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Morphological study of the pineal gland of *Alouatta belzebul*

[*Estudo morfológico da glândula pineal de* Alouatta belzebul]

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ABSTRACT

The pineal is a neuroendocrine gland responsible for the synthesis and release of melatonin. It is present in the brain of vertebrates, but its morphology and location vary considerably among species. For the species *Alouatta belzebul*, although some anatomical aspects of the nervous system have been described, there is no information on the morphology and histological composition of this gland. Thus, the present study aimed to describe the morphological, morphometric, and histological aspects of the pineal of *Alouatta belzebul*. Seven adult specimens were dissected from which the location of the gland in relation to the surrounding brain structures was described, and its length and width were measured. Histological slides were then prepared and stained using hematoxylin-eosin and PAS techniques. It was observed that the pineal of *Alouatta belzebul* is located superior and cranial to the cerebellum, superior to the superior colliculi and below the splenium of the corpus callosum and was classified as sub-callosal. It had an average length of 2.6mm and an average width of 1.14mm. Histologically the gland is composed of irregular strands of pinealocytes and gliocytes. The pinealocytes showed pigments similar to melanin.

Keywords: morphology, neuroanatomy, primate, pineal gland

RESUMO

A pineal é uma glândula neuroendócrina responsável pela síntese e liberação de melatonina. Está presente no encéfalo dos vertebrados, mas sua morfologia e localização variam consideravelmente entre as espécies. Para a espécie Alouatta belzebul, *apesar de terem sido descritos alguns aspectos anatômicos do sistema nervoso, não há informações sobre a morfologia e composição histológica desta glândula. Assim, o presente estudo teve como objetivo descrever os aspectos morfológicos, morfométricos e histológicos da pineal de* Alouatta belzebul. *Foram dissecados sete encéfalos de espécimes adultos dos quais se descreveu a localização da glândula em relação às estruturas encefálicas circunvizinhas e foram realizadas medidas de comprimento e largura da mesma. Em seguida foram preparadas lâminas histológicas e coradas pelas técnicas de hematoxilina-eosina e PAS. Observou-se que a pineal de* Alouatta belzebul *localiza-se superior e cranialmente ao cerebelo, superior aos colículos superiores e abaixo do esplênio do corpo caloso e foi classificada como subcalosa. Apresentou comprimento médio de 2,6mm e largura média de 1,14mm. Histologicamente a glândula é composta por cordões irregulares de pinealócitos e gliócitos. Os pinealócitos apresentaram pigmentos semelhante à melanina.*

Palavras-chave: glândula pineal, morfologia, neuroanatomia, primata

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INTRODUCTION

The species *Alouatta belzebul* is endemic to Brazil and occurs in the Amazon floodplain forest, the floodplain forest of Marajó and fragments of the northern Atlantic Forest. Regarding the nervous system of this species, the spinal cord, vascularization of the encephalon and venous sinuses of the dura mater have been described. However, the morphological aspects of the pineal gland have not been described (Souza-Terra *et al.*, 2018; Sabec-Pereira *et al*., 2020a, 2020b). According to the Red List of Brazilian Fauna threatened with extinction classification updated in 2018, the species *A. belzebul* is categorized as vulnerable.

The pineal gland, also known as the pineal body or cerebral epiphysis, is a structure found in the vertebrate brain whose location and anatomy vary significantly between species (Klein, 2015). In humans, non-human primates, and ungulates the gland is located deep, in the epithalamus, and maintains contact with the third ventricle through the pineal recess. However, in rodents, part of the pineal is found more superficially, between the cerebellum and cerebral cortex connected by a rod to a deeper and smaller portion, in the epithalamic region (Mano and Fukada, 2007; Falcon *et al.*, 2009).

