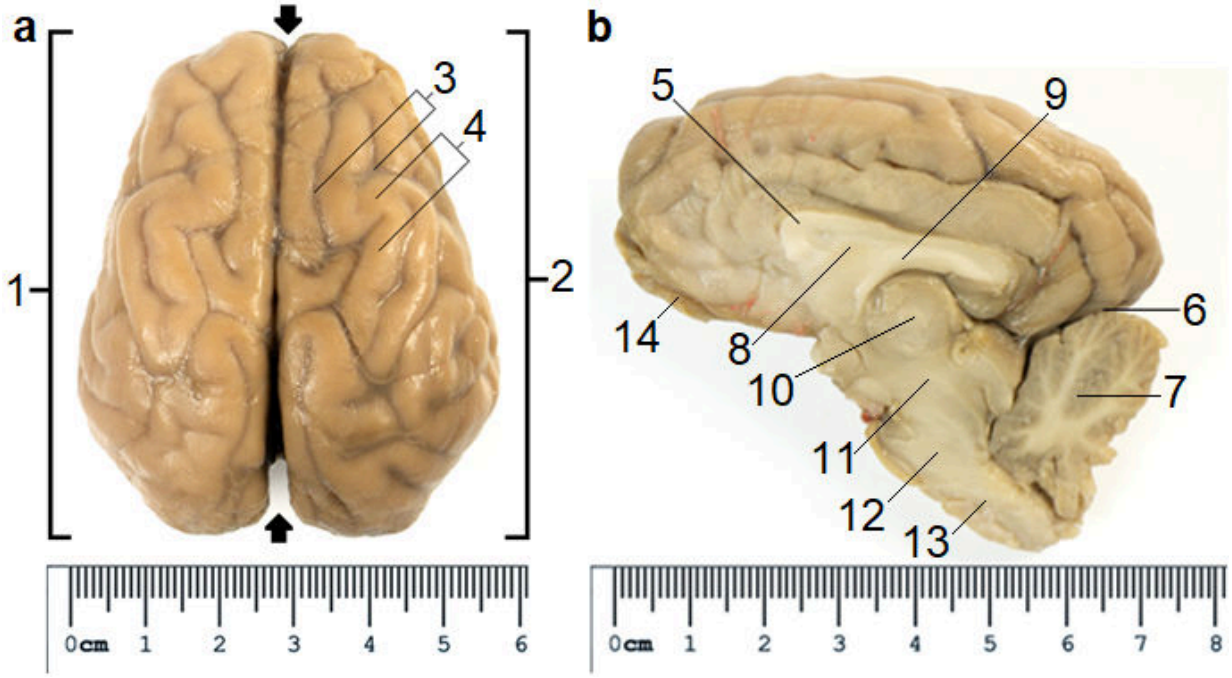

Figure 1. Boar brain and cerebellum.

a = dorsal view of the brain of one of the dissected wild boar specimens, highlighting: longitudinal fissure (arrow); left hemisphere, 1; right hemisphere, 2; grooves, 3; gyrus, 4; b = medial view of a cerebral hemisphere of one of the dissected wild boar specimens, highlighting: corpus callosum, 5; transverse fissure of the brain, 6; cerebellum, 7; septum pellucidum, 8; fornix, 9; thalamus, 10; midbrain, 11; pons, 12; bulb, 13; olfactory bulb, 14. Source: the authors.

Considering the basic structure of each cerebral hemisphere, if the central region is excluded, which is formed by an alternation of basal ganglia and white matter nerve fibers, most of the neuronal bodies of this organ are located in the superficial gray matter, already reported here as cerebral cortex. However, when considering the evolutionary history, structural characteristics and functionality of this part of the nervous system, some authors name it as pallium and divide it into three regions, according to their development and specialization: the paleopallium or paleocortex, which is the sector more primitive and essentially related to smell, located in the basal part of the brain; the archopallium or archicortex, which occupies the second position in antiquity, located more internally and not visible on the surface, related mainly to the limbic system, a neural component for regulating visceral motor activities, emotional behavior and memory; and the neopallium or neocortex, the largest, most important and most recent sector of the cortex, located predominantly in the dorsal, lateral and medial areas of the cerebral hemispheres, where the main gyri and sulci of the brain are located (KÖNIG; LIEBICH, 2016; DYCE ; SACK; WENSING, 2010).

According to Dellmann and Mcclure (2008a), the sum of the paleopallium and the archopallium results in a region called rhinencephalon, made up of the allocortex, that is, a

cortical tissue that is not uniform in the distribution of neurons; The neopallium has a more regular neuronal distribution, which justifies the use of the name isocortex by some authors for this region. However, for Dyce, Sack and Wensing (2010), the use of the term rhinencephalon, a brain for processing “smell”, is assumed inappropriately by some anatomists. They argue that this classification should not be maintained, as it disregards other functions attributed to this region.

Both König and Liebich (2016) and Dyce, Sack and Wensing (2010) mentioned that the paleopallium is mainly made up of the two olfactory bulbs, which receive olfactory nerve fibers from receptors in the nasal mucosa; by the caudal continuations of the olfactory bulbs, called olfactory peduncles or common olfactory tracts, which are divided into a medial and a lateral component, where they delimit a region called the olfactory triangle; and by the large piriform lobe, a prominence lateral to the hypothalamus that receives fibers from the lateral olfactory tract. The separation of the paleopallium and the neopallium is made by the rhinal groove, which is most easily seen in a lateral view, although it is also visible in the ventral view. Such structures were easily visualized in a ventral view of the brain of the wild boars dissected in this work, with the shape and size of each of them varying, including between the hemispheres of the same organ (figure 2a).

The archopallium, described by König and Liebich (2016), is located in the most medial part of each hemisphere and makes up part of the so-called limbic system, a set of interconnected cortical and subcortical structures mainly related to emotional behavior and its influences, in especially olfactory impulses. This region is made up of the cingulate gyrus, hippocampus and also the piriform lobe, a part already described as part of the paleopallium; It is worth noting that these cortical structures establish functional links with the subcortical part of the limbic system, which includes components of the diencephalon, midbrain and the amygdaloid body. The hippocampus, located internally to the piriform lobe, is a gyrus that establishes connections with other telencephalic structures to influence the endocrine system, viscera and emotions. All these elements were evident in the prepared and dissected boar pieces, with significant variation in their shapes and dimensions (figure 2b).

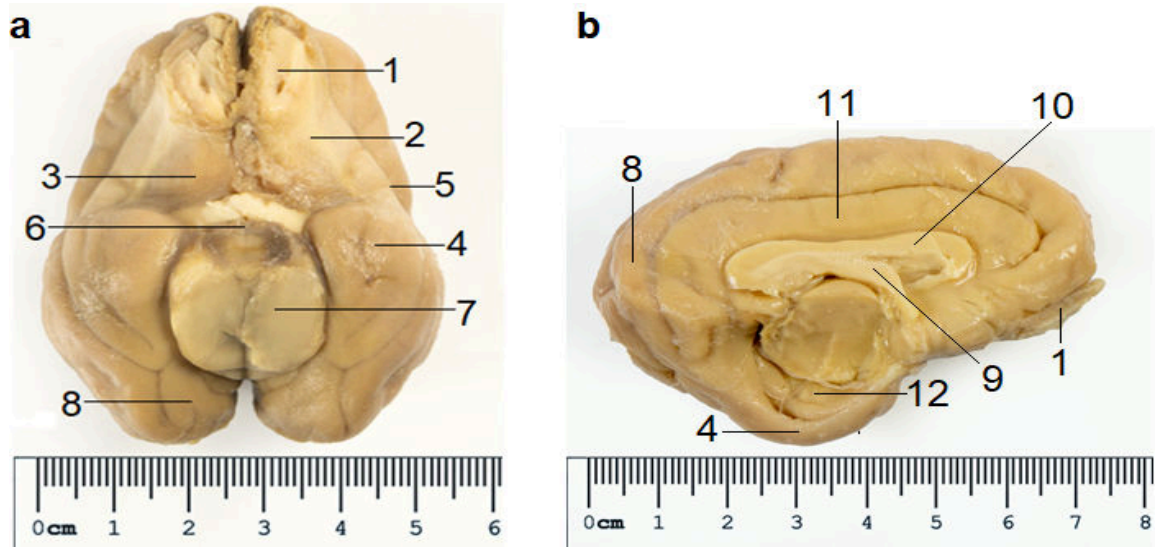

Figure 2. Boar brain.

a = Ventral view of the brain of one of the dissected wild boar specimens, highlighting: olfactory bulb, 1; common olfactory tract or olfactory peduncle, 2; olfactory triangle, 3; piriform lobe, 4; rhinal sulcus, 5; hypothalamus, 6; midbrain, 7; occipital lobe, 8; b = medial view of a cerebral hemisphere of one of the dissected wild boar specimens, highlighting: olfactory bulb, 1; piriform lobe, 4; occipital lobe, 8; fornix, 9; corpus callosum, 10; cingulate gyrus, 11; hippocampus, 12. Source: the authors.

As already mentioned, in domestic animals, the neopallium constitutes the majority of each cerebral hemisphere in domestic animals, being considered the most developed cortex in higher animals. Its numerous gyres and grooves on the surface give it its characteristic appearance, depending on the animal's degree of evolution and its physical size. It also has two ends that serve as a reference for more specific observations: the rostral or frontal pole, longer, convex and with the ventral surface covered in part by the olfactory bulb; and the caudal or occipital pole, which is wider and establishes close relationships with the colliculi of the midbrain, with the pineal gland of the epithalamus and the dorsal surface of the cerebellum, through the tensor of the cerebellum (DELLMANN; McCLURE, 2008a). All boar brains dissected for this work showed the anatomical characteristics discussed above (figure 3a).

The study of the neopallium can be facilitated by dividing it into four lobes, that is, regions that bring together several gyri and grooves, named after the cranial bones that protect them. They are: the frontal lobe, where the motor areas that give rise to the efferent tracts of the cortex are located; the parietal lobe, containing sensitive or somesthetic areas, being the site of reception of the afferent pathways; the temporal lobe that houses the auditory area; and the occipital lobe, where visual stimuli are processed (KÖNIG; LIEBICH, 2016). In the wild boar brains observed in this research, such regions can also be separated.

The domestic swine brain, in general, looks more like the brain of a primate than the same organ in a mouse. However, the porcine telencephalon has an ovoid shape, a less evident curvature and a less developed rostral pole when compared to a primate. Its rostral pole is smaller than the caudal pole and has a well-developed olfactory system (SAULEAU et al., 2009, our translation). The same characteristics were seen for the wild boars dissected in this study (figure 3a and 3b).

For Dellmann and Mcclure (2008b), the telencephalon of domestic swines varies more in shape compared to horses and ruminants. Interestingly, unlike other species, the Sylvian fissure, on the side of the hemisphere, is not particularly prominent in the region; a similar situation was seen in the wild boars in this research (figure 3b). Another pertinent observation, made by Dyce, Sack and Wensing (2010), considered that the pattern of gyri and sulci is usually constant in one species, but different in others; and that, despite being useful references for researchers in the functional study of certain areas of the cerebral cortex, the names of many of them are not relevant to students. Thus, it was possible to identify some gyri and grooves in the telencephalons of wild boars (figure 3a and 3b), observed in this research, similar to those described for domestic swines. However, not all of them were easily evident in all the dissected pieces.

