# Brachial plexus and points for anesthetic block in royal sloth and common sloth

[*Plexo braquial e pontos para bloqueio anestésico em preguiça-real e preguiça-comum*]

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## ABSTRACT

This study presents a macroscopic description of the brachial plexus in the royal sloth, *Choloepus didactylus*, and the common sloth, *Bradypus variegatus*, to identify the points for anesthetic block to perform surgical procedures. Six *C. didactylus* and nine *B. variegatus* had their forelimbs dissected, exposing the brachial plexus, and the points for blockage were located subsequently. In general, the formation of the brachial plexus in *C. didactylus* involved C5–T1 nerves, whereas the brachial plexus in a few individuals of the species involved C4–T2. In *B. variegatus*, the brachial plexus was contributed by C6–T2. In both species, the ramifications gave rise to three trunks that joined together to form a common brachial plexus trunk. In the brachial plexus block technique, four accesses were performed: supraclavicular, infraclavicular, axillary, and distal. The results of this study contribute to surgical and anesthetic procedures and research on comparative anatomy among wild animals.

Keywords: *Bradypus variegatus, Choloepus didactylus*, neuroanatomy, Xenarthra

## RESUMO

*Este estudo apresenta uma descrição macroscópica do plexo braquial na preguiça-real,* Choloepus didactylus, *e na preguiça-comum,* Bradypus variegatus, *a fim de identificar os pontos de bloqueio anestésico para realização de procedimentos cirúrgicos. Seis* C. didactylus *e nove* B. variegatus *tiveram seus membros torácicos dissecados, expondo-se o plexo braquial, e posteriormente localizados os pontos de bloqueio. Em geral, a formação do plexo braquial em* C. didactylus *envolveu nervos C5–T1, enquanto o plexo braquial em alguns indivíduos da espécie envolveu C4–T2. Em* B. variegatus, *o plexo braquial foi constituído por C6–T2. Em ambas as espécies, as ramificações deram origem a três troncos que se uniram para formar um tronco comum do plexo braquial. Na técnica de bloqueio do plexo braquial, foram realizados quatro acessos: supraclavicular, infraclavicular, axilar e distal. Os resultados deste estudo contribuem para procedimentos cirúrgicos e anestésicos e pesquisas sobre anatomia comparativa entre animais silvestres.*

*Palavras-chave:* Bradypus variegatus, Choloepus didactylus, *neuroanatomia, Xenarthra*

## INTRODUCTION

The species *Choloepus didactylus*, popularly known as the royal sloth, is found in Venezuela and the Guianas to Ecuador and Peru, passing through northern and northeastern Brazil in the Amazon biome. The species *Bradypus variegatus* known as the common sloth has a wide occurrence in the Neotropical regions of Central and South America (Superina *et al.*, 2010).

Several factors affect animals in the wild, including deforestation, fires, electric shocks, traffic, and car traumas that result in death or fractures (Barreto, 2007). Sloths are among the species most affected by man-made disturbances, and road kills are the main accidents (Glista *et al.,* 2009).

Veterinarians must know the anatomical and physiological characteristics of the species to be cared for (Silva *et al.,* 2018). It is not uncommon to report improperly performed surgical interventions, resulting in an unfavorable prognosis due to the lack of knowledge of the anatomy of wild animals. Anatomical studies can contribute to elucidating several issues involving the biology of an animal, providing a basis for clinical and surgical approaches (Vavruk, 2012).

The brachial plexus is a peripheral structure composed of a network of nerves commonly addressed in clinical, surgical, and anesthetic procedures, such as nerve blocks to perform surgical procedures on the thoracic limb. Brachial plexus block is a local regional anesthesia technique that promotes analgesia, numbness, and trans-and postoperative relaxation of the thoracic limbs by blocking nerve conduction through the infiltration of a local anesthetic (Campoy *et al.,* 2010).

Considering the above, we sought to describe the anatomical aspects of the brachial plexus in *C. didactylus* and *B. variegatus*, describing the origin, composition, and territory of innervation to establish an anatomical model and contribute to the information on the neuroanatomy of wild animals, as well as to propose loco-regional anesthetic blockade points in the thoracic limb.

## MATERIAL AND METHODS

Six animals of the species *C. didactylus*, including four females and two males, and nine *B. variegatus*, including four females and five males, were selected. The animals came from the Botanical Garden Bosque Rodrigues Alves, located in Belém, Pará. The animals died of natural causes and were sent to the Laboratory of Animal Morphological Research of the Federal Rural University of the Amazon, under the authorization of the Biodiversity Authorization and Information System (SISBIO) Nº 23401-7, SEMA - PA Nº 455/2009, and 522/2009.

The animals were thawed under running water. Then, three *C. didactylus* and six *B. variegatus* individuals were fixed in 10% aqueous formaldehyde solution by infusion through the common carotid artery. Fixative infusions were also made intramuscularly and in the body cavities. The other specimens received an

injection of neoprene latex stained in red for better arterial visualization. The dye was administered directly through the right or left common carotid artery or both after prior dissection, followed by fixation with 10% aqueous formaldehyde solution, and preserved for a minimum of seven days.

After the fixation period, bilateral dissection was performed by incision of the skin, subcutaneous tissue, and pectoral muscles until reaching the axillary space, exposing the brachial plexus. Folding the skin of the thoracic region enabled better visualization. Incisions were made close to the sternum, followed by thoracic limb retraction, enlarging the axillary space to facilitate access to the plexus.

The ribs were disarticulated, followed by sternum, trachea, and esophagus removal for better visualization of the cervical vertebrae, cervical nerves, and ventral branches of the cervical and thoracic spinal nerves. Next, the vertebral bodies between the second cervical spinal nerve (C2) and the third thoracic spinal nerve (T3) were removed to completely expose the spinal cord and spinal nerve roots that form the brachial plexus nerves. All the structures were carefully analyzed, identified, and photo documented.

