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Original Article

Anatomy of the spinal cord of *Alouatta belzebul*

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The genus *Alouatta* hosts species popularly known as red-handed howler, presenting wide geographic distribution and being found in several biomes. The objective is to describe the anatomy of the spinal cord of *Alouatta belzebul* specimens, focusing on the topography of the medullary cone, stressing the cervical and lumbar intumescences and cauda equina, to provide anatomical data and compare it with other species to assist in anesthetic and surgical procedures. Four animals were received for scientific research, *post mortem,* from the fauna rescue program of the Hydroelectric Plant of Belo Monte - Pará, and they were fixed in 10% formaldehyde solution. Structures such as the medullary cone, cervical and lumbar intumescence, and cauda equina were photographed (Sony α200-10.2 mpx). After thawing, we measured the specimens and observed a size of 80 to 82 cm from head to toe. After the skin and musculature were removed, it was observed that the spine of all specimens presented 7 cervical, 13 thoracic, 5 lumbar and 3 fused sacral vertebrae. The spinal cord was exposed after the removal of vertebral arches, it has 22 cm length in all animals, presenting the cervical intumescence between C3 and C6 vertebrae, with average of 2.2 cm and lumbar intumescence between T11 and T12 vertebrae, with average of 1.65 cm. The medullary cone is located between T12 and L1 vertebrae, with average of 1.5 cm, and the cauda equina between L1 and S3, with an average of 15 cm. This study has an important role as the basis for epidural anesthesia in the species.

INTRODUCTION

Advances in comparative animal anatomy possess fundamental importance due to the scarcity of information available in the literature. The anatomical descriptions subsidize comparative and evolutionary studies, since through these descriptions one can succeed in anesthetic procedures essential to diagnostic and surgical processes (SLULLITELL, 2008).

Regional anesthetic techniques are used with a suitable safety margin, aiming to anesthetize spinal nerves of the lumbar and sacral regions, thus the location of anesthetic application in the epidural space varies according to the species and the ending site of the spinal cord. The use of sites caudal to the medullary cone makes the application technique safer, consequently avoiding spinal cord injuries and helping professionals who need to perform surgical procedures (GREGORES et al., 2010; SOUZA et al., 2014).

Primates of the genus *Alouatta* are medium-sized mammals, yet they are considered robust animals and well-known for long-range vocalization. Their jaw accommodates a rather large hyoid bone, mainly in males, which forms an oval resonance chamber, responsible for the characteristic vocalizations related to group location and territory defense (HIRSCH et al., 1991). Research about these primates involves diet, vocalization, life style and ecology of the species (AGUIAR et al., 2003; BICCA-MARQUES et al., 2009; BICCA-MARQUES, 2003; CAMARGO, 2005; GRECORIN,

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2006; MARTINS, 2002; NEVILLE et al., 1988). However, information regarding the comparative and/or evolutionary anatomy of these animals is scarce, as well as clinical correlations with the described structures.
The red-handed howler *Alouatta belzebul* is endemic to Brazil, occurring in the states of Amazonas, Pará, Maranhão, Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco and Alagoas (BONVICINO, 1989; RUSSELL et al., 2013). It is a neotropical primate, pertaining to the family Atelidae. Due to the fundamentally folivorous feeding habit (NEVILLE et al., 1988), howlers present slow and discrete behavior and spend more than 70% of their time resting (SOUZA, 2005).

Habitat destruction and fragmentation has placed all *Alouatta* species and subspecies in the endangered species category (BICCA-MARQUES, 2003; CHIARELLO et al., 2008; RUSSELL et al., 2013). Due to the huge threat, howler management tools as translocation and reintroduction are being increasingly observed and successfully applied in *Alouatta* conservation programs (JERUSALINSKY et al., 2010; SOUZA, 2005). The management techniques require ecological, behavioral and morphological data of the target species (STERLING; BYNUM; BLAIR, 2013).