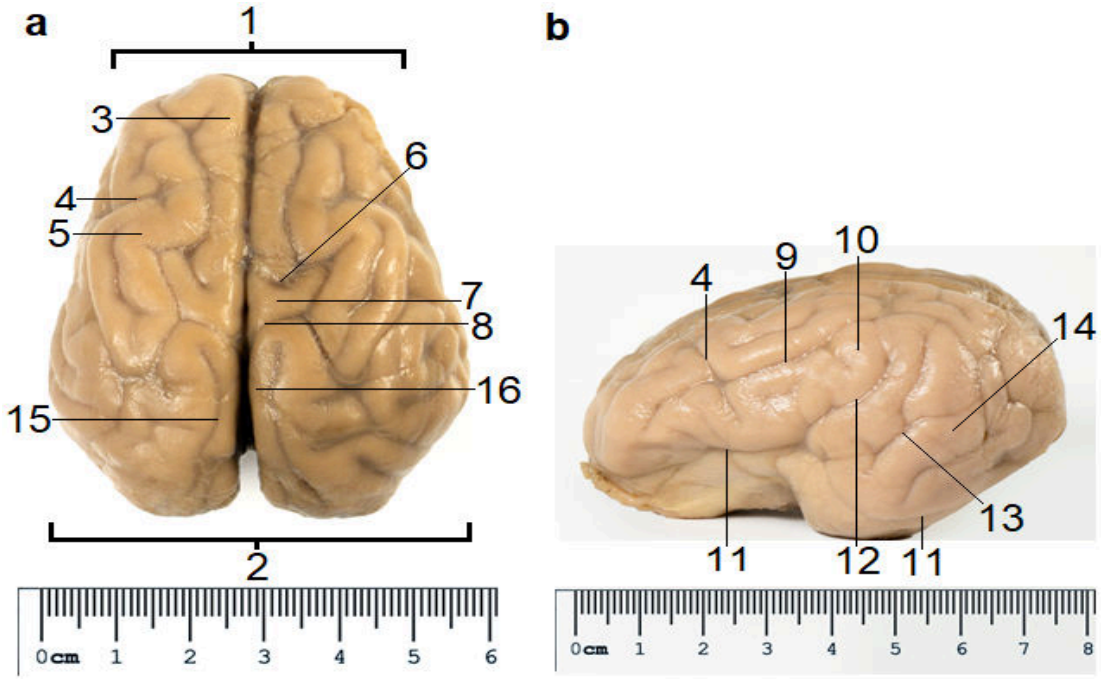

Figure 3. Boar brain.

a = dorsal view of the brain of one of the dissected boar specimens, highlighting: rostral or frontal pole, 1; caudal or occipital pole, 2; sigmoid gyrus, 3; diagonal groove, 4; coronal gyrus, 5; crossed groove, 6; cross turn, 7; coronal sulcus, 8; marginal sulcus, 15; marginal gyrus, 16; b = lateral view of a cerebral hemisphere of one of the dissected wild boar specimens, highlighting: diagonal sulcus, 4; suprasylvian groove, 9; sylvian gyrus, 10; rhinal sulcus, 11; Sylvian fissure, 12; ecto-sylvian groove, 13; ecto-Sylvian gyrus, 14. Source: the authors.

4. CONCLUSIONS

In general, the boar's cerebral cortex was very similar to that of the domestic swine, with discrete and specific variations in the size and shape of some anatomical structures. The similar characterization of basic components, such as the corpus callosum, the longitudinal and transverse fissures of the brain, the shape and size of the rostral and caudal poles, the division into lobes and the most relevant components of the archopallium and paleopallium regions, can be explained because they are the same species, only the subspecies varies, which would not justify the presence of major changes.

The biggest difference in the wild boar's telencephalon, when compared to the domestic swine, was shown in the anatomical structures of the neopallium, especially the gyri and sulci on the dorsal and lateral surfaces of the brain. Many of them were absent or difficult to see, some with very varied shapes and sizes, even among wild boar specimens. One explanation for this condition may be the fact that this area of the brain is the most influenced by the animal's habits and the environment in which it lives.

It is expected that the work can contribute to the improvement of the related scientific collection, so that neurosciences benefit from the results found and the information produced serves as support for neurobiological research.

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CADAVERIC PRESERVATION TECHNIQUES IN ANATOMICAL TEACHING

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ABSTRACT
Anatomy is a basic Science that forms the foundation of multiple graduation courses in biological sciences, health sciences, and even human sciences. Due to its essentiality and the complexity of its content, either in animal or human anatomy - and comparative anatomy, the learning process presents several challenges to be overcome so that students can comprehend and consolidate the knowledge. Different methodologies can be applied to reinforce anatomical learning, depending on the appropriate fixation and conservation of real anatomical structures. The present chapter aims to present and evaluate the fixation of anatomical parts using formaldehyde, and further preservation following different approaches, such as preservation in formaldehyde, sodium chloride, and glycerin. The possibility of preserving pieces at dry, cryodehydration, is also presented. Under each form of conservation, the description is provided along with advantages, disadvantages, and applications of use in the anatomy laboratory. The described techniques for fixation and preservation in anatomy have the purpose of appropriately conserving the morphological characteristics of cadaveric material, as close as possible to the in vivo tissue. It is also desired that the preserved specimens, either from animal or human material, do not exhibit significant color, texture, and flexibility changes. Substantial methods of fixation and preservation thus provide high-quality material for future professionals, who demand concrete knowledge of body morphology to become competent and complete in their professional career.
Keywords: Morphology, formalin, glycerol, saline and cryodehydration.

1. INTRODUCTION

Anatomy is basic science and is crucial for the foundation of diverse graduation courses, which include biological, health, and human sciences. To know and to recognize each part of the body, either animal or human and to be able to identify possible morphological

variations are the focus of research and anatomical teaching. They also contribute to maintaining anatomy as a dynamic and vivid science. To guarantee the teaching-learning process of anatomy, it is recommended, whenever possible, to present a collection of real anatomical pieces to students, so that a three-dimensional visualization is possible. This also offers a first contact with the body and helps prepare the forming professional. Yet, several challenges in the process of anatomy teaching can interfere with an effective consolidation of the subject; thus, there is a constant need for operative methodologies that can be used to improve the teaching-learning process, as well as for the development of new strategies (FORNAZIERO et al., 2021).

Access to fresh cadaveric material is very restricted and out of reality for most teaching and research institutions. Even in animal anatomy, where it is possible to have access to fresh pieces, it is still demanding and sporadic, and it is not guaranteed that any animal part, of any desired species, will be available when necessary. Therefore, assertive fixation and preservation methods are necessary, so that the cadaveric pieces can retain their morphological characteristics and be used for several years, and even for a full decade.

Under certain circumstances, depending on a cultural change of teachers and institutions, it is possible to implement significant adaptations in the laboratory routine, so that the practical learning-teaching process of anatomy is facilitated. Some of these adaptations include the availability of cadaveric pieces in good conservation state; association between real pieces and technology; and pedagogic strategies to stimulate ethical behavior and a critical view of students towards the body and its parts. It is thus mandatory that a sum of all available instruments is conducted so that the knowledge can be better assimilated (FORNAZIERO et al., 2010). Teachers and academics must use their best efforts to employ their creativity so that a didactic anatomical material is obtained, and that such material is not harmful to health when manipulated while also supporting environmental changes, such as temperature and humidity, in particular in laboratories that do not possess climate control.

Our working group is in constant engagement in the effort to improve and provide robust fixation and preservation techniques. The goal is to work with methods that are effective at reaching its audience of undergraduate and resident students, and also teenagers and schoolteachers of subjects related to anatomy. The present chapter aims at summarizing and presenting, objectively, how to preserve cadaveric pieces that had been previously fixated using formaldehyde, using different methodologies for conservation.

2. MATERIALS AND METHODS

The Department of Anatomy, at the Center of Biological Sciences (CCB) of the State University of Londrina (UEL), in north Parana, Brazil, develops multiple important teaching, research, and extension projects. The ongoing projects aim to propose new methodologies, test already published techniques, and reproduce concepts widely known for their efficacy to provide helpful didactic material, such as the preparation of animal and human anatomical pieces. Among the activities with an academic focus is the extension project entitled "Orientation and concession of anatomical pieces of the Department of Anatomy to high and middle school teachers," which enables the dissemination of anatomical knowledge and allows opportunities for access to the university environment for middle and high school students from the surrounding area.

Regarding the Federal University of Jataí (UFJ), located in southeast Goias, Brazil, there are also several ongoing teaching, research, and extension projects focused on enhancing the teaching-learning process of students linked to the Laboratory of Human and Comparative Anatomy. One of the main developed activities is student tutoring, where veteran students, approved after selection, can offer tutoring during extra-curricular periods to students currently taking the discipline. This type of activity supports anatomy learning and reinforces the content ministered by the teachers.

Anatomy teaching depends on the visualization of the body, and students should have access to a laboratory that possesses high-quality materials. To do so, efficient fixation and conservation protocols are necessary to obtain good final pieces. In addition, research projects can be developed to validate teaching strategies and techniques conducted by the teaching community and to evaluate the effectiveness of school tutoring for academic performance. There is a constant effort from teachers to provide differentiated forms of ministration of the content so that anatomy is taught in a meaningful form to the student. It is more deductible, then, that the discipline will be more easily incorporated into the student's routine and contribute more effectively to the formation of the future professional.

Various techniques can be applied in the diversity of anatomy teaching according to how the structures are prepared; in this sense, in the present chapter the fixation of cadaveric pieces is presented using formaldehyde, and the subsequent conservation is presented using four different techniques. First, it discusses the conservation in formaldehyde; then, the conservation in hyper-saturated salt solution (sodium chloride), in glycerin; and finally, the cryodehydration, in which the pieces are kept under dry conditions. The tools that can be

used to obtain and preserve humid and dry cadaveric pieces are described and presented below.

2.1. PREPARATION OF ANATOMICAL PIECES FROM FRESH MATERIAL

2.1.1. Fixation Of Pieces Using Formaldehyde

Fresh cadaveric tissue is the ideal model to simulate surgical procedures, due to its proximity to the living tissue (CAREY et al., 2014; TURNER; MELLINGTON; ALI, 2005). However, the high demand for anatomical pieces and the unfeasibility of regular fresh material, in particular regarding human material, makes fixation a necessity. Fixated anatomical pieces can be used for prolonged periods (MARTINS; SAKALEM, 2022), and enable the visualization of the main morphological characteristics of the tissue, including color, consistency, and flexibility (RODRIGUES, 2010). Such characteristics are crucial for the development of several procedures, including cadaveric dissection.

Dissection has become obsolete in developed countries after the implementation of technological resources such as simulation and other approaches that replace real pieces, supporting alternative strategies to anatomical learning. Nevertheless, it can be argued that, although alternative tools present themselves as valuable resources, the practical experience obtained with the dissection of real anatomical parts cannot be completely replaced (KERBY et al, 2011; ANYANWU; UGOCHUKWU, 2010). To obtain an effective dissection using conserved tissue, the gold-standard method for fixation is formaldehyde, also known as methanol or formic aldehyde.

