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Original Article

The lumbosacral plexus in two *Didelphis* species (Didelphidae, Didelphimorphia): origin and nerve distribution

Variação morfológica do plexo lombossacral em duas espécies de *Didelphis* (Didelphidae, Didelphimorphia)

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Abstract

Morphological studies provide knowledge that allow us to understand how animals interact with the natural environment or the captivity. The goal of this study was to describe the origin and antimeric distribution of lumbosacral plexus nerves in *Didelphis aurita* and *D. albiventris*. Fourteen adult cadavers of *D. aurita*, seven males and seven females, and 13 adult cadavers of *D. albiventris*, nine males and four females were used. The specimens were sexed, identified, fixed and dissected until the origins of the lumbosacral plexus nerves were exposed. Data were represented as absolute frequency and simple percentage. The lumbosacral plexuses derived a trunk for the femoral and obturator nerves from the ventral branches of L3-L4 (75%) in *D. aurita*, and in *D. albiventris* the femoral nerve of L3-L4 (73.1%) and the obturator nerve of L3-L4 (61.5%). In both species, formation of a lumbosacral trunk derived from L5-L6-S1 occurred in 78.6% of *D. aurita* and 61.5% of *D. albiventris*. The origin and distribution of lumbosacral plexus nerves of the studied species present similarities with domestic and wild eutherian mammals.

Keywords: hindlimb, innervation, marsupial, opossum.

Resumo

Estudos morfológicos fornecem conhecimentos que permitem entender o modo como os animais interagem com o ambiente natural ou em cativeiro. O objetivo desse estudo foi descrever a origem e distribuição antimérica dos nervos do plexo lombossacral de *Didelphis aurita* e *D. albiventris*. Foram utilizados 14 cadáveres adultos de *D. aurita*, sete machos e sete fêmeas, e 13 cadáveres adultos de *D. albiventris*, nove machos e quatro fêmeas. Os espécimes foram sexados, identificados, fixados e dissecados até a exposição das origens dos nervos do plexo lombossacral. Os dados foram representados em frequência absoluta e percentual simples. Os plexos lombossacrais derivaram um tronco para os nervos femoral e obturatório dos ramos ventrais de L3-L4 (75%) em *D. aurita*, e em *D. albiventris* o nervo femoral de L3-L4 (73,1%) e nervo obturatório de L3-L4 (61,5%). Nas duas espécies, ocorreu formação de um tronco lombossacral derivado de L5-L6-S1 em 78,6% no *D. aurita* e em 61,5% no *D. albiventris*. A origem e distribuição dos nervos do plexo lombossacral das espécies estudadas apresentam similaridades com os mamíferos eutérios domésticos e silvestres.

Palavras-chave: membro pélvico, inervação, marsupial, gambá.

1. Introduction

Marsupial mammals are distributed in the Americas and Australasia. In the New World, most species that compose this group belong to the order Didelphimorphia Gill, 1872, which consists of only one family, Didelphidae Gray, 1821 (Rossi et al., 2006, 2012). This one features the genus *Didelphis* Linnaeus, 1758, which comprises the largest living American marsupials. They are medium-sized species, nocturnal, omnivorous and have a long, prehensile tail (Rossi et al., 2006).

Didelphis albiventris Lund, 1840, presents, in Brazil, a wide geographic distribution, occurring in the great biomes of the country, including a Caatinga, Cerrado (savannah-like), Pantanal, Atlantic Forest and Pampa (grassland-like vegetation) (Cerqueira, 1985; Lemos and Cerqueira, 2002; Rossi et al., 2006; Paglia et al., 2012). It has a body length of 30.5-89 cm and a tail length of 29-43 cm, black ears at the base and pinkish-white on the distal half, and a prehensile tail with up to two proximal thirds.

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Didelphis aurita Wied-Neuwied, 1826, occurs in eastern Brazil associated with the Atlantic Forest, southwestern Paraguay and northeastern Argentina. It has a body length of 67-80 cm and a tail length of 31-41 cm, black ears in the basal portion and white in the distal end and a glabrous tail (Rossi et al., 2006; Paglia et al., 2012).