To identify the anesthetic blockade points in the thoracic limbs of *C. didactylus* and *B. variegatus*, we used the techniques described by Martins *et al.* (2016), which used human studies to identify the reference points, positioning, and access to the blockade points of the brachial plexus through supraclavicular, infraclavicular, and axillary accesses in *Sapajus libidinosus*. In addition, the nerve branch block technique has been described and used in cats (Klaumann and Otero, 2013). To demonstrate the process in *B. variegatus*, we used a 20G hypodermic needle (1.1 mm x 32 mm) that was inserted at the identified points in a specimen positioned in dorsal decubitus with one antimere intact and one antimere dissected to expose the nerves, also showing the needle insertion point.

In *C. didactylus*, a 16G catheter (1.7 mm x 45 mm) was used to demonstrate supraclavicular, infraclavicular, and axillary access. The animal was positioned in the lateral decubitus position for the supraclavicular and infraclavicular blocks

2

*Arq. Bras. Med. Vet. Zootec.*, **v.76, n.5, 2024**

and the dorsal decubitus position with arms extended for the axillary and distal blocks. We used a 21G (0.80 mm x 30 mm) and 22G (0.70 mm x 25 mm) hypodermic needle for distal anesthesia.

The protocols used in this study were approved by SISBIO of the Brazilian Institute of Environment and Renewable Natural Resources with protocol number 62674-1 on June 8, 2018, and by the Ethics Committee on the Use of Animals (CEUA) of the Federal Rural University of the Amazon under protocols 037/2018 (CEUA) and 23084.020585 / 2018-34.

## RESULTS AND DISCUSSION

*Choloepus didactylus* showed seven cervical vertebrae, and the number ventral branches of spinal nerves involved in the formation of the brachial plexus varied between five and seven pairs. In general, all animals showed the participation of C5, C6, C7, C8, and T1 in brachial plexus formation, and in two males and one female, C4 also participated, whereas, in two females, the plexus also had the contribution of T2 (Fig. 1a).

Endo *et al.* (2013) conducted a study with two *C. didactylus* and observed the same number of cervical vertebrae in addition to the participation of C5–T1 in the formation of the brachial plexus. In *Choloepus hoffmanni*, six cervical vertebrae have been reported, and the brachial plexus is derived from C4–C7 nerves, with no thoracic branches participating in the composition of the brachial plexus (Giffin and Gillet 1996). As in the abovementioned study, only two specimens of *C. didactylus* were used. Therefore, no variations were observed regarding the number ventral branches of spinal nerves involved in the formation of the brachial plexus in agreement with the observations made in our study.

All *Bradypus variegatus* specimens showed the same characteristics, and no variation was observed within the species. The species presented anatomical particularities in the brachial plexus compared to other Xenarthras and arboreal species, such as primates, due to the unusual number of cervical vertebrae and the additional cervical spinal segments in comparison to other mammals. In this species, we determined the occurrence of nine cervical vertebrae, and the brachial plexus was derived from branches of the cervical spinal nerves C6, C7, C8, C9, and C10, and the thoracic nerves T1 and T2 (Fig. 2a).

In studies on *B. variegatus*, Amorim Júnior *et al.* (2003) also described plexus formation with seven nerve branches. However, with the participation of the 4th cervical nerve branch up to the 1st thoracic nerve branch (C4–T1), the authors considered that the animals had only eight cervical vertebrae, which justifies the difference in the participation of nerve branches among the same species. In *Bradypus torquatus* (Cruz *et al.*, 2013), the occurrence of nine cervical vertebrae and the origin of the brachial plexus between C7–T2 have been described. The exact anatomical features were characterized by Bielik (1937) in *Bradypus tridactylus*, whereas Giffin and Gillet (1996) described the plexus derived from C6–T1, resembling the common sloth in the 1st cranial branch of plexus formation.

Studies involving other Xenarthras pointed out similarities to *C. didactylus* and diverged from *B. variegatus*, where *Tamandua tetradactyla* (Cruvinel *et al.*, 2012), *Priodontes maximus* (Fernandes *et al.*, 2015), *Euphractus sexcinctus* (Lima *et al.*, 2021) showed seven cervical vertebrae and brachial plexuses formed from C5–T1.

The unusual arrangement of cervical vertebrae in sloths can be explained by evolutionary development, and the number variation is correlated directly with changes in pelvic position (Hautier *et al.*, 2010). Genus *Choloepus* can show variation in the number of cervical vertebrae, with 5–6 cervical vertebrae in *C. hoffmanni* or 6–7 vertebrae in *C. didactylus*. In the three-toed sloth (*Bradypus sp.*), this variation occurs between eight or nine cervical vertebrae (Hautier *et al.*, 2010). However, despite the variable number of cervical vertebrae, the sloth follows the same pattern of emergence of the first cervical spinal nerve pair (C1) between the occipital and atlas and the last cervical spinal nerve pair (C10) between the last cervical vertebra (ninth vertebra) and the first thoracic vertebra (Bielik, 1937).

The two males of *C. didactylus* showed the same pattern of brachial plexus origin between C4 and T1, and the females did not show a specific origin pattern. Unlike that in *B. torquatus*, no differences were observed between the sexes

(Cruz *et al.,* 2013). No differences were observed when comparing the antimeres of *C. didactylus*, like that observed in other species (Cruz and Adami, 2010; Cruvinel *et al.,* 2012; Fernandes *et al.,* 2015). No interspecific or antimeric variations were observed in the origin and distribution of the plexus of *B. variegatus*, although differences between antimeres have been reported by Santos-Sousa *et al.* (2016) in *Macaca mulatta* and in humans, where a high incidence of variations occurs, including antimeric asymmetries (Malukar and Rathva, 2011).

The spinal nerves of *C. didactylus* gave rise to three trunks. The cranial trunk was constituted by segments C4, C5, and C6 in two males and one female and by segments C5 and C6 in others. The middle nerve trunk in the six sloths was formed only by segment C7 and, the caudal nerve trunk in two females originated from segments C8, T1, and T2, whereas in the other two females and two males, this trunk was formed by C8 and T1 segments (Fig. 1a and 1b). In *B. variegatus*, the cranial nerve trunk was constituted by segments C6–C8, mid-trunk by segment C9, and caudal trunk by segments C10–T2. The three nerve trunks were united and formed a common brachial plexus trunk in all the specimens (Fig. 2a).