Formaldehyde is the most used chemical compound to fixate cadaveric pieces (BRENER, 2014). Silva and colleagues (2016) have reported that up to 96% of medical schools in Brazil use real cadaveric pieces in practical anatomical lessons and that 83,3% of them use formaldehyde as a fixating agent. There is a predominance of the use of formaldehyde in all regions of the country.

Aldehydes present a carbonyl group (C = O) attached to the end of the carbon chain, and methanol is the main aldehyde, being the most commonly used (SOUZA, 2024). This chemical compound was discovered in 1867 and soon began to be used as a fixating agent, which is an essential substance for the maintenance of cadaveric parts. To date, the formaldehyde solution in concentrations commonly varying between 3 and 10% has been widely used due to its easy availability, simplicity of technique, and time to prepare the pieces

(MARTINS-COSTA et al., 2022). The acquisition cost is low, and formaldehyde possesses a high tissue penetration rate (about 6 mm in 12 hours), which makes the fixating process very quick (DE OLIVEIRA, 2014). After the initial fixation and penetration of formaldehyde into the cadaveric tissues, the piece can be preserved using different techniques, either in formaldehyde itself or using alternative methods.

Formaldehyde, in its gaseous form, is colorless and possesses a pungent and irritating odor. It can also cause tearing when in high concentration in the environment (WORLD HEALTH ORGANIZATION, 2002). As years pass by, researchers involved with the health area have endeavored to reduce the amount of formaldehyde required for the fixation of cadavers, to make dissection more convenient and interesting for the students (GOSOMJI et al., 2018). Investigations conducted by the Department of Health and Human Services (DHHS), International Agency for Research on Cancer (IARC), and Environmental Protection Agency (EPA) have confirmed that the use of formaldehyde is carcinogenic for humans and animals (WORLD HEALTH ORGANIZATION, 2002). Formaldehyde presents destructive effects on microscopic structures, such as DNA, leading to alterations that can cause issues in DNA identification of cadaveric pieces (CARVALHO, 2009; FONTOURA et al., 2020). Thus, despite its relevance and efficacy as a fixating agent, and as a conservative agent, as will be shortly discussed, there is a great incentive to reduce and minimize formaldehyde use.

2.2. PRESERVATION OF HUMID MATERIAL

2.2.1. Formaldehyde

The usual concentration of the solution used to preserve anatomical pieces tends to be the same as that used for fixating tissues. To do so, commercially acquired formaldehyde (37% formalin) is diluted until the desired percentage (FONTOURA et al., 2020). In our research group, we use 3.7% formalin, resulting from a dilution of 1 part original solution to 9 parts water. Anatomical pieces are then kept submersed in the solution, and even in the case of whole corpses and the impossibility of constant return of the specimens to the tank, it is possible to keep them allocated on laboratory stretchers, with regular restitution of the preserving solution. Considering that the pieces are kept with a surrounding solution and covered with plastic canvas or any other material to prevent a fast evaporation of formaldehyde, it is possible to keep the tissues preserved and stop any undesired biological growth and consequent material damage, and autolysis.

The regular maintenance of pieces preserved in formaldehyde demands constant repositioning of the solution, usually every 15 days because of evaporation. Also, exposed pieces during lectures or dissection and related activities must be closely monitored and sprayed with the solution to guarantee their durability and avoid dryness and tissue damage. It is possible to humidify the pieces using sprayed water, up to three times a day, to keep them moist and avoid dryness; in this case, considering that the water will dilute the solution, it is necessary to add more formaldehyde to keep the correct percentage (FONTOURA et al., 2020).

As an advantage of using formaldehyde as a preservative agent, the technique presents easy maintenance and is still the most accessible technique to conserve cadaveric pieces (OLIVEIRA, 2021). The solution presents no significant effect on the color of pieces, and the 10% solution can maintain the original color for at least six months (GOSOMJI et al., 2018). Due to its action as a disinfectant, antiseptic, germicidal, fungicidal, and bactericidal even at low doses, formaldehyde is very effective in preventing the spread of microorganisms that could cause tissue damage (OLIVEIRA, 2021).

As a precautionary measure for this technique, there must be a constant verification of formaldehyde levels and concentrations. Even so, because formaldehyde has a long lifespan, it is very effective at avoiding the proliferation of microorganisms and tissue autolysis and is a good approach to conserving material. In this way, conserved pieces present good preservation of external and internal organ anatomy, and this allows for a good and easy demonstration of structures. Another advantage relates to the total cost of the technique: considering the most employed formaldehyde concentration as a preserving agent, of 3.7%, and considering it is commercially acquired as 37% formalin, one litter can generate approximately 10 litters of solution (MARTINS-COSTA et al., 2022).

Despite formaldehyde being able to effectively fixate and preserve the macroscopical morphology, in the long term, it causes the darkening of pieces (MARTINS-COSTA et al., 2022). Also, since formaldehyde can penetrate very easily into the tissue, it increases its absolute weight (MARTINS-COSTA et al., 2022), which can result in increased difficulty in manipulating larger pieces, which are already heavy per se. For students, researchers, and teachers working directly with anatomical pieces preserved in formaldehyde, the solution presents a strong and adverse odor and is irritative, causing tearing and burning of eyes and mucous membranes in general (FONTOURA et al., 2020). As a result, there is an inconvenience generated for those who manipulate the pieces.

Investigations aiming at evaluating the resistance of microorganisms to formaldehyde indicate that *Klebsiella sp.* and *Salmonella sp.* are more susceptible than *Staphylococcus sp.*; and that fungi are more resistant than bacteria, with *Aspergillus niger* being more resistant than *Candida albicans*. This higher resistance of species from the genus Aspergillus may indicate a matter of concern, considering that this genus presents a predominance for closed environments, and whose spores can penetrate and contaminate structures very easily (DE OLIVEIRA, 2014). Formaldehyde is also an environmental pollutant and can trigger deep impacts at the moment of disposal if done incorrectly (WORLD HEALTH ORGANIZATION, 2002; MARTINS; SAKALEM, 2022).

It is important to mention that formaldehyde presents a cumulative effect and high toxicity potential to all living beings, along with a potential carcinogenic and mutagenic effect, as already mentioned. Thus, cadaveric material preserved in formaldehyde poses a great threat to the health of students and professionals involved in its manipulation, either directly through exposure to the solution or indirectly by the propagation of pathogenic agents. Every possible preventive care, such as the use of face shields and good-quality gloves, along with the use of adequate clothing, must be taken when handling pieces preserved in formaldehyde.

2.2.2. Sodium Chloride

Due to all the disadvantages of the use of formaldehyde as a laboratory compound, there is a constant and active search for alternative methods to preserve cadaveric material. The use of hyper-saturated salt solution (sodium chloride) is a recent proposition, but it has been implemented and well-accepted in many anatomical laboratories. For this technique, anatomical pieces are kept submerged in a 30% salt solution, and preserved pieces exhibit tissue aspects very similar to fresh pieces (MARTINS-COSTA et al., 2022).

Preservation in sodium chloride also depends on the immersion of the piece in the solution until the moment of use. To prepare the solution, both pure salt (NaCl) and commercial saline can be used (DE OLIVEIRA, 2014). In a recent performance evaluation, 30% of solutions of pure saline and commercial saline presented equivalent results, with commercial saline generating slightly better morphological preservation than pure saline (BRANT, 2023).

Sodium chloride is a natural compound, of low cost, non-toxic, and easily disposable. Previous studies investigating its effectiveness as a preservative agent have shown an

absence of fungal and bacterial contamination of cadaveric pieces maintained in hyper-saturated saline solution, and the preservation of the morphology of the evaluated structures (DE OLIVEIRA, 2014). Pieces that had been used for five years did not present contamination or decomposition. In addition, the solution does not give off strange or inconvenient odors nor issues toxic steam, making the laboratory environment more safe and pleasant (DE OLIVEIRA, 2014; MARTINS-COSTA et al., 2022).

The macroscopical aspect of the pieces preserved in saline resembles the quality of fresh material. In comparative investigations using pieces preserved in ethanol, saline, or formaldehyde, the ones that had been preserved in saline did not fluctuate in weight, in contrast to the ones kept in formaldehyde, which presented an increase in total weight. Also, although the overall dimensions of evaluated structures decreased by a few millimeters compared to fresh measurements and to pieces kept in formaldehyde, no visible difference was found regarding the morphology of the structures (MARTINS-COSTA et al., 2022).

Furthermore, the final color of the pieces tends to be more faithful to the real color of the structure, when compared to formaldehyde and glycerin as preservative agents. There is no need to wash the pieces between uses, but all used pieces must be returned to the tanks or vats after exposure in lectures. The total cost of using salt solution for preservation is less than one-tenth of the cost of using formaldehyde. Also, salt is non-toxic and non-flammable (DE OLIVEIRA, 2014). Pieces kept in saline present a high rate of hydration and malleability, more than formalized ones. Studies using pieces of locomotor apparatus indicate that those preserved in saline present less joint and muscle stiffness in comparison with pieces preserved in formaldehyde (BRANT, 2023). When working directly with pieces maintained in saline, it becomes clear to the handler that the pieces are well-hydrated and more flexible. In studies that evaluated the experience of those involved with dissecting and comparing cadavers preserved in formaldehyde or saline solution, participants affirmed that cadavers preserved in saline were not only more suitable for dissection but also presented a closer aspect to fresh tissues. A percentage of participants also claimed that the experience of dissecting cadavers maintained in saline was remarkably similar to the experience of operating on living patients (HOMMA et al., 2019).

There are also downsides to using saline as a preservative agent. Salt is corrosive, and it is necessary to pay attention to metal stretchers and general materials used in the anatomy laboratory to prevent oxidation. Also, since salt solution penetrates the tissues, during the removal of pieces from tanks and vats for exposure in class or for study and dissection, much of the solution is also taken with the piece, which leads to possible waste.

This disadvantage can be overcome as conducted in our laboratory, by using small vats to separate desired pieces from the tank and allowing them to rest for some minutes in the vats before moving them to the stretchers. This allows the saving of the solution and its return to the tank.

Because of saline's high hydrating potential, there is also the need for constant verification of the state of the cadaveric pieces, to avoid dryness. In addition, the solution evaporates under higher temperatures, and the maintenance of humidity must be conducted several times a day depending on the climate; also, pieces should not be exposed to air for longer hours (DE OLIVEIRA et al., 2014). Evaporation also can cause the precipitation of salt crystals outside the tanks and vats, and even on the pieces, indicating the necessity of constant verification to avoid structural and tissue damage (MARTINS-COSTA et al., 2022).

There have also been reports of the appearance of pathogens, such as bacteria and fungi, in a persistent way in vats containing hyper-saturated saline solution (BRANT, 2023). The same authors have shown that tissue stains and tissue darkening may appear; and, that a rate of autolysis is present, even after the initial fixation using formaldehyde (BRANT, 2023). In conclusion, a hyper-saturated sodium chloride solution presents itself as a viable alternative to formaldehyde as a preservative agent, but the pieces and the laboratory tools must be constantly verified to guarantee the quality of the material.

2.2.3. Glycerin

Glycerol ($C_3H_8O_3$), commercially known as glycerin, was discovered in 1779 by the Swedish chemist Karl W. Scheele. Over one century later, in 1884, the Italian anatomist Carlo Giacomini started using glycerin to preserve cadaveric pieces, creating the Giacomini method of glycerinating (KARAM et al., 2016). Glycerinating is a well-employed technique up to current days, especially because it is a colorless solution with a viscous aspect and is capable of dehydrating structures while presenting an antiseptic effect. These conditions are related to the ease of handling, the possibility of keeping pieces in dry environments, and the ability to keep cellular integrity (SILVA et al., 2016).