Species of the *Didelphis* genus seem, according to Vieira and Camargo (2012), to present a greater variation in the use of the three vertical stratification of a forest, within the group of marsupials. These animals are likely to adapt to particular habitat conditions. They observed that the occupation of different forest strata, as well as the use of the aquatic environment, depends on the development of specializations of morphological structures in the postcranial skeleton directly linked to locomotion (Vieira and Camargo, 2012).

The postcranial axial skeleton contains the spinal cord that provides sensory and motor innervation mainly to the neck, trunk, tail and, above all, the appendicular skeleton. Aspects of this anatomy are well established in domestic animals. Therefore, the lumbosacral plexus gives rise to the main nerves of the pelvis and pelvic limbs, which are the femoral, obturator, cranial gluteal, caudal gluteal, sciatic and pudendal nerves, with variations in their origin and distribution among domestic animals (Dyce et al., 2019; König and Liebich, 2021). Despite that, the knowledge of neuroanatomy in wild animals is scarce, especially regarding the formation of nervous plexuses in didelphids.

Studies on the ecology of roads in different Brazilian states (Oliveira and Silva, 2012; Orlandin et al., 2015) have observed that among the mammals most impacted by being run over on highways are specimens of the *Didelphis* genus. These data reinforce the need to increase anatomical information, in order to enable better treatment conditions in cases of trauma and fractures, contributing to animal welfare and conservation of this species, as observed in the study by De Oliveira et al. (2023).

The goal of this research is to describe the origin and distribution of lumbosacral plexus nerves in *Didelphis aurita* and *D. albiventris*. Such knowledge aims to expand the knowledge of comparative neuroanatomy and support eventual procedures in wild animal medicine.

2. Materials and Methods

For this study, 14 adult cadavers of *D. aurita*, seven males and seven females, from the Serra dos Órgãos National Park (PARNASO), located in the Serra do Mar in the Mountain Region of the State of Rio de Janeiro, including the Municipalities of Teresópolis, Petrópolis, Magé and Guapimirim, located in the Atlantic Forest biome, and which were donated to the Departamento de Anatomia Animal e Humana of Universidade Federal Rural do Rio de Janeiro (UFRRJ). For *D. albiventris*, 13 adult cadavers were used, seven males and two females, from the collection of the Animal Anatomy Laboratory of the Federal University of Pampa (UNIPAMPA), from the Pampa biome, and two males and two females from the Mammals Collection of National Museum /UFRJ, from the Atlantic Forest biome. All specimens have different histories of death from natural causes. The Ethics Committee on the Use of Animals of the Federal Rural University of Rio de Janeiro approved the research under protocol number 018/2017.

In the Laboratório de Ensino e Pesquisa em Morfologia de Animais Domésticos e Silvestres of UFRRJ, animals from PARNASO were thawed in running water, sexed and identified by placing a numbered plastic label. With the aid of a precision metal measuring tape, the rostrum-sacral length of each animal was measured, taking as a reference the end of the snout to the insertion of the tail.

The cadavers were placed in the right lateral position to access the thoracic aorta, after resection of the intercostal muscles and ribs. The artery was cannulated and the fixation was performed by injecting a 10% formalin solution. Intramuscular and body cavity infusions were also performed with the solution for better fixation. After the procedures described, the specimens were placed in low density polyethylene boxes with a capacity of 500 liters containing a 30% formaldehyde solution.

The specimens coming from UNIPAMPA underwent the same procedure in the laboratory of origin; and those from the Museu Nacional/UFRJ were preserved in a 70°GL alcohol solution.

Using basic dissection materials, the abdominal cavity was opened through a longitudinal incision in the *linea alba*, disarticulation of the pubic symphysis and skin folding. Exposing the abdominal and pelvic cavities, the viscera were removed, as well as the adipose tissue and part of the sublumbar musculature, partially exposing the nerves originated from the plexus under study.

Nerve structures were dissected, allowing visualization of the ventral branches of the spinal nerves, their communications, the originating nerves and their distribution. The findings were documented through schematics made in CorelDraw 2020 software and digital photographs (Nikon D7200 24.2 MP).