The formation of three well-defined nerve trunks resembles that observed in the anteater (Cruz *et al.,* 2012) and other primates described (Cruz and Adami, 2010; Kikuchi *et al.,* 2010; Santos *et al.,* 2016; Santos-Sousa *et al.,* 2016; Falcão *et al.,* 2017). In *M. tridactyla* (Souza *et al.,* 2014), *B. torquatus* (Cruz *et al.,* 2013), and *B. tridactylus* (Bielik, 1937) two trunks were formed from the origin and branches of the brachial plexus and in *Saimiri sciureus* (Araújo *et al.,* 2012) and *Sapajus libidinosus* (Alcântara, 2018) four trunks were present. The organization of the brachial plexus into nerve trunks has not been identified in several other mammalian species, mainly domestic (Souza *et al.,* 2014), but is the typical arrangement of the primate brachial plexus, as reported in new and old-world primates (Miller, 1935).

Cruz *et al.* (2013) reported that trunk formation results from the contribution of all nerve branches forming the brachial plexus, highlighting the importance of the anatomical knowledge of the brachial plexus aimed at surgical approaches and emphasizing that trauma or injuries to the common trunk can cause serious problems, e.g., limb paralysis. The exact configuration was observed in *C. didactylus*, where most nerves originate from the common brachial plexus trunk.

After the common trunk formed in both species, the nerves were organized into dorsal and ventral fascicles, from which most of the nerves originated (Fig. 1b and 2c). The same conformation was described in *B. torquatus* (Cruz *et al.,* 2013) and *B. tridactylus* (Bielik, 1937), which resembles the description attributed to the plexus of man, defined as the lateral and posterior fascicles. The fascicles of the brachial plexus originate from the anterior and posterior divisions of the trunks (Costabeber *et al.,* 2010) and are referred to as a reorganization of these trunks after their passage through the axilla, inferiorly to the clavicle and scapula, and superiorly to the first rib (Aumüller, 2009).

The major nerves that make up the brachial plexus of *C. didactylus* and *B. variegatus* are the suprascapular (NSP), subscapular (NSB), axillary (NA), radial (NR), cutaneous medial antebrachial (NCMA), ulnar (NU), and thoracodorsal (NTD) nerves (Fig. 1c and 2b). In *B. variegatus*, in addition to those mentioned above, we also located the musculocutaneous median nerve (NMMC) and the long thoracic nerve (NTL) (Fig. 2b and 2d). In *C. didactylus*, in addition to those mentioned above, the following nerves were observed: the caudal cutaneous antebrachial nerve (NCCA) (Fig. 1d), median nerve (NM), and musculocutaneous nerve (NMC) (Fig. 1c and 1d). The origin and innervation territory of the nerves that constitute the brachial plexus of *B. Variegatus* and *C. didactylus* are summarized in Table 1. In addition, in *P. maximus*, Fernandes *et al.* (2015) described the same nerves, except for NCCA and NCMA, in which the lateral thoracic nerve was observed. In *T. tetradactyla*, Cruz *et al.* (2012) described the same nerves as in *P. maximus*, with the addition of the pectoral nerve. *Lagothrix lagothricha* also has a pectoral and a long thoracic nerve, the latter being similar to that identified in *B. variegatus* (Cruz and Adami, 2010).

4

*Arq. Bras. Med. Vet. Zootec.*, v.76, n.5, 2024

Table 1. Brachial plexus of *Choloepus didactylus* and *Bradypus variegatus*, nerves, origin, and innervation territory. fd: dorsal fascicle, fv: ventral fascicle, m.: muscle.

| Nerve | Origin *C. didactylus* | Origin *B. variegatus* | Innervation Territory *C. didactylus* | Innervation Territory *B. variegatus* |
|---|---|---|---|---|
| Suprascapular Nerve | Cranial trunk | Cranial trunk | m. supraspinal | m. infraspinal and supraspinal |
| Subscapular Nerve | Cranial trunk | Common trunk | m. subscapular | m. subscapular |
| Axillary Nerve | Common trunk (*fd*) | Common trunk (*fd*) | m. subscapular; m. round major | Lateral face (crosses subscapular and teres major muscle) |
| Radial Nerve | Common trunk (*fd*) | Common trunk (*fd*) | m. triceps brachii. m. brachialis; m. biceps brachii m. extensor carpi radialis m. common digital extensor; m. extensor carpi ulnaris; m. flexor carpi ulnaris; digits | m. triceps brachii, m. brachioradialis, m. extensor digitorum extensor digitorum lateralis, extensor carpi ulnaris, digits |
| Median Musculocutaneous Nerve | ---------------- | Common trunk (fv) | ---------------- | m. biceps brachii, m. pronator pronator, flexor digitalis superficialis flexor digitorum superficialis, m. abductor digitorum longus I, digits |
| Median Nerve | Common trunk (*fv*) | ---------------- | m. superficial digital flexor; m. ulnar flexor of the forearm; digits | ---------------- |
| Ulnar Nerve | Common trunk (*fv*) | Common trunk (*fv*) | m. extensor carpi ulnaris; m. flexor carpi ulnaris; skin of the distal forearm and dorsal region of the hand; dorsal aspect of the hand; digits | m. flexor carpi radialis, m. ulnar flexor carpi, extensor carpi extensor carpi ulnaris, digits |
| Musculocutaneous Nerve | Common trunk (*fv*) | ---------------- | m. coracobrachial; m. biceps brachii; digits | ---------------- |
| Medial Cutaneous Nerve of the Forearm | Common trunk (*fv*) | Common trunk (*fv*) | skin of the medial aspect of the elbow and forearm | Transverse pectoralis fascia, fascia of the forearm forearm and elbow fascia |
| Thoracodorsal Nerve | Common trunk (*fd*) | Medium trunk | latissimus dorsi muscle | latissimus dorsi muscle |
| Caudal Cutaneous Nerve of the Forearm | Common trunk (*fv*) | ---------------- | triceps brachii medial head | ---------------- |
| Long Thoracic Nerve | ---------------- | Cranial trunk | ---------------- | m. serratus thorax |

The NSP of *B. variegatus* and *C. didactylus* arose from the cranial trunk, similar to that described in other xenarthros (Cruz *et al.,* 2012, Souza *et al.,* 2014) and primates (Cruz and Adami, 2010; Kikuchi *et al.,* 2010; Araújo *et al.,* 2012; Santos-Sousa *et al.,* 2016; Souza Júnior *et*

*al.,* 2018). In *C. didactylus*, NSP innervates only the supraspinal muscle. In *B. variegatus*, NSP supplies the supraspinatus and infraspinatus muscles. In *B. torquatus* (Cruz *et al.,* 2013) and *L. lagothricha* (Cruz and Adami, 2010), NSP also innervated the deltoid muscle, and in *T.*

*tetradactyla* (Cruz *et al.,* 2012), NSP supplied only the supraspinal muscle, as was observed in *C. didactylus*. *Macaca mulatta* participates in the innervation of the subscapular, supraspinal, and infraspinal muscles (Santos-Sousa *et al.,* 2016).