The collection and analysis of cerebrospinal fluid (CSF) present a safe, viable and effective means of access and evaluation of the nervous system, for diagnosis and prognosis of its diseases, such as encephalopathies and myelopathies that affect the *Alouatta* (GAMA et al., 2005; TRANQUILIM et al., 2013). According to Bailey and Vernau (1997), this test can detect diseases in the central nervous system with reasonable sensitivity and low specificity and thus provide a general index of health of the system.

The use of anesthesia in wildlife management is a common practice in veterinary medicine, in which safe and effective drugs are used to contain the animal during the procedures (WOLFE-COOTE, 2005). Epidural anesthesia is a frequently used technique because of its good margin of safety and ease, showing itself as an effective and practical alternative in cases that the animals present risk factors to inhaled or intravenous anesthetics. This technique is used to anesthetize the spinal nerves of the lumbar and sacral regions and data about the application procedure and anatomy of the manipulated species are necessary (CARVALHO, 2004; GREGORES et al., 2010).

The place of application of the anesthetic in the epidural space varies according to the species, being related to the ending site of the spinal cord. However, the use of sites caudal to the medullary cone makes the application safer, thus avoiding spinal cord injuries (GREGORES et al., 2010). The objective of the present study was to describe the anatomy of the spinal cord of *A. belzebul*, emphasizing in its structure the vertebrae and intumescences location, as well as the morphology of the medullary cone, in order to contribute with anatomical bases to the practice of epidural anesthesia in this species.

MATERIALS AND METHODS

In the present study, four male *A. belzebul* specimens were collected from the Wild Animals Triage Center linked to the fauna rescue program of the Hydroelectric Plant of Belo Monte under license nº 473, frozen and subsequently donated to the Federal University of Goiás - Regional Jataí.

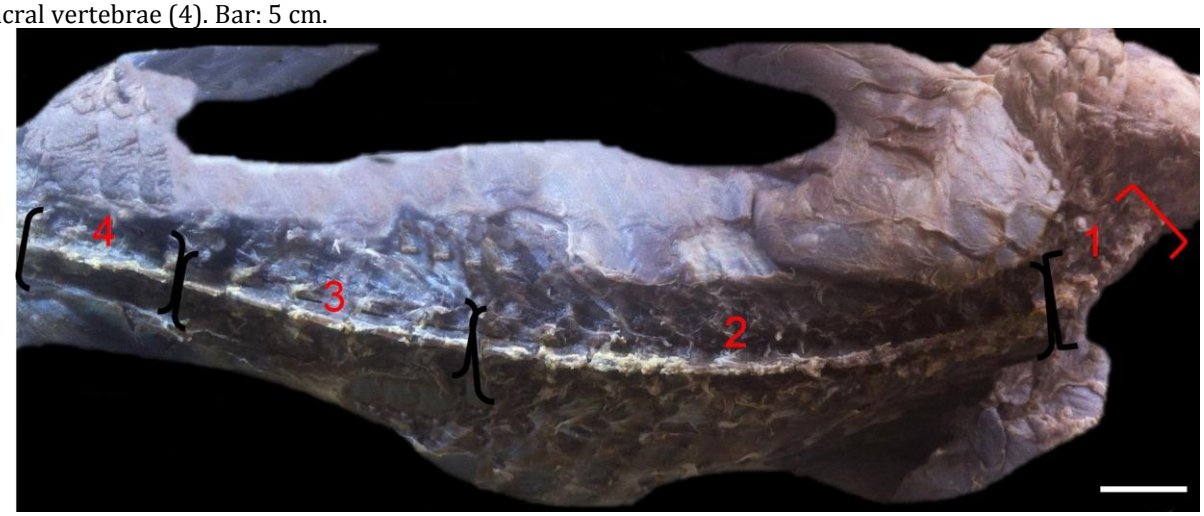
Prior to fixation, the specimens were thawed for partial dissection, starting an incision at the dorsal midline to remove the skin, from the cranial region to the base of the tail, separating it from the tissue underneath. After the skin was removed, the animals were fixed in 10% formaldehyde solution and kept in tanks with the same solution for preservation. Once fixed, the dorsal musculature and the vertebral arches were removed to expose the spinal cord. Then the cervical and lumbar intumescences, the medullary cone and cauda equina were exposed and measured with a pachymeter and photo documented with a Sony α200-10.2 mpx digital camera. The project was approved by the Ethics Committee in Animal Experimentation (CEUA) with protocol nº 083/17.