Glycerinating enables the maintenance of tissues humid without the need for submersion, as seen in saline or formaldehyde preservation. This technique is vastly employed in medical schools throughout Brazil to preserve human cadavers employed in practical anatomical lessons (SILVA et al., 2016). One major point is that glycerin can replace formaldehyde since glycerinating can be conducted on recently-fixated pieces but also in

pieces that had been fixated in formaldehyde for longer periods (KARAM et al., 2016; FORTUNA et al., 2020).

For the Giacomini method of glycerinating, pieces are previously fixated in formaldehyde at 10% and then kept in formaldehyde at 5% for a year, as described by Silva et al. (2017). To initiate the glycerinating process, the pieces are washed and remain submersed in 95% ethanol for five days. Then, the ethanol solution is renewed, and pieces are maintained for a further five days and submerged. After this, pieces can be allocated in tanks with pure glycerin. Once the pieces submerge into the bottom of the tank, they can be removed and allowed to drain off the glycerin excess. After this process, pieces are placed on stretchers in the shade to dry. Once dry, pieces are ready for use either in classes or for dissection. To guarantee the preservation of the material, pieces must be regularly bathed in glycerin to avoid material deterioration (LIMA et al., 2022).

The use of glycerin to preserve cadaveric pieces presents multiple advantages, and the main one relates to the low oral and dermal toxicity potential, as opposed to formaldehyde, which is irritating and carcinogenic. In addition, glycerin is antibacterial and antifungal, effects that are of utmost importance for tissue conservation. The solution also offers ease of handling since the pieces are lighter, and the solution does not evaporate easily (flash point: 97.3 ºC). This guarantees the possibility of keeping pieces in a dry environment, which is also safe for users due to low steam liberation (FORTUNA et al., 2020).

The major disadvantage of using glycerin as a preservative agent relates to its cost. One litter of glycerin costs approximately three times the cost of one litter of formaldehyde. Due to its viscosity, the cleaning and maintenance of stretchers is more complex. Also, over time, pieces kept in glycerin tend to become darkened in a more notorious way than pieces kept in formaldehyde, which impairs the visualization of anatomical structures. Finally, glycerin is not effective as a fixating agent, which raises the need for the use of formaldehyde for the first steps of glycerinating, preventing anatomy laboratories from eliminating the use of formaldehyde (FORTUNA et al., 2020).

2.3. PRESERVATION OF DRY MATERIAL: CRYODEHYDRATION

Cryodehydration, or cryopreservation, is another accessible and inexpensive alternative to preserve cadaveric tissue. This technique enables the maintenance of specimens at dry and odorless, reducing the use and the exposure to formaldehyde. Cryodehydration consists of consecutive cycles of freezing and thawing, and results, at the

end of the process in a reduction of 60 to 70% in total weight. The final piece preserves the anatomy of the structure, maintaining the key morphological aspects of cavitary and parenchymal organs, muscles, and even whole body sections (CAMPOS et al., 2022).

In the technique, the fresh organs are previously washed and dissected to remove excessive fat tissue; the organs are also weighted to estimate the mean weight loss as the dehydration process occurs. Next, a fixating agent is injected, such as formaldehyde (3.7%) or ethanol (99.5%), with a disposable needle and syringe to a volume of 10% of the initial weight of the organ. Cavitary (hollow) organs must be first filled with cotton embedded with the fixating agent to allow the original organ architecture to be maintained as dehydration occurs (MARTINS; SAKALEM, 2022; FERREIRA et. al., 2023). During the fixating phase, organs remain immersed in the solution inside a vat for at least 20 days. After this period, pieces are washed, and dissected once again to remove any remaining fat or connective tissue, and excess water is removed with absorbent paper. Cavitary organs must have the cotton filling replaced by new, clean, dry cotton pads.

Before the freezing-thawing cycles begin, the organ must be once again weighted, and then they can be frozen at -18°C in a horizontal freezer for at least 12 consecutive hours. After this period, organs are removed and thawed at room temperature in the shade, or under ventilation by a forced air-ventilated greenhouse (at 25°C) for seven consecutive hours (MARTINS; SAKALEM, 2022). The use of a greenhouse is not mandatory but helps to reduce the total days needed to complete the cryodehydration, maintain stable conditions for the thawing process, and standardize final specimens. After thawing, depending on the thickness of the organ wall, it is indicated that a thin layer of liquid glycerin is applied using a soft bristle brush. The application of glycerin before returning the organ to freezing aims at smoothing the material (MARTINS; SAKALEM, 2022). The freezing process followed by the thawing cycle is repeated until the total organ weight has been reduced to 60 to 70% of the initial weight, as already mentioned.

To better follow the whole process, daily weighting is recommended. The technique can be considered effective, and the cycling can be interrupted, when the weight variation is constant for several consecutive days, resulting in a weight variation of up to 5% (MARTINS; SAKALEM, 2022). Once the piece has been cryodehydrated and the final specimen is ready, liquid varnish can be applied using a soft bristle brush. The pieces can then be kept in plastic boxes or vats with lids (MARTINS; SAKALEM, 2022; FERREIRA et. al., 2023). The resulting piece is extremely light, which facilitates transport and handling.

This method can also be highlighted because of its low cost. The only necessary apparatus to perform cryodehydration is a freezer for the freezing process, while thawing can occur naturally at room temperature. Usually, cavitary organs demand less total time to achieve their final phase in comparison with parenchymal organs (CURY; CENSONI; AMBRÓSIO, 2013; CAMPOS et al., 2022; MARTINS; SAKALEM, 2022). The result is cryopreserved organs with visual aspects similar to the fresh material, and almost completely odorless. Regarding maintenance, cryodehydration does not require substance restitution since the piece is kept completely dry. Occasionally, the varnish used to lacquer the piece may need to be reapplied after a few years of use.

As a disadvantage, cryodehydration demands a prolonged time to generate the final pieces, in particular when no greenhouse is employed for the thawing phase. Also, since it is a sort of dehydration, it causes an inevitable tissue refraction due to the water loss, and this is more accentuated when formaldehyde is not the fixating agent used. However, this is less evident for cavitary organs. Another significant alteration refers to the color and stiffness of the organ; the pieces are usually yellowish or whitish due to dehydration and the absence of fat tissue and present a hard aspect. In case the pieces are not constantly verified, it is also possible that they are predated by insects and exhibit corrosion; because of this fragility, it is best to keep the pieces in dry storage, free from insects (CURY; CENSONI; AMBRÓSIO, 2013; CAMPOS et al., 2022; MARTINS; SAKALEM, 2022). In our laboratory, we usually maintain the pieces inside boxes covered with airtight lids to avoid humidity and insert naphthalene mothballs to prevent insects. Cryodehydration is a simple technique, of low cost, easy to replicate, and which enables the preparation of anatomically relevant pieces. The technique also enables the reduction of formaldehyde use, offering better working conditions and turning the anatomy laboratory into a more efficient and fascinating environment (CURY; CENSONI; AMBRÓSIO, 2013; CAMPOS et al., 2022; MARTINS; SAKALEM, 2022).

3. RESULTS AND DISCUSSION

Every technique here presented to preserve cadaveric pieces presents different and specific indications, and advantages and disadvantages. Each anatomy laboratory must evaluate its possibilities before deciding on which technique is more suitable for its reality and objectives. To facilitate the comparison among the three methods, Figure 1 presents pictures of real Hearts fixated in formaldehyde and preserved according to the different techniques: formaldehyde (Figure 1A); saline (Figure 1B); glycerin (Figure 1C), and cryodehydration

(Figure 1D). In addition, Table 1 presents a direct comparison of the methods here presented, with the advantages and disadvantages of each one.

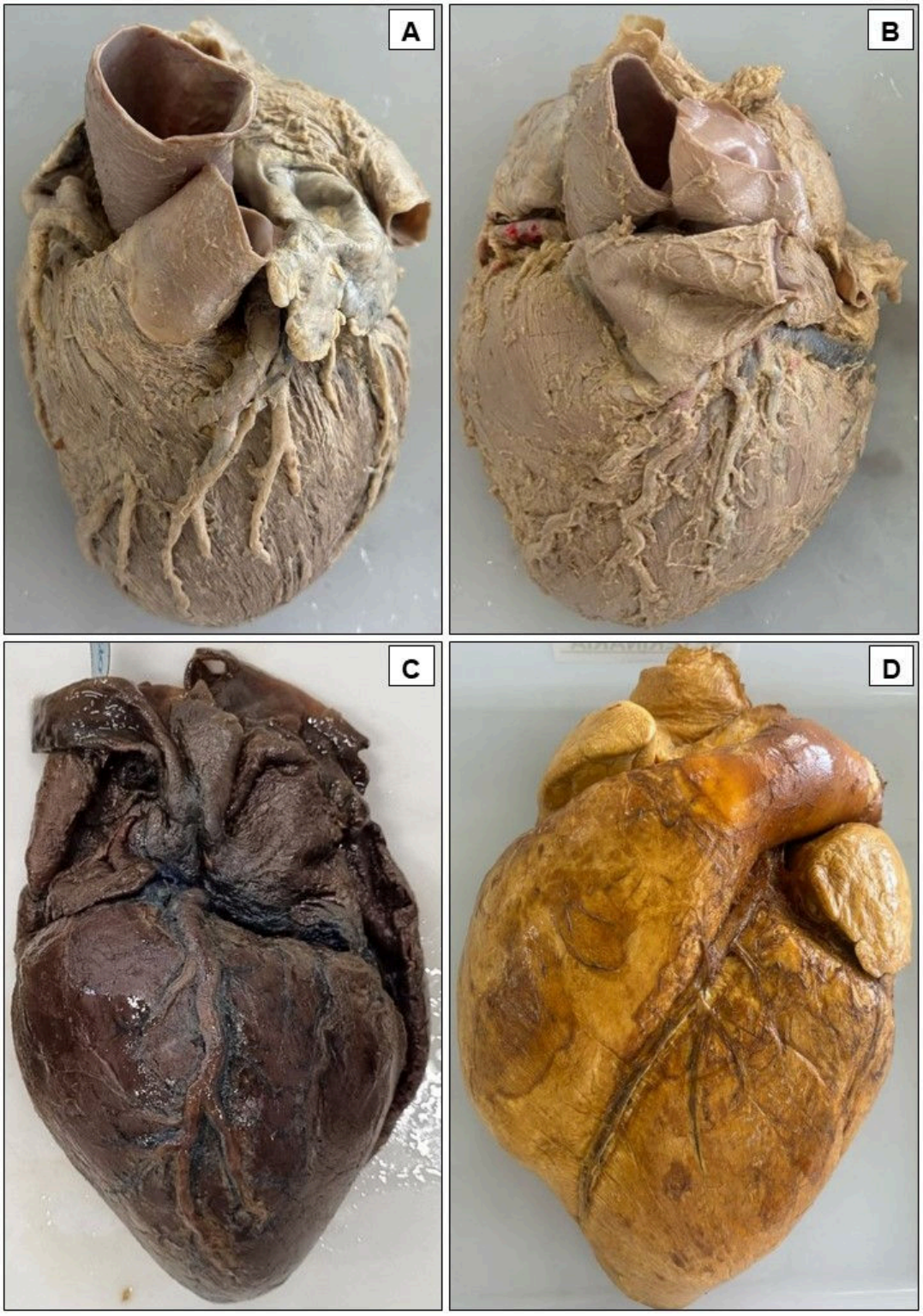

Figure 1. Heart fixated using 3.7% formaldehyde and preserved according to different methods.
A) Preservation in formaldehyde (human); B) Preservation in sodium chloride (human); Preservation in Glycerin (human); Cryodehydrated heart (horse). Personal collection.