In this study, the formation of the lumbosacral plexus consisting of the femoral, obturator, sciatic, cranial gluteal and caudal gluteal nerves will be considered, as these are of greater relevance for locomotion (Lacerda et al., 2006; Aydin, 2009; Aydin, 2010; Cardoso et al., 2013; Cruz et al., 2014; Oliveira et al., 2014; Tonini et al., 2014; Araújo-Júnior et al., 2016; Oliveira et al., 2016; Senos et al., 2016; Silva, 2017; Dyce et al., 2019; König and Liebich, 2021; De Oliveira et al., 2023). In addition, the pudendal nerve was included because it is associated with the lumbosacral trunk and innervates the perineal muscles.

The nomenclature adopted was based on the Nomina Anatomica Veterinaria (ICVGAN, 2017).

The results of the origin and antimeric distribution of nerves were expressed as absolute frequency and simple percentage for *Didelphis aurita* and *D. albiventris*.

3. Results

In *D. aurita* and *D. albiventris*, six lumbar vertebrae and three sacral vertebrae were observed, the absence of the *tensor fasciae latae* muscle and the *sartorius* muscle consisted of a single belly on each limb. In both species, the lumbosacral plexus was formed by the derivations of the ventral branches of the spinal nerves to form the femoral, obturator, sciatic, cranial gluteal, caudal gluteal and pudendal nerves. The most cranial nerve in the plexus was the femoral, and the most caudal one was the pudendal.

3.1. *Didelphis aurita*

The lumbosacral plexus was composed by the ventral branches of the spinal nerves from the third lumbar vertebra (L3) to the first sacral vertebra (S1) in 25 antimeres (89.3%) and from the fourth lumbar vertebra (L4) to S1 in three antimeres (10.7%), being this variation found only in males (Table 1).

3.2. I. Origin

The absolute and the frequencies percentage of the ventral branches of the spinal nerves that formed the lumbosacral plexus nerves of *D. aurita* are shown in Table 2.

The femoral and obturator nerves had a common origin from a trunk derived from the ventral branches of the L3-L4 spinal nerves in 21 antimeres (75%), being 12 antimeres of females (85.7%) and nine antimeres of males (64.3%); in four of L3-L4-L5 (14.3%), being two antimeres of females (14.3%) and two of males (14.3%); and one antimer of male of L4 (3.6%). In two antimeres (7.1%), both from the same male animal, the ventral branches of the L4 and L5 emitted branches to the femoral and obturator nerves, not forming a trunk. Therefore, the origin of the femoral and obturator nerves was from L3 to L5, with a predominancy of the ventral branches of L3 and L4. The ventral branch of L4 participated in the composition of the origin of the femoral and obturator nerves in 28 antimeres (100%), L3 in 25 (89.3%) and L5 in six antimeres (21.4%) (Figure 1).

The lumbosacral trunk presented gave to sciatic, cranial gluteus, caudal gluteal and pudendal nerves. It was composed by the ventral branches of the spinal nerves from L5 to S1 in 22 antimeres (78.6%), 12 in females (85.7%) and ten in males (71.4%); and from L4 to S1 in six antimeres (21.4%), two in females (14.3%) and four in males (28.6%). Thus, the origin of the lumbosacral trunk was from L4 to S1, with a predominancy of the ventral branches of L5, L6 and S1. The ventral branches of L5, L6 and S1 participated in the composition of the lumbosacral trunk in 28 antimeres (100%) and L4 in six antimeres (21.4%).

The variations observed in the origins of the femoral, obturator and lumbosacral trunk nerves in *Didelphis aurita* are shown in Figure 2.

3.3. II. Distribution

The femoral nerve, after its origin, ran between the *psoas minor* and *psoas major* muscles up to the height of the iliacus muscle, where it followed distally on the medial surface of the thigh. At the level of the middle third of the femur, it distinguished as a saphenous nerve to supply the region of the medial face of the leg. In its short course, the femoral nerve emitted branches to the *psoas major, iliacus, quadratus lumborum, sartorius, quadriceps femoris* and *gracilis* muscles. In four antimeres (14.3%), the femoral nerve also emitted a branch to the *pectineus* muscle (Figure 3).

The obturator nerve, detached from the common trunk with the femoral nerve, followed caudally to the lumbar region, under the *psoas minor* muscle, and on the lateral margin of the pelvic cavity, crossed the obturator foramen. On the medial aspect of the thigh, it discharged branches to the adductor muscles of the thigh (*pectineus, adductor* and *gracilis*) and to the obturator externus muscle.