The pineal gland develops from a saccular evagination of the roof of the diencephalon, posterior to the midline of the third ventricle. The diverticulum gives rise to a solid mass of cords, or cordlike clusters of pinealocytes and glial-like support cells, surrounded by connective tissue derived from the meninges and carrying blood vessels and nerves to the pineal gland. The histological composition of the already developed pineal gland comprises some cell types such as pinealocytes, microglia, astrocytes, and endothelial cells. The pinealocytes are secretory cells that rest on a basal lamina, have cellular extensions that end in bulbous expansions, of which some end near capillaries. In addition, they have areas of ribbon synapse, characterized by dense lamella surrounded by an areola of vesicles (Mays *et al.*, 2018; Lumsden *et al.*, 2020).

Another histological feature of the pineal, observed in some species, is the presence of melanin pigments in pinealocytes (Busolini *et al.*, 2017). In addition, in several species, defined areas of calcification, called calcareous concretions, are also observed, resulting from the secretion of extracellular matrix by pinealocytes, in which calcium phosphate crystals are deposited. The incidence varies among species and individuals (Przybylska-Gornowicz *et al.*, 2009). Functionally the pineal acts as a neuroendocrine organ and is responsible for the synthesis of melatonin that regulates the circadian rhythm in mammals and the estrous cycle in some species (Bolat *et al.*, 2018). In addition, it is currently considered a regulator gland that modifies the activity of the adenohypophysis, neurohypophysis, pancreatic islets, parathyroid, adrenals, and gonads (Koshy and Vettivel, 2001).

Finally, the objective of this work was to describe the macroscopic anatomy, morphometric aspects, and histological composition of the pineal gland of *A. belzebul*.

MATERIAL AND METHODS

Seven adult specimens of *A. belzebul*, without apparent brain lesions, four males and three females were used, collected during the rescue and salvage of terrestrial fauna, during the vegetation suppression activities for the implementation of the Belo Monte hydroelectric plant - Brasília-DF, governed by IBAMA process No. 02001.001848/2006-75 and authorization No. 473/2014. After rescue, the animals were frozen and sent to the Human and Comparative Anatomy Laboratory of the Federal University of Jataí, kept under freezing until the beginning of the fixation process. This study was submitted to the Ethics Committee on Animal Use/CEUA-Jataí and approved under protocol 031/19.

For the fixation procedure, the animals were thawed at room temperature for twelve hours on aluminum tables and covered with cloths. Then, the bodies were submitted to intramuscular, subcutaneous, and intracavitary injections of a 10% aqueous formaldehyde solution and submerged in tanks with the same solution, in which they remained for at least 72 hours. After the fixation period, trichotomy was performed on the dorsal surface of the primates' heads, followed by dissection of the skin and subcutaneous tissue using a scalpel, scissors and

anatomical tweezers, starting at the temporal region in a craniocaudal direction. After folding the skin together with the intrinsic muscles of the temporal region, the cranial cap was removed with an oscillatory saw (Dremel® 3000) in the cranium-caudal direction, from the height of the frontal bone to the occipital bone, to maintain brain integrity.

Then the meninges were carefully removed with the help of tweezers, scissors, and scalpel to expose the brain, which was dissected until accessing the pineal gland. Then the glands were measured with an MTX® digital pachymeter. A digital camera was used to photograph the exposed gland and later its location and position in the brain, its shape and dimensions were described.

After dissection, the pinnae were again submitted to 10% formaldehyde solution for 48 hours to ensure fixation and to prepare histological slides. Then, the samples were dehydrated in alcoholic solution in increasing concentrations from 70% to 100%, diaphanized in xylol, impregnated in paraffin and embedded. For the preparation of slides, serial sections were made, with approximately 5µm thickness and stained with hematoxylin-eosin and PAS (Tolosa and Behmer, 2003). The observation and analysis of the histological sections were performed using a Leica DM 750 optical photomicroscope with an ICC50 (HD-521420221) built-in digital camera.