Table 1. Different preservation techniques of cadaveric material.

	Type of technique	Advantages	Disadvantages
Humid techniques	**Formaldehyde**	Low cost; Good preservation of morphology; Easy maintenance.	Irritative (eyes and mucous membranes); Carcinogenic; Environment pollutant; Pieces become heavier (solution absorption); Need to verify formaldehyde concentration.
	Sodium chloride	Meager cost; Easy maintenance; Good preservation of morphology; Non-toxic;	Oxidates metallic materials; Pieces become heavier (solution absorption); Need to verify solution level.
	Glycerin	Non-toxic; Easy maintenance; Maintain cellular integrity; No need to keep parts submerged in solution.	Higher cost; Darkened pieces; Difficulty in distinguishing anatomical structures.
Dry technique	**Cryodehydration**	Dry and light pieces; Non-toxic; Enables manipulation with no gloves.	Demands more time to prepare the pieces; Color alteration (yellow/white shades).

7. CONCLUSION

Each method of preservation presents multiple advantages and disadvantages. In addition, the techniques here presented are the ones we have contact with, but they are not the only possibilities for cadaveric conservation. There are several recent approaches of valuable contributions to anatomic learning, such as plastination. It is thus especially important that each laboratory of anatomy and the professionals involved investigate and evaluate the possibilities, to decide for the best suiting method for its workspace, infrastructure, and goals.

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MORPHOMETRIC STUDY OF SKULLS FROM THE DEPARTMENT OF HUMAN ANATOMY AT THE FEDERAL UNIVERSITY OF UBERLÂNDIA

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ABSTRACT

The aim of this study was to investigate the morphological characteristics of skulls belonging to the collection of the Laboratory of Human Anatomy at the Federal University of Uberlândia, thus forming a database for future research. After being washed with neutral detergent and bleached in an aqueous solution of hydrogen peroxide, the 153 skulls were air-dried and subjected to material evaluation, including all those with intact cranial points for subsequent measurements. Craniometry was performed to determine ancestry after sex determination through cranioscopy. Cranioscopy revealed 122 (79.74%) male skulls and 31 (20.26%) female skulls. The calculation of the cranial index (CI) classified them into dolichocephalic, sub-dolichocephalic, mesaticephalic, sub-brachycephalic, and brachycephalic categories, with the majority being brachycephalic, indicating Mongoloid ancestry, both in males (47.06%) and females (13.73%). According to the IV.Pe index, skulls were categorized into platycephalic, mesocephalic, and hypsicephalic, with the majority of male (39.22%) and female (9.81%) skulls being mesocephalic, indicating Caucasoid ancestry. The IV.Po index differentiated skulls into eurycephalic, mesocephalic, and leptencephalic, with the latter being more prevalent in both males (38.56%) and females (13.73%), indicating Negroid and Caucasoid ancestries. Skulls were further categorized into leptorrhine, mesorrhine, and platyrrhine based on the nasal index (IN), with platyrrhine skulls being more prevalent in both males (47.71%) and females (12.42%), indicating Negroid ancestry. Given the breadth of the subject, we were able to contribute to other areas by providing a database for future research.

Keywords: Cranioscopy, Craniometry and Forensic Anthropology.

1. INTRODUCTION

Establishing the identity of a human being has been an unrelenting and challenging goal for many years. Identification refers to the set of procedures to individualize a person or an object. In all spheres of human relations, whether in social interaction or in legal matters, human identification plays an important role. It is through the process of recognizing an individual that it becomes possible to establish an identity, which can be understood as the set of physical, functional, or psychological characteristics, whether normal or pathological, that individualize a particular person. The need for human identification is rooted in the innate social nature of humans and in the desire to differentiate oneself from others (BIANCALANA et al., 2015; CAPP, 2017; ETCHEGOYEN, 2018).

Forensic anthropology is an area of knowledge, and its main object of study is the identification of the human being. It has been studied and improved since the fourteenth century through methods and techniques aimed at achieving the goal of determining an individual's identity. Identifying recent and intact bodies is not as intricate a task for experts, as they have all the biological material necessary for the recognition of crucial characters, responsible for establishing the identity of a human being. However, identification becomes complicated when corpses are charred, skeletonized, incomplete, or in an advanced state of decomposition (CAPP, 2017; CABRAL, 2019; SOUZA; SOARES, 2019).

Skeletal characteristics are often used in forensic anthropology, whether for estimating biological variables or for identifications. There are different methods for identifying corpses; however, skull analysis is one of the most viable means (KONIGSBERG; ALGEE-HEWITT; STEADMAN, 2009; NUNES; GONÇALVES, 2014; PONCE, 2016).

One of the four pillars of the anthropological protocol is the estimation of biological sex, which defines the initial stage of the identification process, as age and ancestry estimation patterns cannot be properly determined without this assessment. Therefore, in any discussion of race within forensic anthropology, it is necessary to also consider sex (JOHNSON et al., 1989; KIMMERLE; ROSS; SLICE, 2008; KONIGSBERG; ALGEE-HEWITT; STEADMAN, 2009; CABRAL, 2019).

According to Konigsberg, Algee-Hewitt, and Steadman (2009), it is important to recognize the difference between the concepts of race and ancestry. The former is a socially constructed mechanism for self-identification and group participation and therefore a biologically meaningless concept, while the latter is a scientifically derived descriptor of the

biological component of population variation and can be estimated in anthropological practice using morphological signatures of population history representing a complex accumulation of genetic variation shaped by generations of microevolution and environmental pressures.

Parts of a skeleton, such as the pelvis, femur, tibia, humerus, and radius, are useful in determining sex (JOHNSON et al., 1989; SANGVICHEN et al., 2007; NUNES; GONÇALVES, 2014). However, skull investigation, through cranioscopic and craniometric techniques, can be used to assist in defining the sex and ancestry of individuals.

The human skull is located at the upper end of the vertebral column, forming a bony box, the cranial vault, which consists of 22 bones, of which only the mandible is movable and is connected to the rest of the skull by a synovial joint; the other bones are joined by fibrous joints that, in the skull, the sutures are its notable features. The skull can be divided into two major portions: the Neurocranium and the Viscerocranium. The former, superior and posterior, larger, houses the brain; the latter, anterior and inferior, smaller, is related to organs of two systems, the digestive and respiratory. The Viscerocranium is commonly known as the face. The skull functions to protect the brain, special sensory organs, and the cephalic parts of the respiratory and digestive systems. Additionally, it allows for the insertion of head and neck muscles, contains foramina for the passage of blood vessels, nerves, and cerebrospinal fluid, has openings essential for air and food passage, and presents maxillae, mandible, and teeth necessary for chewing (DANGELO; FATTINI, 2007; FERREIRA, 2018; SOUZA; SOARES, 2019).

The human skull is complex and presents a series of peculiarities; it is composed of structures with distinct characteristics that succeed each other throughout life, variable quantity of bones among individuals, differences related to ethnic groups, and exhibits significant sexual dimorphism. Except for the pelvis, the skull is widely considered the best indicator for sex diagnosis. In addition to sexual dimorphism, there are also notable differences regarding ancestry (HU et al., 2006; ALMEIDA JÚNIOR et al., 2013; NUNES; GONÇALVES, 2014; BIANCALANA et al., 2015; FERREIRA, 2018; SOUZA; SOARES, 2019.

Sex determination by the skull can be performed through visual inspection, a non-metric method (cranioscopy), observing the morphology of bone features, or by metric analysis (craniometry), based on measurements of its anatomical structures (BIANCALANA et al., 2015).

Cranioscopy, as a visual inspection technique, based on the observation and description of the cranial bone shapes, where the examination of certain characteristics allows sexual distinction in about 70% of the cases analyzed. Craniometry involves measuring the

dimensions of bones, and to be performed, the skull is divided into planes, delimiting its superior, inferior, anterior, posterior, left, and right portions (NUNES; GONÇALVES, 2014; SOUZA; SOARES, 2019).

From these divisions, specific points, the craniometric points, are determined, which have a standardized definition worldwide. Based on these points, linear measurements are taken, and these are compared to a database. These measurements can be used for sex determination, ancestry, and possible stature and age estimation.

Some skeletal structures serve as reference for metric and non-metric methods used for defining human sex in anthropological examinations and in cases of medicolegal investigation (MANOEL, 2009). For such procedures, preference is given to cranial structures due to the varieties that can be evaluated and their known characteristics with sexual and chronological correlations. Additionally, studies have reported that craniometry is a rapid and efficient method for evaluating morphological characteristics, such as aspects related to ethnic groups, sex, age, genetic factors, dietary habits, temporal and regional variations, which can modify the shape and size of bone structures.

The skull is also considered the most informative component of the skeleton for analyzing ancestral traits, as it is the best-preserved part after death. If the analysis cannot be done with the aim of determining a single ancestral origin, individuals of white (leucoderm), black (melanoderm), or yellow (xanthoderm) skin can at least be differentiated (NUNES; GONÇALVES, 2014).

The science that attributes racial origin based on cranial characteristics is called craniofacial anthropometry. Forensic anthropologists perform identification by developing a biological profile, since skulls belonging to the same racial group have common characteristics; however, as races become increasingly mixed, cranial identification becomes more difficult. There are three main groups: Caucasian, Mongoloid, and Negroid (PONCE, 2016).

The Human Anatomy Laboratory of the Federal University of Uberlândia (UFU) has a collection of skulls, which were acquired over its more than 50 years of existence through an agreement with the Human Pathology sector of UFU and donations. However, these skulls had no identification related to sex and/or ancestry. Thus, the present study aimed to verify the morphological characteristics of the skulls belonging to the collection of the Human Anatomy Laboratory of the Department of Human Anatomy (DEPAH) of the Institute of Biomedical Sciences (ICBIM) of UFU. By identifying, through cranioscopy, the skulls of female and male sexes, classifying them, through craniometry, into groups of leucoderms or

Caucasians (white), xanthoderms or Mongoloids (yellow), and melanoderms or Negroids (black).

2. MATERIALS AND METHODS

A total of 158 skulls belonging to the collection of the Human Anatomy Laboratory of the Department of Human Anatomy (DEPAH) of the Institute of Biomedical Sciences (ICBIM) of the Federal University of Uberlândia (UFU) were examined. These skulls were washed with neutral detergent and then bleached for 48 hours in containers containing a 20% aqueous solution of hydrogen peroxide. Subsequently, they were removed from this solution and left to dry in the shade. Once completely dry, the skulls received two coats of acrylic sealer (Coral ™ Paint Gloss), for waterproofing and protection of cranial structures. The study was conducted in accordance with the CONEP (National Commission of Ethics in Research) standards, with exemption from registration in the Biobank, under number 25000.071424/2017-36.