The data described for cervical intumescence, lumbar intumescence, medullary cone (base and apex) and cauda equina, in their length and location, were compared with the literature available of other wild primates and mammals, according to the NAV (2017).

RESULTS AND DISCUSSION

After the dissection of the dorsal region, from the cranial region to the base of the tail, the spine of *A. belzebul* was evidenced and showed for all the specimens: 7 cervical vertebrae, 13 thoracic vertebrae, 5 lumbar vertebrae and 3 fused sacral vertebrae (Figure 1). A similar result was found by Silva et al. (2013) for *Callithrix jacchus* and Cordeiro et al. (2014) for *Sapajus libidinosus*. However, Lima et al. (2011a) observed a total of 9 lumbar vertebrae for *Saimiri sciureus*, and Martins et al. (2013) observed 7 lumbar vertebrae for *Saguinus midas*.

Figure 1 – *A. belzebul* specimen presenting: 7 cervical vertebrae (1); 13 thoracic vertebrae (2); 5 lumbar vertebrae (3); 3 fused sacral vertebrae (4). Bar: 5 cm.



Measurements of the body size of *A. belzebul* present an average of 48 cm from the apex of the head to the sacrum of the animal, and 81 cm from the apex of the head to the feet. The spinal cord of *A. belzebul* presented on average 22 cm in length, from C1 to T11 vertebrae (Figure 2A). There are few descriptions of primate spinal cord measurement in the literature. Silva et al. (2013) described for *C. jacchus* (common marmoset) 10,04 cm for females and 10,59 cm for males. Although *C. jacchus* and *A. belzebul* have a similar number of vertebrae, *C. jacchus* is a smaller, which is in agreement with the size of the spinal cord in *A. belzebul* being twice its size in *C. jacchus*.

The cervical intumescence (IC) of *A. belzebul* is located between C3 and C6 vertebrae, measuring on average 2.2 cm in length (Figure 2B and Table 1).

Figure 2 – A. Dorsal view of *A. belzebul*, highlighting the region of the Spinal Cord (ME - average of 22 cm in length) and Cauda Equina - 15 cm (CE). Bar 5: cm. B. Cervical region highlighting the Cervical Intumescence (IC). Bar: 1 cm.