RESULTS AND DISCUSSIONS

Although the animals were from wildlife rescue and the initial conditions of preservation of the cadavers could influence the state of the encephalon, the glands, as well as the encephalic structures, were present in a good state of preservation in all the specimens analyzed. In other work with *A. belzebul*, specimens were used under the same conditions, kept frozen, and it was possible to conduct anatomical and histological studies (Sabec-Pereira *et al*., 2020a, 2020b, Pereira *et al*., 2020).

Regarding the anatomical characteristics, the pineal was observed as a unique structure, well developed, and located in the region corresponding to the median sagittal plane, between the occipital lobes of the cerebral hemispheres, superior and cranially to the cerebellum, superior to the superior collicles and below the splenium of the corpus callosum. It is in direct contact with the tissue of the third ventricle, resting on the mesencephalic roof, and connected by two transverse bundles of fibers that connect the gland to the commissure of the habenulae (Fig 1).

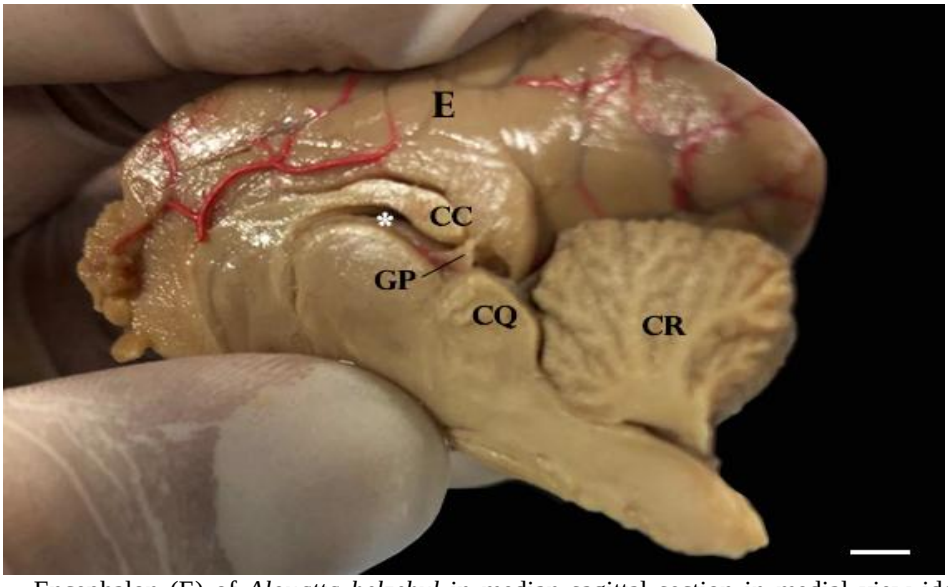

Figure 1 – Encephalon (E) of *Alouatta belzebul* in median sagittal section in medial view identifying cerebellum (CR), third ventricle (*), pineal gland (GP) and its sub-callosal anatomical position, below the splenium of the corpus callosum (CC), cranially to the quadriplegic bodies (CQ). Bar = 1cm

The location of the pineal, in relation to the splenium of the corpus callosum, allows us to classify it as subthalmos, according to the proposal of Blin and Maurin (1956). According to the authors, the relation of the gland with the anatomical region allows its observation as supracalosa, subcalosa and postcalosa. Considering this classification criterion, the pineal is subcalosa in carnivores and primates, corroborating the findings in *A. belzebul*, while the postcalosa is found in cattle, horses, mules, donkeys, goats, and sheep (Vollrath, 1981). In rodents, considering the location of the pineal for classification may be flawed, because the gland shows great variation in shape and position, so it may occupy a sub or supracalosa position or both (Branco *et al*., 1997), a fact not observed in this study.

Macroscopic studies of the buffalo pineal gland have shown it to be large, globose in shape, divided into two lobes (right and left) and situated between the rostral colliculi (Carvalho, 1997). This division into lobules was not found in *A. belzebul*, corroborating the findings in cattle and dogs, since the connective tissue trabeculae of the parenchyma are not numerous (Leeson and Lesson 1976).