The lumbosacral trunk was composed by the sciatic, cranial gluteus, caudal gluteal and pudendal nerves, which crossed the greater sciatic foramen and was distributed on the lateral aspect of the pelvic limb

Table 1. Absolute (AF) and percentage frequency (PF) of the ventral branches of spinal nerves that participate in the composition of the lumbosacral plexus of *Didelphis aurita*.

VENTRAL BRANCHES	FEMALES		MALES		TOTAL	
	AF	PF	AF	PF	AF	PF
L3 - S1	14	100	11	78.6	25	89.3
L4 - S1	-	-	3	21.4	3	10.7

Table 2. Absolute (AF) and percentage frequencies (PF) of the origins of the lumbosacral plexus nerves of *Didelphis aurita*.

NERVE	ORIGIN	FEMALES		MALES		TOTAL	
		AF	PF	AF	PF	AF	PF
Femoral and obturator	L3-L4	12	85.7	9	64.3	21	75
	L3-L4-L5	2	14.3	2	14.3	4	14.3
	L4	-	-	1	7.1	1	3.6
	L4-L5	-	-	2	14.3	2	7.1
Lumbosacral trunk	L5-L6-S1	12	85.7	10	71.4	22	78.6
	L4-L5-L6-S1	2	14.3	4	28.6	6	21.4

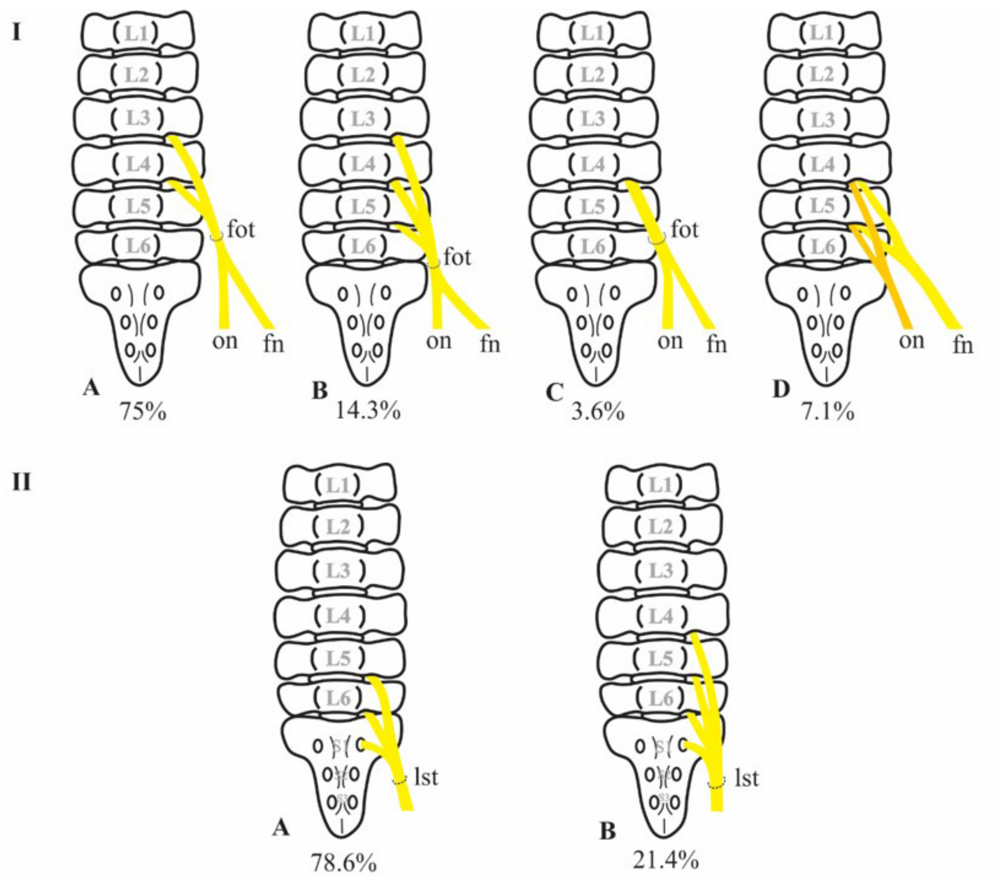

Figure 1. Schematic representation of the origins (I) of the trunks (fot) formed by the femoral (fn) and obturator nerves (on) and (II) of the lumbosacral trunk (lst) in *Didelphis aurita*. I: (A) L3-L4; (B) L3-L4-L5; (C) L4; (D) L4 and L5. II: (A) L5-L6-S1; (B) L4-L5-L6-S1.