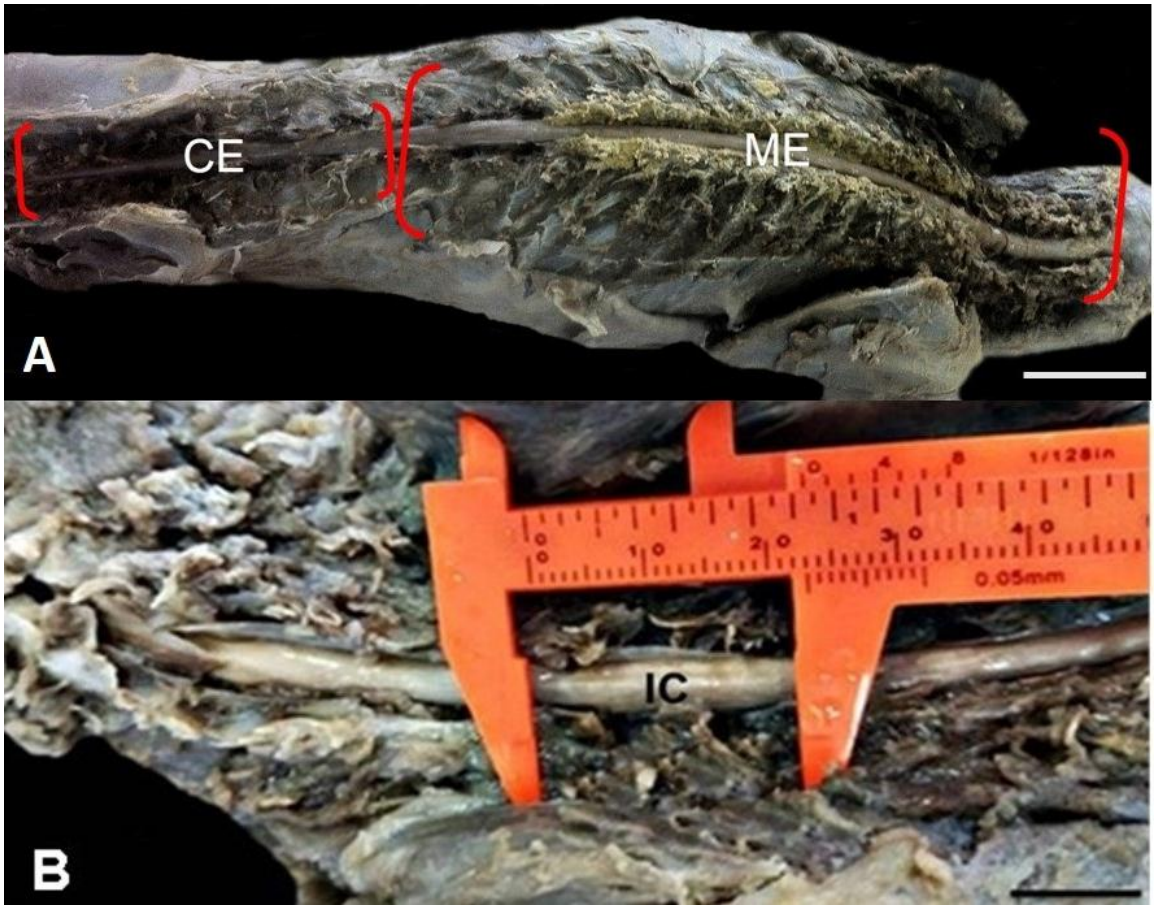


Table 1 describes the results of IC measurement of *A. Belzebul* and other primate and mammal wild species. The comparative results of the IC of *A. Belzebul* are not similar to the topography of the IC of other primates and wild mammals observed in the literature.

Table 1 – Measurement of the Cervical Intumescence of *A. belzebul* and other species of wild primates and mammals.

Species	Base	Apex	Lenght	Reference
Red-handed howler (*Alouata belzebul*)	C3	C6	2.2 cm	
Common marmoset (*Callithrix jacchus*)	C4	T5	1.34 cm females, 1.39 cm males	Silva et al., 2013
Gray brocket (*Mazama gouazoubira)*	C4	T1	Not reported	Lima et al., 2010
South American coati (*Nasua nasua*)	C4	T1	Not reported	Gregores, 2006
Red-footed tortoise (*Geochelone carbonaria)*	C5	T1	Not reported	Carvalho et al., 2011

In *A. belzebul*, the lumbar intumescence (IL) is located between T11 and T12 vertebrae, with an average size of 1.65 cm in length (Figure 3). Compared with tayra, crab-eating raccoon and other primate species, the IL of *A. belzebul* presents its location more cranially. However, among the primates studied, a certain similarity is observed regarding the position and length of lumbar intumescence (Table 2).

Table 2 – Measurement of the Lumbar Intumescence of *A. belzebul* and other species of wild primates and mammals.

Species	Base	Apex	Lenght	Reference
Red-handed howler (*Alouata belzebul*)	T11	T12	1.65 cm	
Common squirrel monkey (*Saimiri sciureus*)	L6	L7/L8	3.3 cm	Lima et al., 2011a
Common marmoset (*Callithrix jacchus*)	T12	L1	1.64 cm females, 1.73 cm males	Silva et al., 2013
Tayra (*Eira barbara*)	L3	L4	Not reported	Branco et al., 2013
Crab-eating raccoon (*Procyon concrivorus*)	L3	L4	1.8 cm	Souza et al., 2014

The medullary cone (CM) of *A. belzebul* is located between T12 and L1 vertebrae, measuring 1.5 cm in length (Figure 3A and 3B). The cauda equina is located between L1 and S3 vertebrae, presenting 15 cm in length (Figure 2A and 3C).