An assessment of the material was performed considering the presence of intact craniometric points; therefore, skulls that had fractures or loss of anatomical structures at these points were excluded. Consequently, out of the 158 skulls in the collection, 153 (96.84%) were included, and five (3.16%) were excluded from the present study.

After the material assessment, cranioscopy was performed for sex classification. This was carried out based on double observation, meaning that the described bone features (Table 1 and Figure 1) were analyzed twice, by two different observers on different days, to avoid biases.

Table 1. Relationship between cranial anatomical features and sex

Bone Features	Male	Female
Frontal bone	More posteriorly inclined	More vertical
Glabella	More prominent	Less prominent
Supraorbital margins	Blunt edges	Sharp edges
Superciliary arches	Prominent	Not prominent
Frontonasal articulation	Greater angle	Lesser angle
Mastoid processes	More developed	Less developed
Styloid processes	Longer and thicker	Shorter and thinner
Occipital condyles	Long and narrow, More robust	Short and wide, Less robust

Source: adapted from Vanrell (2009

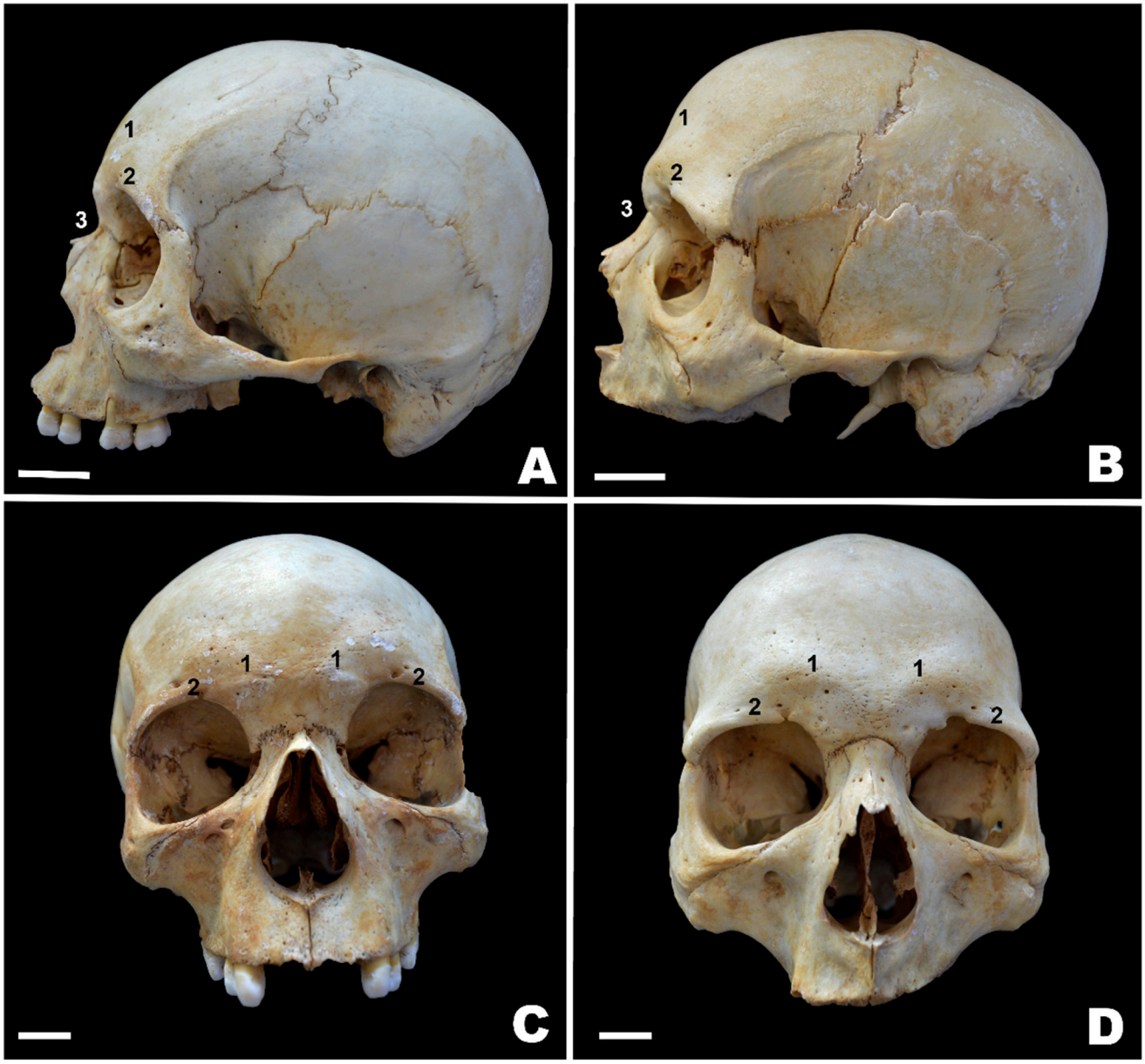

Figure 1. Cranioscopy.
(A) Left lateral view of male human skull. (B) Female: frontal bone (1), glabella (2), angle formed by frontonasal articulation (3), mastoid process (4). (C) Anterior view of male human skull. (D) Anterior view of female human skull: superciliary arches (1), supraorbital margins (2). Scale bar: 1 cm. Source: the authors

The craniometric points examined (Table 2 and Figure 2) followed the definitions of Croce and Croce Júnior (2012) and Pereira and De Mello e Alvim (1979). For craniometry, a digital caliper (Mitutoyo®, Kanagawa, Japan) with a precision of 0.01mm was used. To obtain results with maximum reliability, two measurements were taken by a single examiner at different times.

Table 2. Craniometric points used

Craniometric Point	Definition
Basion (ba)	Midpoint on the anterior margin of the foramen magnum.
Bregma (b)	Convergence point of the sagittal, metopic, and coronal sutures.
Euryon (eu)	Most lateral point of the neurocranium. Location varies, can be on the parietal or temporal bone. Varies between individuals and populations
Glabella (g)	Frontal protuberance between the two superciliary ridges (arches) and above the frontonasal suture.
Lambda (l)	Intersection point of the sagittal and lambdoid sutures.
Nasion (n)	Intersection point of the internasal and frontonasal sutures.
Nasospinale (ns)	Lowest point on the inferior border of the piriform aperture..
Opisthokranion (op) / Metalambda	Most posterior point of the cranial vault in the median plane.
Subspinale (ss) / Subnasal	Most concave point on the sagittal plane, between the prosthion and the anterior nasal spine.

Source: Perreira, de Mello e Alvim (1979 and Croce e Croce Júnior (2012)

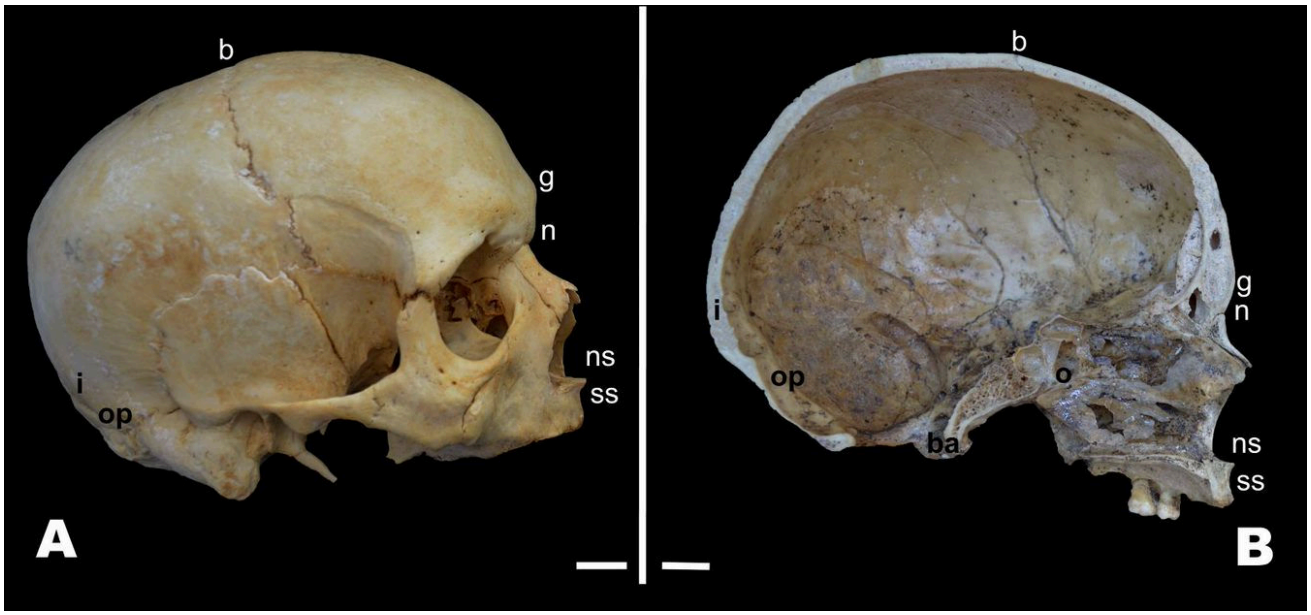

Figure 2. Cranioscopy.
(A) Illustration of the right lateral view of a human skull. (B) Illustration of the medial view of a human skull. Scale bar: 1 cm. Legend: Craniometric points: bregma (b), basion (ba), glabella (g), lambda (l), nasion (n), nasospinale (ns), opisthocranion (op), subspinale (ss). Source: the authors.

For skull classification, measurements were taken of the following segments: maximum length, maximum width, basiobregmatic height, maximum nasal width, nasospinal height (Table 3 and Figure 3).

Table 3. Cranial segments measured in craniometry

Cranial Segment	Definition
Maximum length	Distance between glabella and opisthocranion.
Maximum width	Distance between the two euryon points.
Basiobregmatic height	Distance between basion and bregma.
Maximum nasal width	Greatest horizontal width of the piriform aperture
Nasospinal height	Distance between nasion and nasospinale point

Source: Pereira, de Mello e Alvim (1979) and Croce and Croce Júnior (2012).