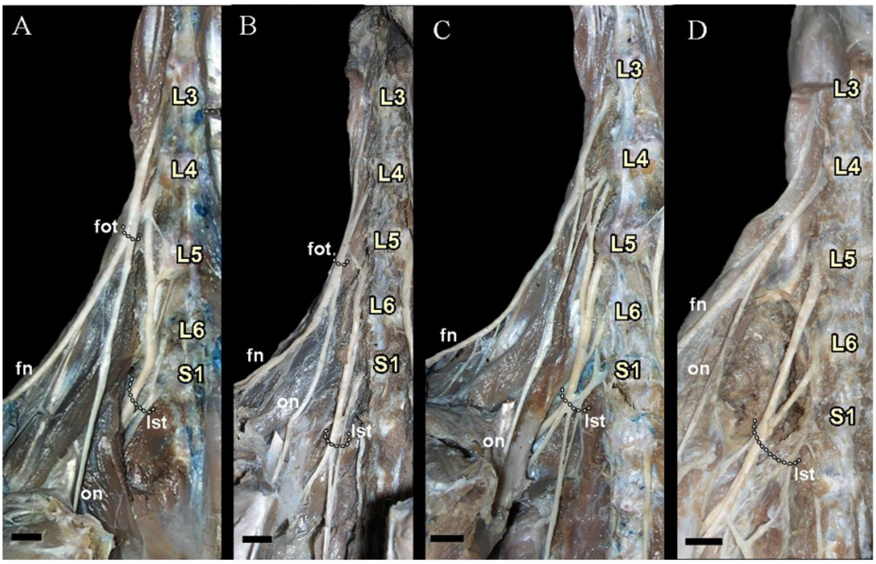

Figure 2. Digital photomacrography of the ventromedial face of the right pelvic limb of adult specimens of *Didelphis aurita* showing the variations in the origins of the lumbosacral plexus nerves. L3 – third lumbar vertebra, L4 – fourth lumbar vertebra, L5 – fifth lumbar vertebra, L6 – sixth lumbar vertebra, S1 – first sacral vertebra, fot – femoral and obturator nerves trunk, fn – femoral nerve, on – obturator nerve, lst - lumbosacral trunk (A) Male, fot: L3 and L4, lst: L4, L5, L6 and S1; (B) Female, fot: L3 and L4, lst: L5, L6 and S1; (C) Male, fn: L3 and L4, on: L3 and L4, lst: L4, L5, L6 and S1; (D) Male, fn: L4, on: L4, lst: L5, L6 and S1. Scale bar = 10mm.

(Figure 4). The most cranial nerve of the lumbosacral trunk was the cranial gluteal nerve, which innervated the *gluteus medius* and *gluteus profundus* muscles in all antimeres studied.

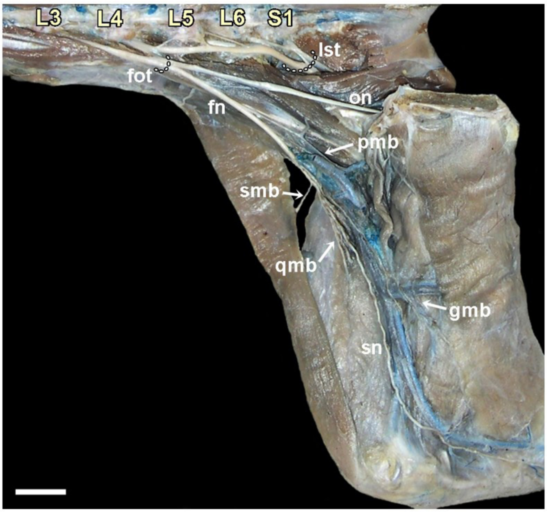

Figure 3. Digital photomacrography of the medial aspect of the right pelvic limb of an adult male specimen of *Didelphis aurita*. L3 – third lumbar vertebra, L4 – fourth lumbar vertebra, L5 – fifth lumbar vertebra, L6 – sixth lumbar vertebra, S1 – first sacral vertebra, fn – femoral nerve, on – obturator nerve, sn – saphenous nerve, lst – lumbosacral trunk, pmb – psoas major muscle branch, smb – sartorius muscle branch, qmb – quadriceps muscle branch, gmb – gracilis muscle branch. Scale bar = 10mm.