The pineal gland of marsupials can be seen as a slight bulbar evagination, slightly below and immediately behind the habenular commissure. Longitudinal median sections of the brain and the habenular region more clearly reveal its relationship to the habenular and posterior commissures. It consists of a simple evagination of varying degree of the inter commissural portion of the roof of the third ventricle (Mançanares, 2004).

Considering its close relationship with the third ventricle, it is also possible to classify it as a proximal or type A gland (Vollrath, 1981), data that corroborate the findings of the pineal gland of buffaloes (*Bubalus bubalis*), nail monkeys (*Sapajus libidinosus*), (Carvalho-Barros, 2006) and in *A. belzebul*. The consistency of the pineal glands analyzed was gelatinous in 100% of the specimens. As for its coloration, it is similar to the gray substance of the whole brain in *A. belzebul*.

The three-dimensional shape of the pineal glands verified in this study was glossoid (similar to a human tongue), being divided into apex, body and base; the apex was directed to the superior colliculi, the body is the intermediate region and connects the apex to the base (Fig 2). It is arranged with the base inserted in the region of the habenules, constituting there an infundibular structure once that region is invaded by the pineal recess of the III ventricle. When observed in dorsal view, the pineal insertion in the region of the habenulae simulates, in both antimeres, a peduncle arrangement (Fig 3).

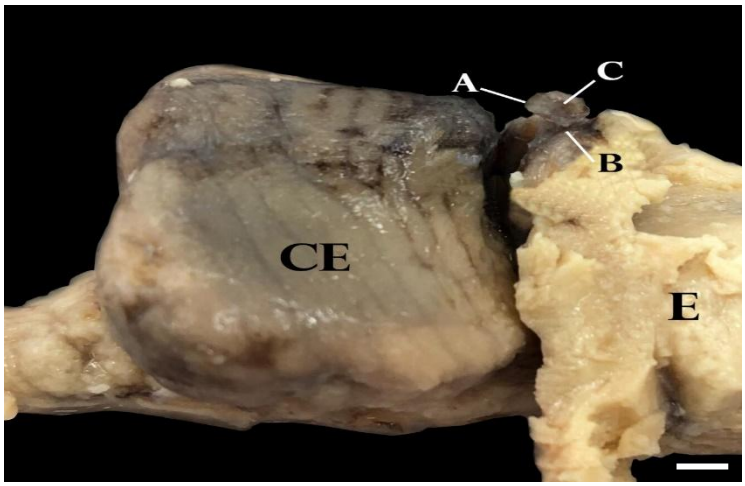

Figure 2 - Lateral image of the *Alouatta belzebul* pineal gland exposure, presenting a glossoid shape and divided into portions: base (B), body (C) and apex (A). CE= cerebellum; E= encephalon. Bar: 0.7 cm

The *Didelphis* pineal structure, according to Maçanares (2004) is very peculiar and has a unique inverted "U" shape, which differs from findings in rodents that have, according to Branco *et al.* (1997) and Vollrath (1981), a rod shape and, in snakes, that according to Holanda-Barros (2002), have an ovoid shape.