Figure 3 – A, B and C: Macro-photography of the dorsal view of the thoracolumbar region of *A. belzebul*, highlighting the Lumbar Intumescence region (IL); Medullary Cone region (CM); Cauda Equina region (CE). In red, the position of the vertebrae (T11, T12, L1, L5, S1 and S3) is observed. In B, the pachymeter to assist CM measurement. Bar: 1 cm.

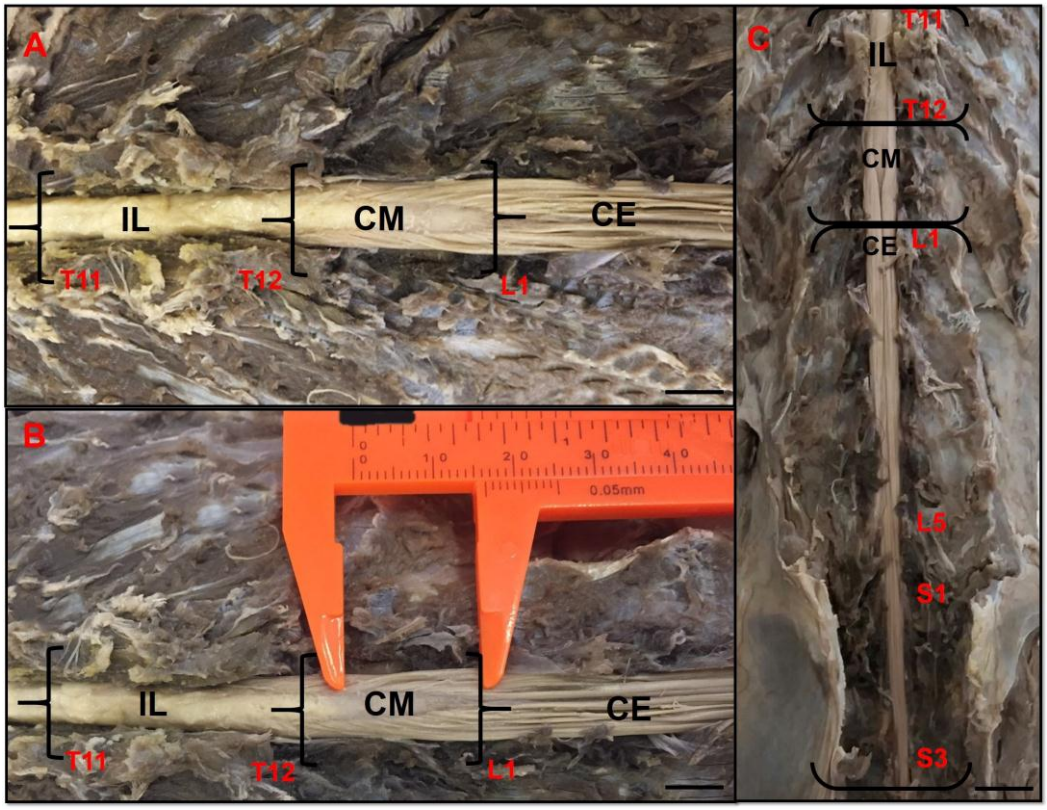


Table 3 describes the results of CM measurement of *A. belzebul* and other primates. The comparative results of CM of *A. Belzebul* do not present similarity to the topography of CM of other primates described in the literature. Table 4 describes the results of the CM measurement of *A. belzebul* in relation to other wild mammal species described in the literature.

Table 3 – Measurement of the medullary cone of *A. belzebul* and other species of Primates.