In addition, in *C. didactylus*, NSB originated from the cranial trunk and supplied the subscapularis muscle. However, in *B. variegatus,* NSB originated in the common plexus trunk, before the formation of the fascicles, differentiating itself from NSB origin in all other species in which the origin of the nerve was compared, and divided into three branches to innervate the subscapularis muscle. In *T. tetradactyla* (Cruz *et al.,* 2012) and *M. tridactyla* (Souza *et al.,* 2014), the origin of the nerve from the cranial trunk and innervation of the

subscapular muscle was described, without specifying branches, as observed in *C. didactylus*. Cruvinel *et al.* (2012) differentiated the subscapular nerve into caudal and cranial nerves with distinct nerve origins. In primates, the participation of C6 in the formation of the subscapular nerve has been described in all individuals, as the nerve originates from the cranial trunk in primates (Cruz and Adami, 2010; Santos *et al.,* 2016; Santos-Sousa *et al.,* 2016). The participation of C7 in the formation of this nerve has been described in *M. mulatta* (Santos-Sousa *et al.,* 2016) and in the innervation of the subscapular and teres major muscles, as well as in *Cebus apella* (currently known as *Sapajus apella*) (Ribeiro *et al.,* 2005) and *L. lagothricha* (Cruz and Adami, 2010).
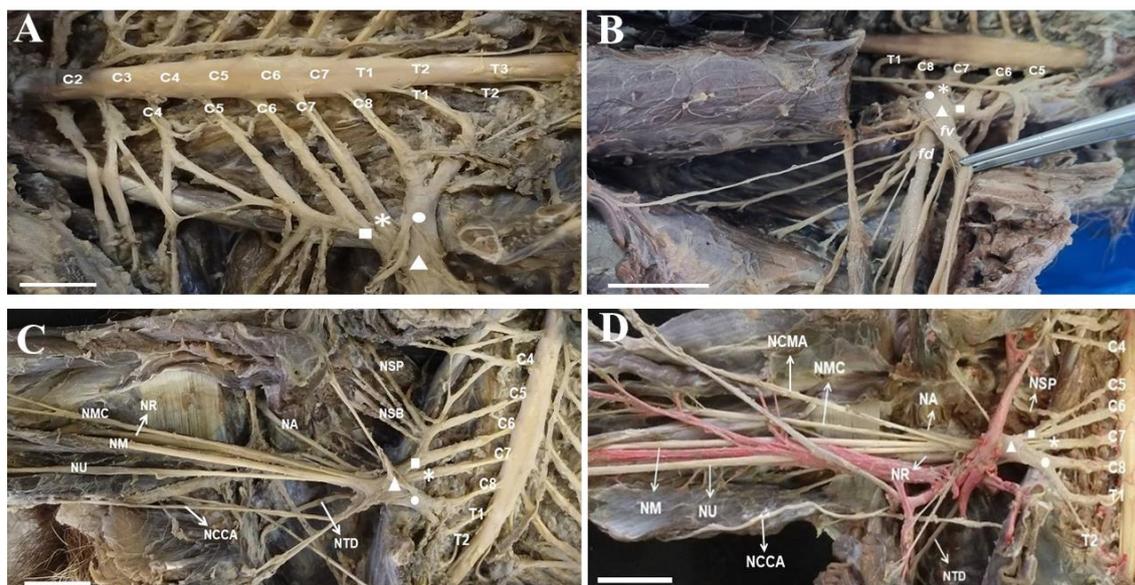