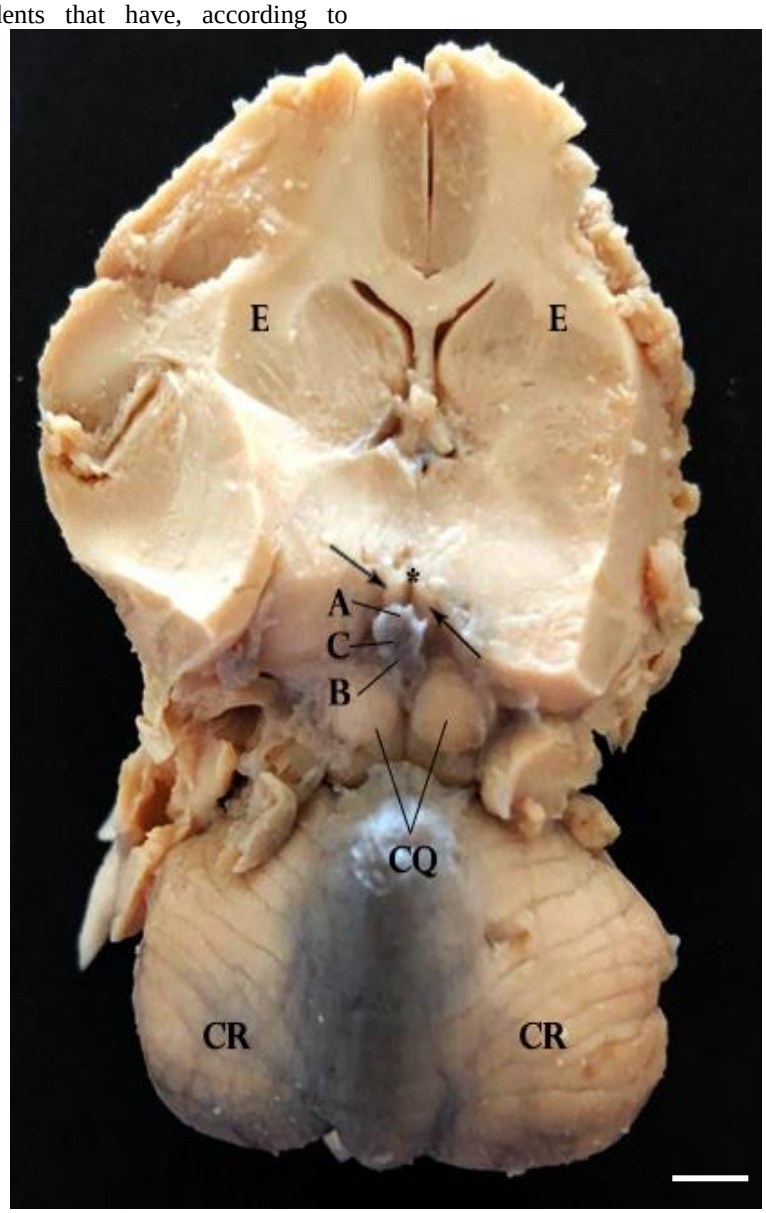

Figure 3 - Cross section of the cerebral hemispheres of *Alouatta belzebul* observing the pineal gland divided into base (B), body (C) and apex (A). Third ventricle (*), pineal recess (arrow), cerebellum (CR), quadriplegic bodies (CQ) and encephalon (E). Bar: 1cm

The designation of the parts of the pineal gland in capybaras (apex, shaft, and base) proposed by Silvino (1994), due to the elongated shape of the gland, is also observed in humans, although the part called shaft is called body by him, as well as in *A. belzebul*. As for the location of the gland, the literature is unanimous in stating that the pineal gland rests between the quadruplet bodies, and as for its connections (peduncles), these vary in number and position in the species. It was also found that in the monkeys *Sapajus libidinosus* and *A. belzebul*, the connections with the pia mater and meningeal extensions of the base are present, constituting the main means of

attachment of the pineal, as suggested by Quay (1970).

In gorillas, Hartmann and Straus Junior (1932) describe that the pineal is attached by a broad base to the dorso-posterior border of the thalamus-mesencephalon and extends horizontally in the sulcus between the cranial quadruplet bodies. Novotná *et al.* (1966) for *Macaca mulatta*; Knight *et al.* (1974) for *Cercopithecus aethiops*; Simmons (1976) also for *Cercopithecus aethiops* state that the pineal is attached by a rod to the roof of the diencephalon or commissura habenularis. Quay (1970) cites that in the Orangutan, the main mass of the commissura habenularis is contained within the pineal body, as it is dispersed over a wide area near the posterior commissura, ventrally to the corpus callosum. For humans, Testut and Latarjet (1979) state that the pineal is located under the knee of the corpus callosum and is kept in position by a series of adhesions to the pia mater and a series of extensions from its base to neighboring structures.