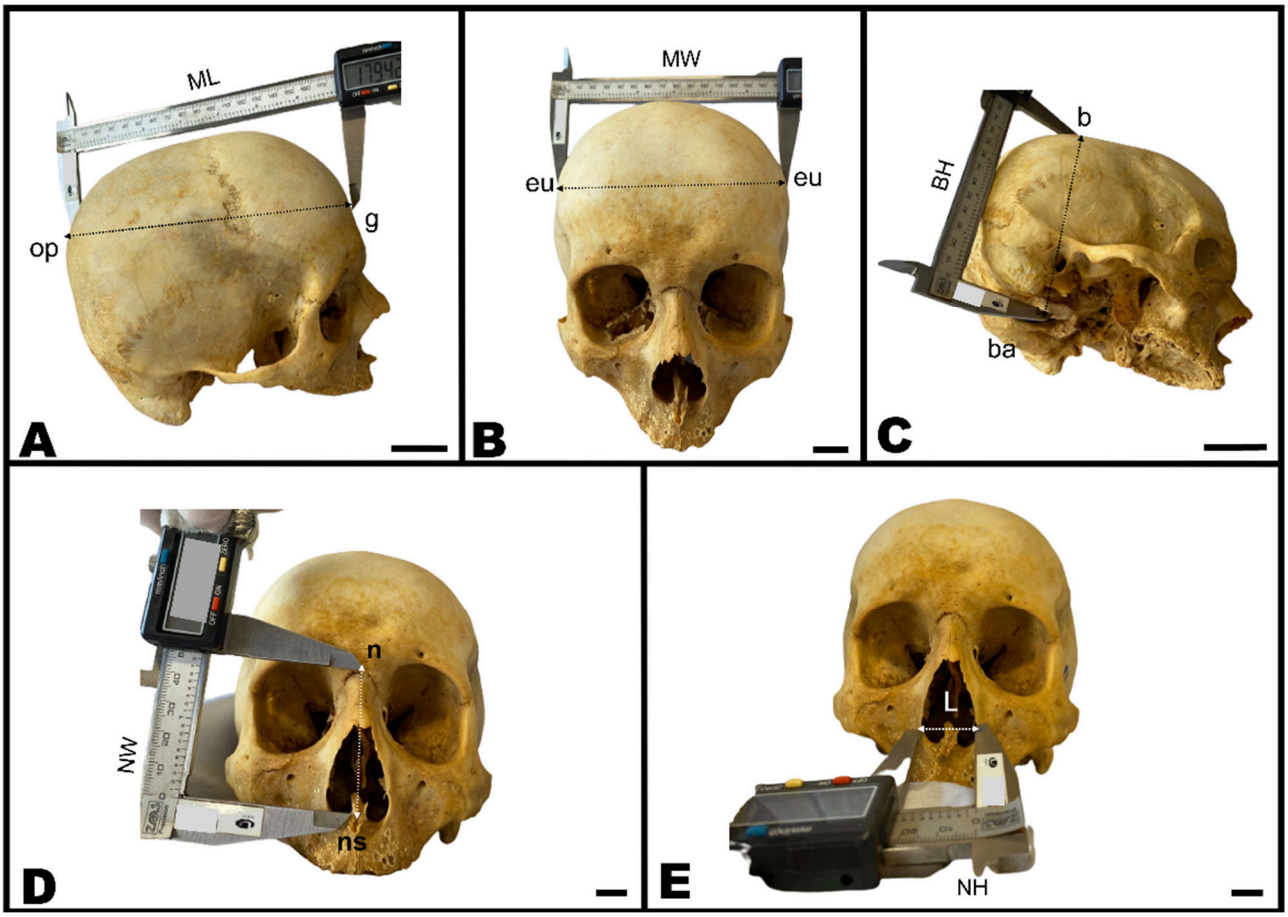

Figure 3. Skull.

(A) Illustration of the lateral view of a human skull demonstrating the maximum length (ML) and the craniometric points. (B) Illustration of the superior view of a human skull demonstrating the maximum width (MW) and the craniometric point. (C) Illustration of the medial view of a human skull demonstrating the basiobregmatic height (BH) and the craniometric points. (D) Illustration of the anterior view of a human skull demonstrating the maximum nasal width (NW). (E) Illustration of the lateral view of a human skull demonstrating the nasospinal height (NH) and the craniometric points. Scale bar: 1 cm. Legend: Craniometric points: opisthocranion (op), glabella (g), euryon (eu), basion (ba), bregma (b), nasion (n), nasospinale (ns). Source: the authors

After the necessary measurements were taken, their absolute values could be evaluated by calculating cranial indices, which are the hundredfold ratio between the measured quantities. The obtained values were compared to pre-established standards, enabling the discrimination of skulls based on their morphological characteristics. The definitions of cranial segments, as well as the parameters of the indices and ancestry, were consulted in the literature (PEREIRA DE MELLO; ALVIM, 1979; VANRELL, 2009; CROCE; CROCE JÚNIOR, 2012) (Table 4 and Figure 4).

The calculated indices were..

Horizontal cephalic index or cranial index or length-width index (HCI)

HCI = (Maximum width x 100) / Maximum length

Vertical index (VI.Pe) VI.Pe = (Basiobregmatic height x 100) / Maximum length

Transverse or posterior vertical index (TVI.Po) TVI.Po = (Basiobregmatic height x 100) / Maximum width

Nasal index (NI) NI = (Maximum nasal width x 100) / Nasospinal height

Table 4. Classification of skulls according to índices.

Cranial index	Classification	Values	Ancestry
ICH	Dolichocephalic	≤ 75	Melanoderms/ Negroids
	Subdolichocephalic	75,01 a 77,77	
	Mesaticephalic	77,78 a 80	Leucoderms/ Caucasians
	Subbrachycephalic	80,01 a 82,33	
	Brachycephalic	≥ 82,34	Xantodermas/ Mongoloids
IV.Pe	Platycephalic	≤ 68,99	Fóssils
	Mesaticephalic	75 a 69	Xanthoderms / Caucasians
	Hypsicephalic	≥ 75,01	Xanthoderms / Mongoloids and Melanoderms/ Negroids
IV.Po	Tapinocephalic	≤ 91,99	Leucoderms/ Caucasians (Central Europeans)
	Metriocephalic	92 a 97,99	Xanthoderms / Mongoloids
	Ectenocephalic	≥ 98	Leucoderms/ Caucasians (northern and southern Europeans) and Melanodermas/ Negroids
IN	Leptorrhine	≤ 47,99	Leucoderms/ Caucasians
	Mesorrhine	48 a 52,99	Xanthoderms / Mongoloids
	Platyrhine	≥ 53	Melanoderms/ Negroids

Source: Pereira, de Mello and Alvim (1979), Vanrell (2009) and Croce and Croce Júnior (2012).

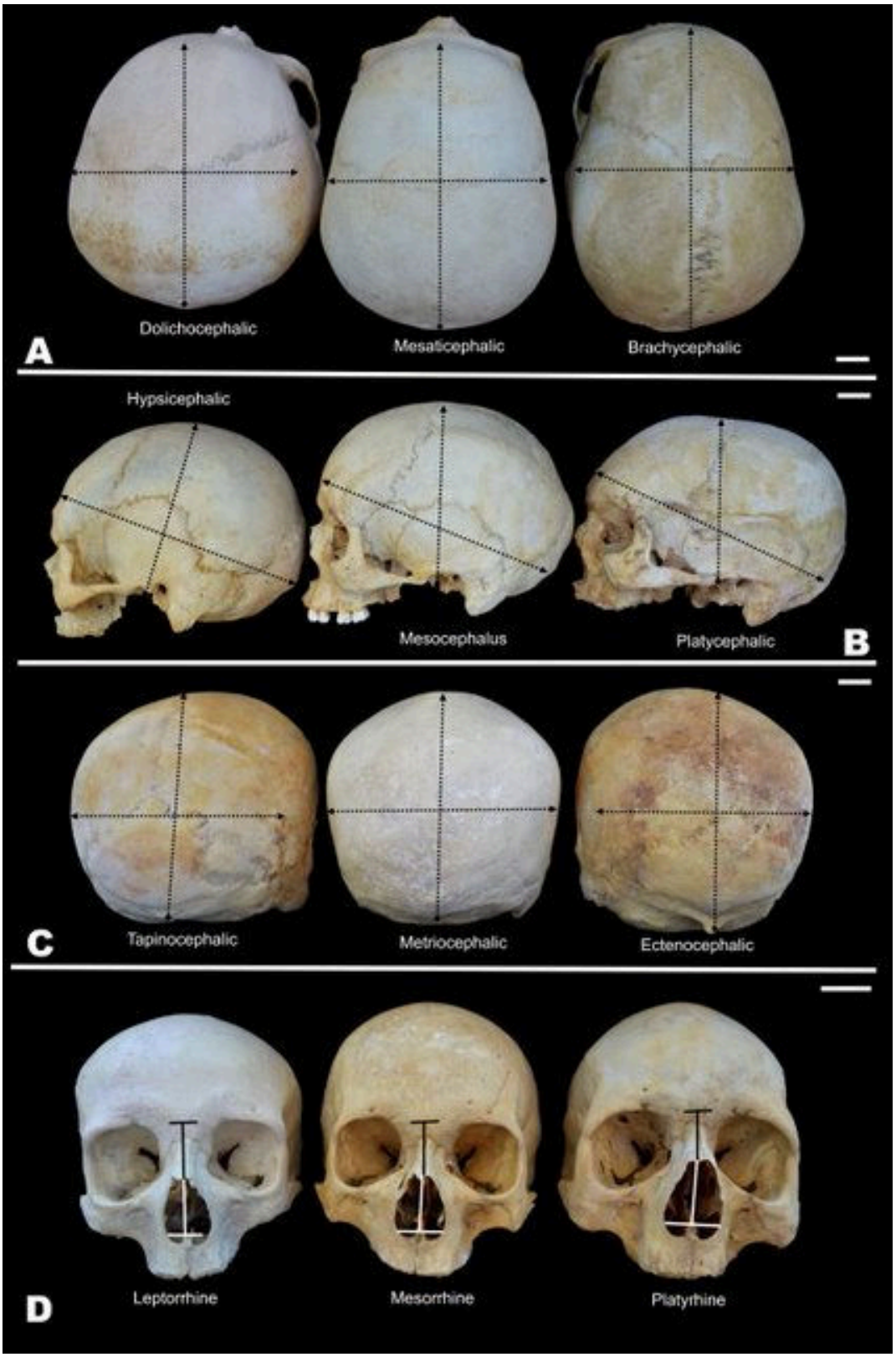

Figure 3. Skull.

Figure 4.(A) Illustration of the superior view of human skulls, demonstrating cranial classification according to the Horizontal Cephalic Index (HCI). (B) Illustration of the lateral view of human skulls, demonstrating cranial classification according to the Vertical Index (VI.Pe). (C) Illustration of the superior view of human skulls, demonstrating cranial classification according to the Posterior Vertical Index (TVI.Po). (D) Illustration of the anterior view of human skulls, demonstrating cranial classification according to the Nasal Index (NI). Scale bar: 1 cm. Source: Modified from Croce e Croce Júnior (2012). Source: the authors

The obtained results were tabulated and subjected to statistical analysis using the BioEstat 5.0 software, adopting a significance level of 95% (P-value < 0.05). Data analysis involved conducting a Proportion Test for two samples.

3. RESULTS AND DISCUSSION

Through cranioscopy, the 153 skulls were analyzed and classified according to sex. Of these, 122 (79.74%) were male and 31 (20.26%) were female (P <0.05). To classify them, eight bony landmarks (frontal, glabella, supraorbital margins, superciliary arches, frontonasal joint, mastoid and styloid processes, and occipital condyles) were evaluated, as the accuracy of sex identification depends on the number and nature of bones examined (FRANCESQUINI JÚNIOR et al., 2007). According to Abe (2000), some scholars believe that sexual dimorphism is better characterized by the shape of cranial bony landmarks rather than by linear or angular measurements, which are based on subjective notions of size.

Craniofacial variations are related to the growth and development of the cranial skeleton and controlled by genetic, geographical, climatic, and racial factors, as they undergo remodeling due to functional influences of masticatory mechanical forces generated by muscles and stimulate the formation of bone tissue directly or indirectly (FARELLA et al., 2003). Morphological differences between male and female skulls are mainly determined by genetic factors rather than nutritional, hormonal, or muscular factors (MANOEL, 2009). Thus, sex determination in human skulls is based on morphological differences, primarily the size and robustness of structures, which may be characteristics of each population and influenced by genetic, environmental, and socioeconomic factors.