The sciatic nerve originated from the lumbosacral trunk as a single nerve in eight antimeres (28.57%), being the most robust of the entire plexus. When crossing the greater sciatic notch, it followed caudally under the *gluteus medius* muscle and, at the height of the greater trochanter of the femur, emitted muscle branches to the *biceps femoris* and *semitendinosus* muscle and divided into its terminal branches: the tibial and common fibular nerves. In 20 antimeres (71.43%), the tibial and common fibular nerves originated close to the greater sciatic notch (Figure 4B), with the tibial nerve being the thickest of the plexus. Both followed distally on the lateral aspect of the thigh, between the *biceps femoris* and *semitendinosus* muscles.

The pudendal nerve originated from the lumbosacral trunk, followed caudally under the *piriformis* muscle and innervated external genital organs and perineal structures.

3.4. Didelphis albiventris

The lumbosacral plexus was composed by the ventral branches of the spinal nerves from the third lumbar vertebra (L3) to the first sacral vertebra (S1) in 23 antimeres (88.5%) and from the fourth lumbar vertebra (L4) to S1 in three antimeres (11.5%) (Table 3).

3.5. I. Origin

The femoral and obturator nerves, for the most part, did not derived from a trunk, each nerve presented its independent origin. In one antimere (3.8%), from a male, the trunk of the femoral and obturator nerves was formed, originating from the ventral rami of the spinal nerves of L3 and L4.

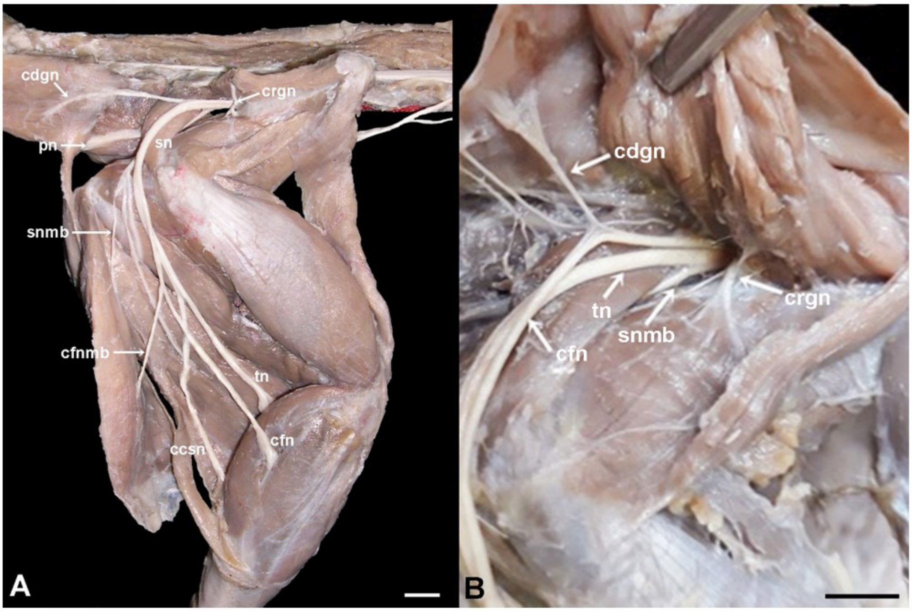

Figure 4. Digital photomacrography of the lateral face of the right pelvic limb of an adult male specimen of *Didelphis aurita* (A and B). sn – sciatic nerve, crgn – cranial gluteal nerve, cdgn – caudal gluteal nerve, pn – pudendal nerve, snmb – sciatic nerve muscular branches, tn – tibial nerve, cfn – common fibular nerve, cfnmb – common fibular nerve muscular branch, ccsn – caudal cutaneous sural nerve. Scale bar = 10mm.