From the point of view of its dimensions, the pineal gland of *A. belzebul* presented an average length of 2.6mm and an average width of 1.14mm. The measurements are compiled in Table 1.

Table 1**.** Length and width measurements of the pineal glands of *Alouatta belzebul*, expressed in millimeters. CP = pineal length; LP = pineal width

Species	Gender	CP	LP
1	M	2.01	1.24
2	M	1.96	1.01
3	M	2.36	1.04
4	M	2.18	1.28
5	F	2.41	1.15
6	F	2.04	1.07
7	F	2.17	1.19
Average		2.16	1.14
Median		2.17	1.15

In their study on the dog pineal (Ellsworth *et al.*, 1985), they did not direct their analysis to the dimensions of the pineal, however they point out that it varied in size in the nine dogs studied and that the diameter was less than or equal to 1mm, thus diverging from the data exhibited by Hullinger (1993) in the treatise entitled *Evans-Miller's Anatomy of the dog* and from the findings of other authors such as Venzke and Gilmore (1940) and Zach (1960).

In gorillas, Hartmann and Straus Junior (1932) observed 2.3 x 2.3mm; Quay (1970), for the Orangutan: 1.7 x 1.1mm; Knight *et al.* (1974), for *Cercopithecus aethiops*: 3.0 x 1.5 mm. However, in relation to the human being, for whom Testut and Latarjet (1979) cite measurements of 7.8 x 4.6mm and Willians *et al.* (1995), 8.0 mm in length, if we consider the body projections between man (± 70 kg) and the monkey *Sapajus libidinosus* (±3.0kg), the relative size of the pineal gland between these two species shows approximate equivalence.

However, it is believed that the relatively average size of the pineal of *A. belzebul* monkey, in view of its small body mass, may be related to the regulatory function of endocrine systems against the action of environmental changes or the organism itself, which earned it the denomination of "regulator of regulators", in relation to all species studied.

Observation of the histological slides (Fig 4) revealed that the *A. belzebul* pineal is composed of pinealocytes with conspicuous, approximately rounded nuclei. These cells are arranged in irregular strands along the parenchyma of the gland, and another cell type, the astrocyte-like gliocytes, appear between them. The cytoplasmic processes of these two cells form a net-like structure between the cells, called the neuropyle. Along the strands of pinealocytes blood vessels are observed in various directions. On the slides stained with PAS, it was possible to observe, in some cells, granules of brownish or dark brown pigments, similar to melanin.