With the extensive miscegenation of the population, the issue of estimating sex for forensic purposes through anatomical characteristics becomes even more important, as parameters from other countries may not be valid (CAPP, 2017). Due to migratory processes, geographic, cultural, climatic variations, and interaction with the environment, each population develops a distinct anthropometric profile, which is why the study of different populations is necessary.

Cabral (2019) confirmed that sexual dimorphism in skeletons occurs especially in the skull, jaw, thorax, and pelvis, with the female skeleton generally being smaller than the male skeleton. The jaw exhibits significant sexual dimorphism, with the degree of assertiveness,

based on jaw characteristics, being even greater than that of the cranial box. In the present study, it was not possible to evaluate the jaws, as they were all disarticulated, making it impossible to identify the skeleton to which they belong.

Ancestry, like sex, can be qualitatively assessed through skulls, with the accuracy advocated by various experts ranging from 77% to 92% (JOHNSON et al., 1989). The calculation of the Horizontal Cephalic Index (HCI) allowed the skulls to be classified as dolichocephalic, subdolichocephalic, mesocephalic, sub-brachycephalic, and brachycephalic, with the majority being brachycephalic in both males (47.06%) and females (13.73%). Additionally, it was possible to associate ancestry with the obtained results (Table 5).

Table 5. Classification of male (M) and female (F) skulls from the Collection of the Human Anatomy Laboratory (DEPAH/ICBIM/UFU), according to the Horizontal Cephalic Index (HCI), their absolute frequency, relative frequency (%), and ancestry. Uberlândia, 2020.

Classification ICH	Sex		Total	Ancestry
	F	M		
Dolichocephalic	2 [a] (1,31%) [c]	6 [a] (3,92%) [c]	8 (5,23%)	Melanoderms/ Negroids
Subdolichocephalic	2 [a] (1,31%) [c]	10 [b] (6,53%)[c]	12 (7,84%)	
Mesaticephalic	3 [a] (1,96%) [c]	12 [b] (7,84%)[c]	15 (9,8%)	Leucoderms/Caucasians
Sub brachycephalic	3 [a] (1,96%) [c]	22[b](14,38%)[d]	25(16,34%)	
Brachycephalic	21[a](13,73%)[d]	72[b](47,06%)[e]	93(60,79%)	Xanthoderms/Mongoloids

Legend: a, b: Different letters in the same row indicate statistical difference (P-value <0.05). c, d, e: Different letters in the same column indicate statistical difference (P-value <0.05). Two-sample proportion test.

The ICH can be associated with the individual's race, indicating that melanoderms (black skin) would be dolichocephalic, meaning they would have a greater ratio between the length and width of the skull, denoting a more elongated head, while leucoderms (white skin) would be mesocephalic, with intermediate measurements, and xanthoderms (yellow skin) would be brachycephalic, presenting wider skulls (NUNES; GONÇALVES, 2014). In this study, this association allowed observing a greater number of xanthoderms/Mongoloids; however, the subdolichocephalic and sub-brachycephalic classes could not be defined, which could alter the result of the ancestry of the skulls in question.

In countries with high rates of miscegenation, such as Brazil, determining ancestry is more controversial because, after more than 500 years, racial classifications based solely on physical characteristics become inaccurate due to the mixture of different ethnic groups over time. Differences between human groups are not pronounced (NUNES; GONÇALVES, 2014). However, in the absence of other clues, it is necessary to identify ancestry through the skull.

Capp (2017) and Sousa and Soares (2019) reiterated that, according to a survey by the Brazilian Institute of Geography and Statistics (IBGE), the Brazilian population has historically consisted of European (77%), African (14%), and Native American (8%) populations, and the proportion of each component varies according to the region of the country. The extent of this mixture may not be visible phenotypically. Additionally, dividing the population into three ancestral groups is a practical approach for population surveys but has been shown to be overly simplistic for other applications.

According to the Vertical Index (IV.Pe), the skulls were classified as platycephalic, mesocephalic belonging to leucoderms/Caucasians, and hypercephalic. The majority of the female (9.81%) and male (39.22%) specimens were mesocephalic and of Caucasian ancestry (49.03%) (Table 6).

Table 6. Classification of male (M) and female (F) skulls from the Human Anatomy Laboratory collection (DEPAH/ ICBIM/ UFU), according to the Vertical Index (IV.Pe), their absolute and relative frequencies (%), and ancestry. Uberlândia, 2020.

Classification IV.Pe	Sex		Total	Ancestry
	F	M		
Platycephalic	4 [a] (2,61%) [c]	9 [a] (5,88%) [c]	13 (8,49%)	Fossils
Mesocephalic	15 [a] (9,81%) [d]	60 [b] (39,22%)[d]	75 (49,03%)	Caucasians /Leucoderms
Hypsicephalic	12 [a] (7,84%) [d]	53 [b] (34,64%)[d]	65 (42,48)	Xanthoderms/Mongoloids and Melanoderms/ Negroids

Legend: a, b: Different letters in the same row indicate statistical difference (P-value <0.05). c, d: Different letters in the same column indicate statistical difference (P-value <0.05). Two-sample proportion test.

Hypsicephalic belong to the Mongoloid and Negroids ethnic groups, mesocranial individuals belong to Caucasoid, and platycephalic are categorized as fossils (VANRELL, 2009). However, in this study, 13 platycranial skulls (8.49%) were identified. This occurrence may perhaps be explained by the fact that existing craniometric tables are based on foreign databases (see ALMEIDA JÚNIOR et al. 2013).

As emphasized by Capp (2017), the Brazilian population, according to genetic studies, cannot be considered a unit in terms of diversity. Except when analyzed at an individual level, the multiplicity of the Brazilian population regarding its Race, region, and genetic ancestry should be taken into account when studying this population. Additionally, the studied collection lacks records of skull ancestry, which complicates comparison with other studies.

The Transverse Index (IV.Po) was examined, and the skulls were classified into dolichocephalic, mesocephalic, and brachycephalic, with the latter being more prevalent in both males (38.56%) and females (13.73%), and of negroids and caucasians ancestry (northern and southern Europeans) (Table 7).

Table 7. Classification of male (M) and female (F) skulls from the Human Anatomy Laboratory collection (DEPAH/ICBIM/UFU), according to the Transverse Index (IV.Po), their absolute and relative frequencies (%), and ancestry. Uberlândia, 2020.

Classifiation IV.Po	Sex F	Sex M	Total	Ancestry
Dolichocephalic	4 [a] (2,61%) [c]	26 [b] (17%) [c]	30(19,61%)	Caucasians/Leucoderms (Central European)
Mesaticephalic	6 [a] (3,92%) [c]	37 [b] (24,18%)[c]	43(28,10%)	Mongoloids/ Xanthoderms
Brachycephalic	21 [a] (13,73%)[d]	59 [b] (38,56%)[d]	80(52,29%)	Negroids/Melanoderms and Caucasians/Leucoderms (Northern and Southern European)

Legend: a, b: Different letters in the same row indicate statistical difference (P-value <0.05). c, d: Different letters in the same column indicate statistical difference (P-value <0.05). Two-sample proportion test.

Vanrell (2009) associated tapinocephalic individuals with Negroid and Caucasian ethnicities (northern and southern Europeans), mesocephalic individuals with Mongoloids, and dolichocephalic individuals with Caucasians (central Europeans).

The results found in this research are consistent with the data described by Biancalana et al. (2015), who also used a sample group from the Brazilian population, which is a mixed, hybrid population deriving its gene pool from different populations. The authors described that the Cranial morphology can be influenced by genetic interactions, in addition to environmental factors and mechanisms such as morphological integration and phenotypic plasticity, and therefore, racial miscegenation may be related to cranial metric variabilities within the same population.

When measuring the Nasal Index, leptorrhine, mesorrhine, and platyrrhine skulls were observed. In both sexes, the number of platyrrhine individuals and Negroid/Melanoderm ethnicities was higher, with 47.71% in males and 12.42% infemales (Table 8).

Table 8. Classification of male (M) and female (F) skulls from the Human Anatomy Laboratory collection (DEPAH/ICBIM/UFU), according to the Nasal Index (IN), their absolute frequencies and relative (%) frequencies, and ancestry. Uberlândia, 2020.

Classification IN	Sex F	Sex M	Total	Ancestry
Leptorrhine	4 [a] (2,61%) [c]	13 [b] (8,50%) [c]	17(11,11%)	Leucoderms/Caucasians
Mesorrhine	8 [a] (5,23%) [c]	36 [b] (23,53%)[d]	44(28,76%)	Xanthoderms / Mongoloids
Platyrrhine	19 [a] (12,42%)[d]	73 [b] (47,71%)[e]	92(60,13%)	Melanoderms/ Negroids

Legend: a, b: Different letters in the same row indicate statistical difference (p-value <0.05). c, d, e: Different letters in the same column indicate statistical difference (p-value <0.05). Two-sample proportion test.

kulls leptorrhine belong to Caucasians, mesorrhine to Mongoloids, and platyrrhine ones African Negroids, Australoids, and fossils (VANRELL, 2009).

We used skulls for which information regarding sex and ethnicity was unknown; the sample consisted of unknown material, and as the author noted, one cannot dispute the likelihood that using measurements collected from a different social class could yield different results. The use of craniometry for determining ancestry is limited in assessing the Brazilian population due to its heterogeneous origin, including European, African, and, to a lesser extent, Asian and Arab, mainly using American standards (NUNES; GONÇALVES, 2014). Hence, it is essential to survey cranial measurements and cranioscopic forms of the Brazilian population.

In Brazil, besides the diversity in demographic and ethnological conformation, the climate is also considered heterogeneous, which is a factor impacting both regional and global craniometric variations, particularly in more diverse climatic regions. This suggests that craniometric analysis extreme regions, namely those with extremely low temperatures and those with extremely high temperatures (CAPP, 2017). Skulls of individuals inhabiting regions with cold and dry climates tend to be broader, whereas in regions with elevated temperatures, skulls tend to be narrower and elongated.

Estimating ancestry from bone analyses is a challenging task considering the admixture of the Brazilian population, which differs from investigations conducted in homogeneous populations (SOUSA; SOARES, 2019). In forensic biology studies, particularly genetics and anthropology aimed at identifying individual and population characteristics, greater precision in data description is necessary, especially in criminal investigations

4. CONCLUSION

The skulls in the collection of the Department of Human Anatomy at the Federal University of Uberlândia comprise both male and female specimens. Using the cranial indices ICH, IV.Pe, IV.Po, and IN, it was possible to classify the skulls according to their ancestry. According to the ICH, the majority of the skulls belong to the brachycephalic group, indicating Mongolic ancestry and male sex. IV.Pe revealed mesocephalic skulls, which suggest Caucasoid ancestry and male sex. IV.Po showed skulls from the tapinocephalic group, indicating a mixture of Caucasoid and Negroid ancestry and male sex. Finally, the IN, that

classified the majority of the skulls belonging to the platyrrhine group, indicating Negroid ancestry and male sex..

This study classified and characterized the skulls, contributing to the construction of a database to be explored by further research. Additionally, it suggests that new investigations be conducted, addressing other anatomical points within the scope of craniometry and cranioscopy.

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