Figure 1. Photomacrographs of the ventral region of the spinal cord and brachial plexus of *Choloepus didactylus*. C4 - 4th cervical spinal branch, C5 - 5th cervical spinal branch, C6 - 6th cervical spinal branch, C7 - 7th cervical spinal branch, C8 - 8th cervical spinal branch. T1 - 1st thoracic spinal branch segment, T2 - 2nd thoracic spinal branch segment, and T3 - 3rd thoracic spinal branch segment. Cranial trunk (■), middle trunk (*), and caudal trunk (●). Suprascapular nerve (NSP), Subscapular nerve (NSB), Axillary nerve (NA), Radial nerve (NR), Median nerve (NM), Musculocutaneous nerve (NMC), Caudal Cutaneous of the Forearm (NCCA), Ulnar nerve (NU) and Thoracodorsal nerve (NTD). **(A)** Origin of the brachial plexus from C4 (between C3 and C4), going to T2 (between T2 and T3). It also shows how they are organized into cranial trunk (■) with the participation of roots C4, C5, and C6; middle trunk (*) formed by C7 and caudal trunk (●) consisting of C8, T1, and T2; and the common trunk (▲) which is the union of the three trunks. Scale bar: 1 cm. **(B)** Photomacrograph of the axillary region of the brachial plexus of *C. didactylus* demonstrating the formation of fascicles from the common trunk (▲) by the ventral (fv) and dorsal (fd) fascicles. Scale bar: 2 cm. **(C)** Photomacrograph of the axillary region of the brachial plexus of *C. didactylus*. Evidence that the NSP and NSB originated from C4 and C5 (cranial trunk). It is also possible to observe the main nerves of the brachial plexus of this species, being the NA, NR, NM, NMC, NCCA, NU, and NTD. Scale bar: 1cm. **(D)** Photomacrography of the axillary region of the brachial plexus of *C. didactylus* illustrating that the NSP originated only from C5 (cranial trunk). It also shows the main nerves of the brachial plexus of this species: NA; NR; NM; NMC; NCMA; NU; NTD and NCCA. Scale bar: 2cm.
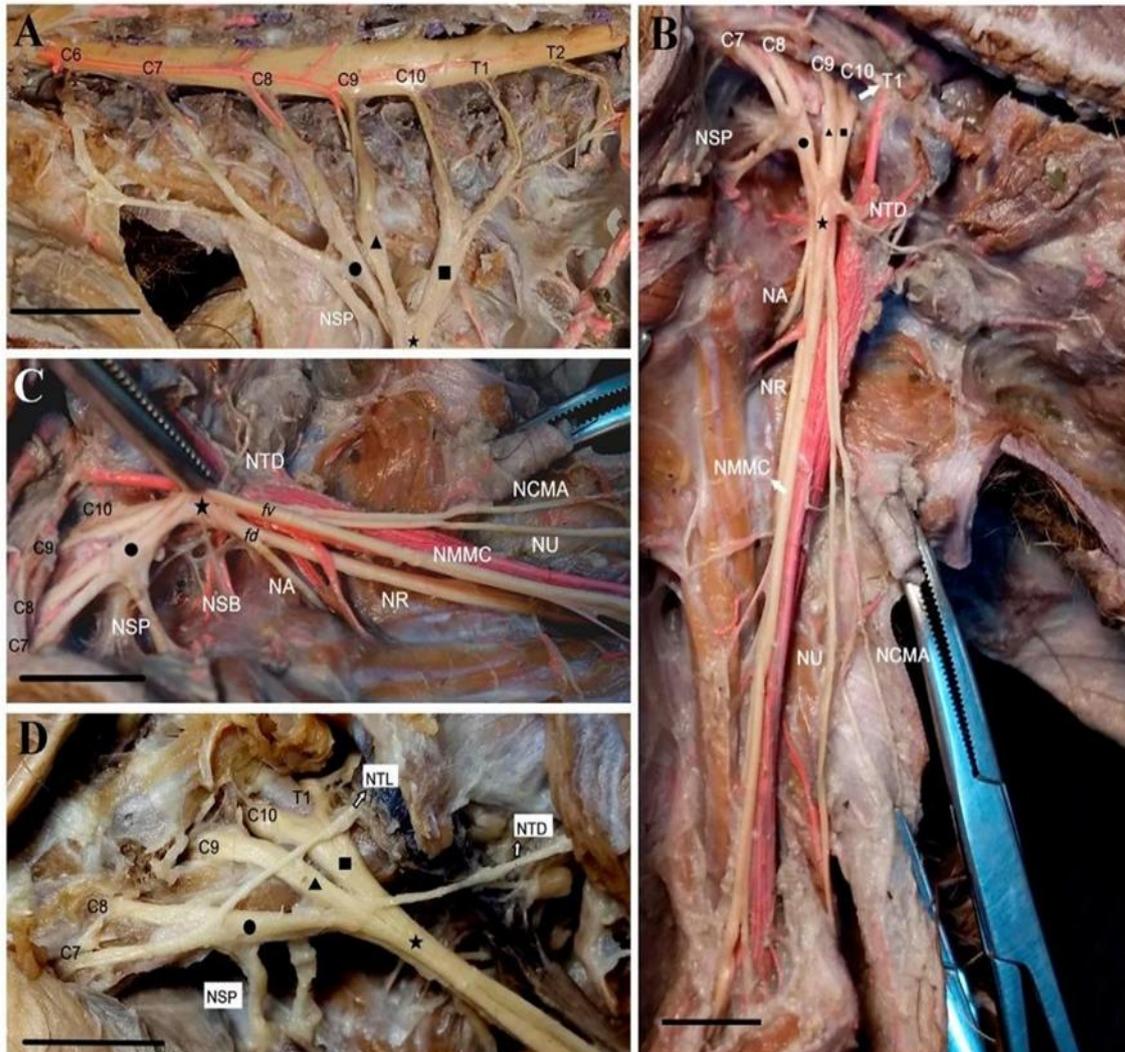
Figure 2. Photomacrographs of the ventral region of the spinal cord and brachial plexus in *Bradypus variegatus*. C6 - 6th segment of the cervical spinal branch, C7 - 7th segment of the cervical spinal branch, C8 - 8th segment of the cervical spinal branch, C9 – 9th segment of the cervical spinal branch. T1 - 1st segment of the thoracic spinal branch, T2 - 2nd segment of the thoracic spinal branch. NSP - suprascapular nerve, NA - axillary nerve, NR - radial nerve, NMMC - median musculocutaneous nerve, NTD - thoracodorsal nerve, NU - ulnar nerve, NCMA - medial forearm cutaneous nerve. Trunks: cranial (●), medial (▲), caudal (■), and common trunk (*). **(A)** Photomacrograph of the ventral region of the spinal cord and its respective segments in B. variegatus showing the origin of the brachial plexus from C6–T2 and its organization into cranial trunks formed by roots C6–C8, medial formed by C9 and caudal formed by C10–T2 that join and form the common trunk. **(B)** Photomacrography of the ventral region of the brachial plexus of *B. variegatus* showing the main nerves, i.e., NSP, NA, NR, NMMC, NTD, NU, and NCMA. **(C)** Photomacrograph of the axillary region of the brachial plexus of *B. variegatus* demonstrating the formation of fascicles from the common trunk (*): ventral fascicle (fv) from which NCMA, NU, and NMMC derive and dorsal fascicle (fd) from which the NR and NA emerge. The NSB is derived from the common trunk (*) and does not participate in the formation of fascicles like the NTD. **(D)** Photomacrograph of the ventral region of the brachial plexus of *B. variegatus* showing the organization of the cranial (●), middle (▲), caudal (■) and common trunk (*) trunks and the origin of the NTL, NSP from the cranial trunk (●) and the NTD from the middle trunk (▲). Scale bars: 1 cm.