The femoral nerve was the most cranial of the plexus and presented origin from the ventral branches of the spinal nerves of L3 and L4 in 19 antimeres (73.1%), being 13 antimeres of males (72.2%) and six of females (75%); from L4 and L5 in three antimeres (11.5%), all males; and of L3, L4 and L5 in three antimeres (11.5%), two of females (25%) and one of males (5.6%). The origin of the femoral nerve was from L3 to L5. The ventral branch of L4 participated in all antimeres (100%), L3 in 23 antimeres (88.5%) and L5 in six antimeres (23.1%) (Table 4) (Figure 5).

The obturator nerve was composed by the ventral branches of L3 and L4 in 16 antimeres (61.5%), being 11 antimeres of males (61.1%) and five of females (62.5%); L4 in six antimeres (23.1%), three males (16.7%) and three females (37.5%); and L4 and L5 in three antimeres (11.5%), all of them male. In one antimere (3.8%), the trunk of the femoral and obturator nerves was formed, originating from the ventral branches of the L3 and L4 spinal nerves. The origin of the obturator nerve was from L3 to L5, with a predominancy of the ventral branch of L4. The ventral branch of L4 participated in the composition of the nerve origin in 26 antimeres (100%), L3 in 17 antimeres (65.4%) and L5 in three antimeres (11.5%) (Table 4) (Figure 5).

The lumbosacral trunk presented origin for the sciatic, cranial gluteus, caudal gluteal and pudendal nerves. The lumbosacral trunk was composed by the ventral branches from L4 to S1 in ten antimeres (38.5%), two in females (25%) and eight in males (44.4%); and from L5 to S1 in 16 antimeres (61.5%), six in females (75%) and ten in males (55.6%). The origin of the lumbosacral trunk was from L4 to S1, with a predominance of the ventral branches of L5, L6 and S1. The ventral branches of L5, L6 and S1 participated in the composition of the origin of the lumbosacral trunk in 26 antimeres (100%) and L4 in ten antimeres (38.5%) (Table 4) (Figure 5).

3.6. II. Distribution

Regarding the distribution of the femoral nerve in *D. albiventris*, it was observed that it followed between the *psoas minor* and *psoas major* muscles and, in all animals, it emitted branches to the *psoas major* and *quadratus lumborum* muscles. The nerves ran to the medial region of the thigh and emitted branches to the *quadriceps femoris*, *sartorius*, *gracilis* and *pectineus* muscles. In the middle portion of the femur, the femoral nerve distinguished as the saphenous nerve, which followed distally to supply the medial aspect of the leg.

After receiving the branches that constituted the obturator nerve, it followed parallel to the tendon of the *psoas minor* muscle, towards the medial face of the body of the ilium bone and before crossing the obturator foramen, it emitted a branch to the *obturator internus* muscle. Upon reaching the medial region of the thigh, this nerve emitted branches to the *obturator externus*, *pectineus*, *adductor* and *gracilis* muscles.

The lumbosacral trunk was the origin of the sciatic, cranial gluteus, caudal gluteus and pudendal nerves. This trunk left the pelvis through the greater sciatic notch and

Table 3. Absolute (AF) and percentage frequency (PF) of the ventral branches of spinal nerves that participate in the composition of the lumbosacral plexus of *Didelphis albiventris*.

VENTRAL BRANCHES	FEMALES		MALES		TOTAL	
	AF	PF	AF	PF	AF	PF
L3 - S1	8	100	15	83.3	23	88.5
L4 - S1	-	-	3	16.7	3	11.5

Table 4. Absolute (AF) and percentage frequencies (PF) of the origins of the lumbosacral plexus nerves of *Didelphis albiventris*.