Species	Base	Apex	Lenght	Reference
Red-handed howler (*Alouata belzebul*)	T12	L1	1.5 cm	
Black-striped capuchin (*Sapajus libidinosus*)	L2	L5	2.47 ± 0.57 cm	Cordeiro et al., 2014
Red-handed tamarin (*Saguinus midas*)	L4	S2	5.14 cm	Martins et al., 2013
Common squirrel monkey (*Saimiri sciureus*)	L7-L8	S3 or Cc1	3.3 cm	Lima et al., 2011a
Common marmoset (*Callithrix jacchus*)	L2	L4 - L5	1.64 cm females, 1.57 cm males	Silva et al., 2013
Common marmoset (*Callithrix jacchus*)	L3	L6	1.4 cm	La Salles et al., 2017

Table 4 – Measurement of the medullary cone of *A. belzebul* and other wild mammals.

Species	Base	Apex	Lenght	Reference
Red-handed howler (*Alouata belzebul*)	T12	L1	1.5 cm	
Brown-throated sloth (*Bradypus variegatus*)	L3	S1	2.7 cm	Lima et al., 2011b
Jaguarundi *(Herpailurus yagouaroundi)*	L6	S2	5 cm	Carvalho et al., 2003
Giant river otter (*Pteronura brasiliensis*)	L2	L4	5.5 cm	Machado; Rosas; Lazzarini, 2009
Tayra (*Eira barbara*)	L3-L4	L6	4.31 cm	Branco et al., 2013
Maned wolf (*Chrysocyon brachyurus*)	L3	L5	6.5 cm	Machado et al., 2002
Crab-eating fox (*Cerdocyon thous*)	L6	S3	10.13 cm	Pinheiro et al., 2011
Crab-eating fox (*Cerdocyon thous*)	L5-L6	L6-L7	16.62 cm	Carreiro et al., 2017
Short-eared dog *(Atelocynus microtis)*	L7	S3	3.9 cm	Saldanha; Branco; Lima, 2011
South American coati (*Nasua nasua*)	L5-L6	S3	5.2 to 5.8 cm	Gregores et al., 2010
Central European boar (*Sus scrofa scrofa*)	L4	S3	34.16 ± 7.23 mm	Santos et al., 2013
South American fur seal (*Arctocephalus australis*)	T5	T7	4.4 cm	Machado; Lesnau; Birck, 2003
Coypu (*Myocastor coypus*)	L3-L5	L4-L6	2 cm	Machado; Cal; Birck, 2009
Ocelot *(Leopardus pardalis)*	L4	S3	8.5 cm	Brigida et al., 2010
Crab-eating raccoon (*Procyon cancrivorus*)	L4	L7	5.35 ± 0.25 cm	Souza et al., 2014

The morphological differences of both the measurement and the topography of the IC, IL, CM and CE among the species described in the literature, show the importance of knowing the location of these structures to apply anesthetics in surgical procedures, which is specific for each species. Silva et al. (2013) states that epidural anesthetics should be applied in the lumbosacral region for *Callithrix jacchus* corroborating the findings of LA Salles et al. (2017) for this species, in *Saguinus midas* it should be applied in the spaces between the vertebral arches of the lumbosacral region (MARTINS et al., 2013), in *Sapajus libidinosus*, in the epidural space of the lumbosacral region (CORDEIRO et al., 2014) and in *Saimiri sciureus* in the girdle and pelvic limbs (LIMA et al., 2011a). For *A. belzebul*, epidural anesthesias should be applied in the lumbosacral region, specifically between L2 and S3 vertebrae.

CONCLUSIONS

To perform epidural anesthesia, it is necessary to take into account the information about the topography and measurement of IC, IL, CM and possible anatomical variations of the spinal cord. It was concluded that the cervical intumescence of *A. belzebul* (IC) is located between C3 and C6 vertebrae, the lumbar intumescence is located between T11 and T12 vertebrae, the cauda equina is located between L1 and S3 vertebrae. The anatomy of the medullary cone of *A. belzebul* shows that its base is located in the T12 vertebra and apex in the L1 vertebra, suggesting that the epidural anesthesia in this species should be performed in the lumbosacral region between the L2 and S3 vertebrae, differing from other species of primates described in the literature.

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