The axillary nerves of *B. variegatus* and *C. didactylus* are derived from the common trunk of the dorsal fascicle, similar to that observed in *B.*

*torquatus* (Cruz *et al.,* 2013). In the observations made in the present study, NA passed between the subscapular and teres major muscles

following the lateral aspect. In *T. tetradactyla*, NA originates from the cranial and middle trunk and supplies the deltoid, infraspinal, teres major, and minor muscles (Cruz *et al.,* 2012). In *L. lagothricha*, had an origin similar to that in *T. tetradactyla*, *Callithrix jacchus*, and *Callithrix penicillate.* However, in *T. tetradactyla*, NA innervated the round major and minor muscles and the deltoid muscle (Cruz *et al.,* 2012). In *C. jacchus* and *C. penicillate*, NA innervated the round major and minor muscles (Santos *et al.,* 2016).

The NRs of the two species studied were derived from the common trunk of the dorsal fascicle, similar to that observed in *B. torquatus* (Cruz *et al.,* 2013), *T. tetradactyla* (Cruz *et al.,* 2012), and *C. jacchus* and *C. penicillata* (Santos *et al.,* 2016). The origin from all three trunks indicates the high functional complexity of this nerve (Souza Júnior *et al.,* 2018). Its formation from the trunks has also been described in other primates (Ribeiro *et al.,* 2005; Cruz and Adami, 2010; Kikuchi *et al.,* 2010; Araújo *et al.,* 2012; Santos-Sousa *et al.,* 2016) and anteaters (*T. tetradactyla* and *M. tridactyla*) (Cruz *et al.,* 2012; Souza *et al.,* 2014).

In *C. didactylus* and *B. variegatus*, NR formed branches on the medial face while sending nerve branches to the triceps brachii muscle, following the lateral face of the arm bypassing the humerus at this point. In *B. variegatus*, the ramification of this nerve occurred in the superficial and deep branches distributed to the muscles of the forearm, innervating the brachioradialis, lateral digital extensor, and ulnar extensor carpi muscles, following the digits. In *C. didactylus*, after bypassing the humerus, the nerve branches to the brachial muscle and biceps brachii, from where the nerve distributes further to the forearm muscles and connects to the musculocutaneous nerve innervating the extensor carpi radialis, extensor digitorum communis, extensor carpi ulnaris, flexor carpi ulnaris, and then to the digits.

In *L. lagothricha* (Cruz and Adami, 2010) and *T. tetradactyla* (Cruz *et al.,* 2012), NR supplied the tensor fascia of the forearm, anconeus, and triceps brachii muscles. In *B. torquatus* (Cruz *et al.,* 2013), the nerve presented the same characteristics as in *B. variegatus*. In *C.*

*didactylus*, NR innervated relatively more muscles than in the other mentioned species.

In *C. didactylus* and *B. variegatus*, NU originates from the common trunk ventral fascicle, similar to that observed in *B. tridactylus* and *B. torquatus* (Bielik, 1937; Cruz *et al.,* 2013). It was followed through the arm without presenting ramifications and passed through the caudal face of the elbow arriving at the forearm. At this point, NU showed differences in innervation patterns between the species analyzed. In *B. variegatus*, NU formed innervations to the flexor carpi radialis, flexor carpi ulnaris, and extensor carpi ulnaris muscles following the digits. In *C. didactylus*, NU distributed branches to the extensor carpi ulnaris, flexor carpi ulnaris, and later to the skin of the distal forearm, dorsum of the hand, and digits. In *B. torquatus* (Cruz *et al.,* 2013), NU showed the same innervation territory as in *B. variegatus*, similar to that in *Saimiri sciureus* and *Macaca mulatta* (Araújo *et al.,* 2012; Santos-Sousa *et al.,* 2016). However, in *L. lagothricha*, NU supplied the medial portion of the triceps brachii muscle (Cruz and Adami, 2010), which in *B. variegatus* and *C. didactylus* was innervated by NR.

In *B. variegatus* and *B. torquatus* (Cruz *et al.,* 2013), NM and NMC followed throughout the thoracic limb, forming a single nerve, NMMC. In *B. variegatus*, NMMC distributed branches in the arm, innervating the biceps brachii muscle cranially to the brachial artery, and arriving in the forearm region, branches innervated the pronator teres, i.e., the medial aspect of the forearm, superficial digital flexor, and long abductor I finger muscles, following the digits. Similar to that found in *Saimiri sciureus* and *Alouatta guariba clamitans* (Araújo *et al.,* 2012; Souza Júnior *et al.,* 2018), with motor innervation directed to the pronator teres, flexor carpi, and digit muscles. In *Lagothrix lagotricha*, the NMMC innervates the biceps brachii muscle (Cruz and Adami, 2010).

In *C. didactylus*, NM originated from the ventral fascicle common trunk, similar to that observed in *Callithrix jacchus*, *Callithrix penicillata* (Santos *et al.,* 2016), and *T. tetradactyla* (Cruz *et al.,* 2012). In *B. variegatus* and *Cercopithecus pygerythrus* (Booth, 1991), union occurs between NM and NMC, which is not observed in *C. didactylus*, where NM followed through the

arm without forming branches and after reaching the forearm, innervates the superficial digital flexor muscle, and ulnar flexor of the forearm following the digits. In *Macaca mulatta* (Santos-Sousa *et al.,* 2016), NM innervates the pronator teres, carpal flexors, brachial muscles, biceps brachii, and triceps brachii muscles in *C. jacchus* and *C. penicillate* (Santos *et al.,* 2016).

The musculocutaneous nerve in *C. didactylus* also originates from the common trunk ventral fascicle, which is different from that observed in *L. lagothricha* (Cruz e Adami, 2010) and *M. mulatta* (Santos-Sousa *et al.,* 2016). In *C. didactylus*, NMC followed the coracobrachialis muscle, biceps brachii, and digits and was in contact with NR. Similar to observations in *T. tetradactyla* (Cruvinel *et al.,* 2012) and *M. mulatta* (Santos-Sousa *et al.,* 2016).

In *T. tetradactyla* (Cruz *et al.,* 2012), NCMA originates before trunk formation, with only T1 contributing, branching into the forearm fascia. In *S. sciureus* (Araújo *et al.,* 2012) it originates at C8 and T1, innervating the skin of the medial side of the forearm. In *C. didactylus*, the origin was in the dorsal fascicle ventral trunk, which formed branches innervating the skin of the medial aspect of the elbow and forearm. In *B. variegatus* the origin was the same as that of *C. didactylus*, however it supplied more regions, innervating the skin tissue even before it branched in the forearm, supplying part of the skin covering the transverse pectoralis muscle and skin of the mediolateral portion of the forearm and elbow skin on the medial side of *B. variegatus*. In *Macaca mulatta*, it is derived from the caudal trunk and innervates the medial skin of the forearm (Santos-Sousa *et al.,* 2016).