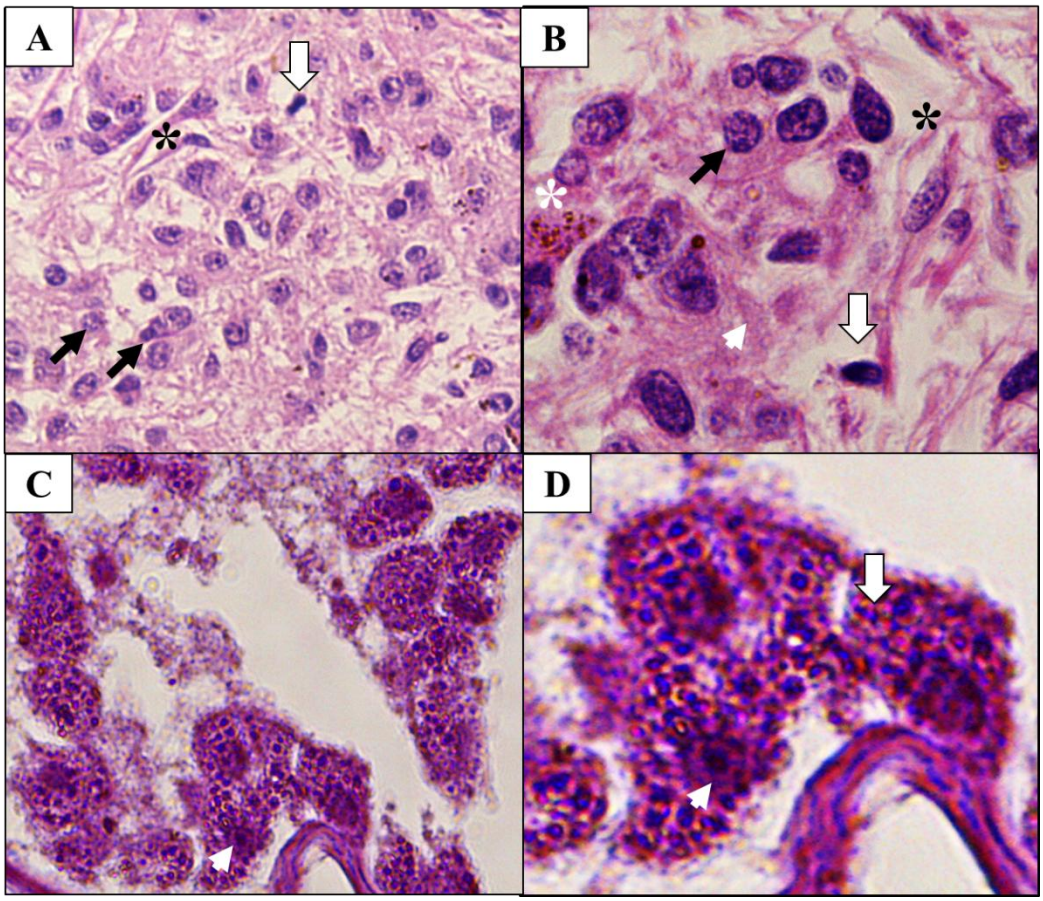

Figure 4. Pineal gland of *Alouatta belzebul*. (A) general histological appearance, with pinealocytes arranged in irregular strands (black arrows), presence of blood vessels (asterisk), astrocyte-like gliocyte (white arrow). HE, 200x. (B) pinealocyte (black arrow), astrocyte-like gliocyte (white arrow), neuropyle (white arrowhead), blood vessel (black asterisk) and pigmentation (white asterisk). HE, 400x. (C) Pinealocyte nucleus (white arrowhead) and cytoplasmic granules. PAS, 400x. (D) pinealocyte nucleus (white arrowhead) and melanin granules in the cytoplasm (white arrow). PAS, 1000x.

The presence of melanin-like pigments in the pineal has been described in different mammals, such as cattle, chinchilla, equine, bat, dog, cat, sheep, and human. However, although histochemical and ultrastructural studies have confirmed that the nature of this pigment is similar to that of melanin, the origin of the cells is controversial. Authors usually report the presence of pigment in pinealocytes, but Regodon *et al.* (1998), proposed that the ultrastructural characteristics of pigmented cells do not coincide with those of pinealocytes and astrocytes, so they could be a third cell type. Koshy and Vettivel (2001) stated that melanin pigments are present in fetal pinealocytes of animals and humans and would continue in the adult cell. Moreover, they demonstrated in their work that the pigment accumulation occurs gradually with increasing age.

Although the actual reasons for the accumulation of melanin-like granules in the pineal are unknown, the phenomenon occurs in different species, demonstrating that there is a common physiological mechanism, as in *A. belzebul*.

CONCLUSION

It was concluded that the pineal gland of *Alouatta belzebul* can be classified as sub-callosal, as it is located superior and cranial to the cerebellum and superior to the superior colliculi and below the splenium of the corpus callosum, similar to what was observed in other primates and carnivores. In addition, the pineal

gland in *A. belzebul* is well developed, with a mean length of 2.6mm and a mean width of 1.14mm. Histological analysis showed that it is composed of pinealocytes arranged in irregular filaments along the parenchyma and astrocyte-like gliocytes. In the pinealocytes were observed pigments, similar to melanin, already described in other species.

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