The NTD in *B. variegatus* is derived from the middle trunk and originates from the C7 root. In *C. didactylus,* it originates from the dorsal fascicle common trunk, similar to that observed in *B. torquatus* (Cruz *et al.,* 2013). In other species, the origin of this nerve was quite varied; Cruvinel *et al.* (2012) described the origin from the lateral trunk, while Cruz *et al.* (2012) observed that the NTD originated from all three trunks of the brachial plexus in *T. tetradactyla*. In the species studied here, the NTD innervated the teres major muscle, and despite the difference in the formation of this nerve between species,

its innervation of the latissimus dorsi muscle was invariable.

The caudal forearm cutaneous nerve originates from the ventral fascicle common trunk and innervates the medial head of the triceps brachii. It was not identified in *B. variegatus*, only in *C. didactylus*, it was also not located in *B. torquatus* (Cruz *et al.,* 2013), nor in *T. tetradactyla* (Cruz *et al.,* 2012), *L. lagothricha* (Cruz and Adami, 2010), *P. maximus* (Fernandes *et al.,* 2015), *E. sexcinctus* (Lima *et al.,* 2021). It is cited in *M. tridactyla* Silva (unpublished data), and its origin is from the ulnar nerve, and the territory of innervation is the medial surface of the arm and the caudal surface of the forearm.

The long thoracic nerve (NTL) also originates from the cranial trunk, similar to that in *M. tridactyla* (Souza *et al.,* 2014). In *B. torquatus* (Cruz *et al.,* 2013), it originated only from the root of C9, in *L. lagothricha*, it originated from C7 (Cruz and Adami, 2010), and in *Cebus apella* (Ribeiro *et al.,* 2005) and was formed by the cranial and middle trunks. NTL was not described in *T. tetradactyla* by Cruz *et al.* (2012), as it was also not observed in *C. didactylus*, but was observed by Cruvinel *et al.* (2012) to be formed by the roots of C6 and C7. In *B. variegatus* and other species described (Ribeiro *et al.,* 2005; Cruz and Adami, 2010; Cruz *et al.,* 2013; Santos-Sousa *et al.,* 2016; Souza Júnior *et al.,* 2018) it is responsible for innervating the serratus thorax muscle.

The brachial plexus block technique in *B. variegatus* resembled the results described by Martins *et al.* (2016) for *Sapajus libidinosus*. In *B. variegatus* it was possible to use the same anatomical reference points for supraclavicular, infraclavicular, and axillary accesses because of the anatomical similarities between the species, such as the positioning of the thoracic limbs, as they are arboreal animals, and the presence of the clavicle, which was the main reference point for locating the blocking points. However, despite *C. didactylus* presenting the similarities mentioned above, we did not use the same anatomical reference point as *Sapajus libidinosus*. This was because it was not possible to locate the clavicle of the animals by palpation. Therefore, we used the humerus joint as the reference for this species.

In the brachial plexus block technique using supraclavicular access in *B. variegatus*, we found a prominent edge of the clavicle as an anatomical reference point, and the needle was introduced at the midpoint of the clavicle in the craniocaudal direction and positioned at a 95° angle (Fig. 3a). The needle had no direct contact with the arterial plexus and approached the common trunk of the brachial plexus (Fig. 3b). In *C. didactylus*, the humerus joint was located as an anatomical reference point, and the catheter was inserted approximately 2 cm away from the humerus joint, at a 95° angle, in the craniocaudal direction, with a depth of approximately 2.5 cm, because we observed that approximately 3 cm would reach the cranial trunk nerves (Fig. 4a and 4b).

In *S. libidinosus*, the supraclavicular route was more adequate for the block because it presented a lower risk of nerve penetration by the needle. In *Alouatta guariba*, the brachial plexus block technique used resembled the supraclavicular approach reported in *S. libidinosus* and adapted from the technique described in humans, using the internal border of the clavicular head as a reference point (Santos *et al.,* 2017). In *C. jacchus* the animal was positioned in the lateral decubitus position to perform the supraclavicular block, similar to *C. didactylus*, but he used the clavicle as an anatomical reference (Almeida *et al.,* 2020).

In the infraclavicular access of *B. variegatus*, we identified the midpoint of the clavicle as the anatomical landmark, and the needle was inserted perpendicular to the skin at 80° angulation in the caudocranial direction (Fig. 3c). The needle approached the caudal edge of the clavicle and was perpendicular to the common trunk of the brachial plexus (Fig. 3d). This technique in *B. variegatus* allowed the needle to be positioned closer to the brachial plexus at the common trunk, suggesting that the application of the local anesthetic by this approach allows diffusion through the nerves with a low risk of injury to the arteriovenous plexus. This approach provides effective anesthesia without adverse effects (Imbelloni *et al.,* 2001).

For infraclavicular access in *C. didactylus*, the humerus joint was used as an anatomical reference point. The catheter was inserted approximately 1.5 cm away from the humeral joint at a 45° angle in the craniocaudal direction

(Fig. 4c). The catheter had no direct contact with the nerve approaching the cranial trunk of the brachial plexus (Fig. 4d). In *B. variegatus* the supraclavicular and infraclavicular access techniques were associated with a lower risk of plexus perforation than axillary access. In contrast, we observed that in *C. didactylus*, the infraclavicular access was closest to one of the nerves of the cervical region, proving to be the riskiest.

For the axillary access of *C. didactylus* and *B. variegatus*, the median line of the axillary fossa was identified as the anatomical landmark, and the catheter and needle (for each species) were inserted perpendicular to the fossa, forming a 90° angle at the height of the initial third of the thorax in the caudocranial direction (Fig. 4e and 3e). In this region, we observed the formation of an arterial plexus that irrigates the thoracic limb close to the nerves. In *C. didactylus* the catheter was at a safe distance from the brachial plexus, and the needle had no direct contact with the nerves approaching the common trunk of the brachial plexus (Fig. 4f). In *B. variegatus* the needle did not approach the common trunk or cross the arterial plexus (Fig. 3f), which did not provide adequate access for such species.

In *B. variegatus* the axillary approach presented a higher risk of injury to the blood vessels owing to the presence of the arteriovenous plexus near the needle access, which increases the risk of arterial perforation. Despite simplified access due to ease of positioning and definition of the application area, the axillary approach was also associated with a higher chance of perforation of important vessels in *S. libidinosus* (Martins *et al.,* 2016). On the other hand, in *C. didactylus*, it was possible to observe that by this access the needle had no contact with the nerves and was at a relatively safe distance from the brachial plexus.

In *B. variegatus* in the distal block approach, the needle was introduced into the subcutaneous space, in the final portion of the distal third of the arm, near the carpal joint, at two points: ventrolateral and ventromedial, on the palmar surface of the hand (Figure 3g). The needle approached the ulnar nerve on the lateral aspect and the musculocutaneous median nerve on the medial aspect (Fig. 3h). *C. didactylus* needles were introduced at a 45° angle into the subcutaneous space at the cranial and caudal edges of the palmar pads, and the needle

approached the median and musculocutaneous nerves at the caudal edge of the ulnar nerve (Fig. 4g and 4h).

In the technique of blocking the distal branches of the ulnar and median musculocutaneous nerves, the main complication is intraneural infiltration (Klaumann and Otero, 2013) because of the proximity of the needle to the nerve, as observed when performing the technique. To avoid this complication, the needle should be of low caliber and inserted sufficiently to pass through the subcutaneous tissue, depositing the anesthetic close to the nerves. In felines, Klaumann and Otero (2013) also reported radial nerve block through a dorsal approach, but in the sloths used in our study, it was not possible to perform it, since the nerve crosses the arm on its cranial side, and we did not find anatomical

reference points that could guide the block safely.

Despite demonstrating that the approaches described for blocking the brachial plexus and distal nerve branches can be used in *B. variegatus* and *C. didactylus*, it was not possible to determine the efficacy of the technique for the treatment of pain in the trans-operative and postoperative periods of thoracic limb surgeries, and the safety of the technique after drug application. To ensure safety in the execution of the block, in addition to the knowledge of anatomy and nerve composition of the plexus, auxiliary techniques, such as peripheral nerve stimulator and ultrasound examination, are necessary to ensure the correct location of the needle and an efficient approach to access (Martins *et al.,* 2016).



Figure 3. **(A)** Brachial plexus block approach from supraclavicular access in *Bradypus variegatus*. **(B)** Positioning of the needle in the supraclavicular access in the brachial plexus dissected from *B. variegatus*. White arrow: brachial plexus. Scale bar: 2 cm. **(C)** Brachial plexus block approach from infraclavicular access in *B. variegatus*. **(D)** Positioning of the needle in the infraclavicular access in the dissected brachial plexus of *B. variegatus*. White arrow: brachial plexus. Scale bar: 1 cm. **(E)** Brachial plexus block approach from axillary access in *B. variegatus*. Scale bar: 4 cm. **(F)** Positioning of the needle in the axillary access in the dissected brachial plexus of B. variegatus. White arrow: brachial plexus. Scale bar: 2 cm. **(G)** Approach to distal thoracic limb block in *B. variegatus*. Scale bar: 2 cm. **(H)** Positioning of the needle in the distal block showing the proximity of the ulnar and median musculocutaneous nerves dissected from *B. variegatus*. Black arrow: median musculocutaneous nerve, red arrow: ulnar nerve. Scale bar: 3 cm.

Figure 4. (A) Positioning of the catheter to perform brachial plexus block by supraclavicular access in *C. didactylus*. Scale bar: 3 cm. (B) Brachial plexus site where the needle is positioned in the supraclavicular access in the dissected limb of *C. didactylus* (white arrow). Location of the clavicle (black arrow). Scale bar: 2 cm. (C) Positioning of the catheter to perform brachial plexus block by infraclavicular access in *C. didactylus*. Scale bar: 4 cm. (D) Brachial plexus site where the needle is positioned in the infraclavicular access in the dissected limb of *C. didactylus* (white arrow). Location of the clavicle (yellow arrow). Scale bar: 4 cm. (E) Positioning the needle to perform brachial plexus block by axillary access in *C. didactylus*. Scale bar: 3 cm. (F) Brachial plexus site where the needle is positioned in the axillary access in the dissected limb of *C. didactylus* (white arrow). Scale bar: 3 cm. (G) Positioning of the needle to perform brachial plexus block by distal access in *C. didactylus*. Needle inserted in the caudal margin of the palmar cushion approaching the ulnar nerve (NU) and in the cranial margin of the palmar cushion approaching the median nerve (NM). Scale bar: 2 cm. (H) Needle inserted in the cranial margin of the palmar cushions approaching the median nerve (NM) and musculocutaneous nerve (NMC). Scale bar: 3 cm.

## CONCLUSION

*C. didactylus* showed divergences and similarities to other sloths. It is similar to anteaters and primates in terms of the number of cervical vertebrae. The brachial plexus in *B. variegatus* has particularities of origin, with the participation of C6–T2, showing that a larger number of branches contribute to the formation of the plexus in this species when compared to other animals.

The organization in the trunks and fascicles that we observed in the two species analyzed showed that most of the nerve branches participated in the formation of the nerves that derive from the plexus and supply the thoracic limb. The anatomical characteristics of the brachial plexus and its derived nerves indicate that anesthesia of the thoracic limb can be performed using the supraclavicular, infraclavicular, and axillary approaches and through distal blockade from the definition of specific anatomical points.

It was not possible to determine the success of the block technique for pain treatment. As it would be necessary to use live animals, we suggest that further research should focus on animal studies *in vivo*, aiming to prove the efficacy of the techniques described. In addition to the association with advanced techniques, such as the use of electrostimulators and ultrasound, these techniques provide greater safety for the performance of the blockades described. These results may contribute to surgical and anesthetic procedures, in addition to research on comparative anatomy among wild animals.

12

*Arq. Bras. Med. Vet. Zootec.*, v.76, n.5, 2024

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*Arq. Bras. Med. Vet. Zootec.,* **v.76, n.5, 2024**

13

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14

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