NERVE	ORIGIN	FEMALES		MALES		TOTAL	
		AF	PF	AF	PF	AF	PF
Femoral	L3-L4	-	-	1	5.6	1	3.8
	L3 and L4	6	75	13	72.2	19	73.1
	L4 and L5	-	-	3	16.7	3	11.5
	L3, L4 and L5	2	25	1	5.6	3	11.5
Obturator	L3-L4	-	-	1	5.6	1	3.8
	L3 and L4	5	62.5	11	61.1	16	61.5
	L4	3	37.5	3	16.7	6	23.1
	L4 and L5	-	-	3	16.7	3	11.5
Lumbosacral trunk	L5-L6-S1	6	75	10	55.6	16	61.5
	L4-L5-L6-S1	2	25	8	44.4	10	38.5

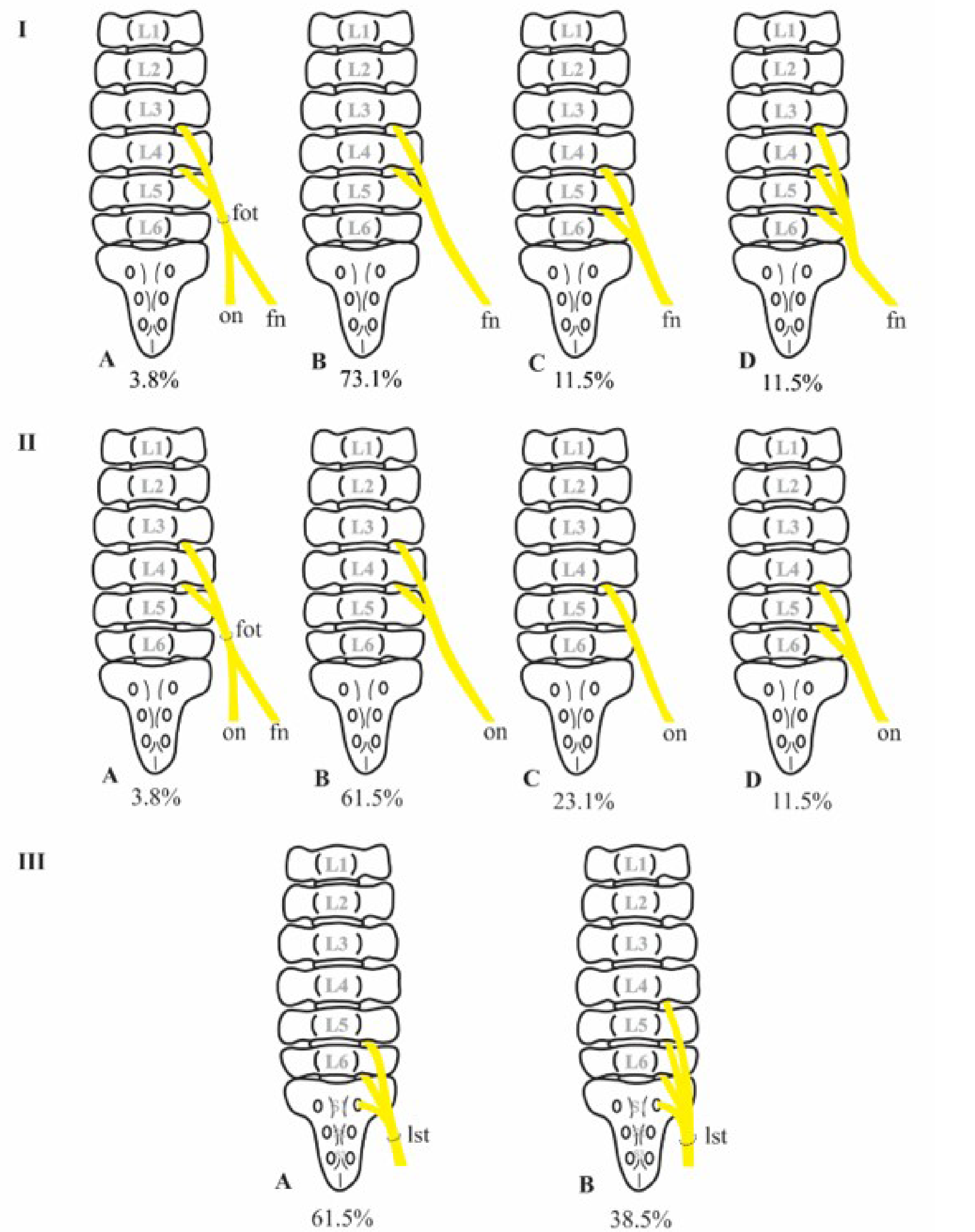

Figure 5. Schematic representation of the origins of (I) femoral nerve (fn), (II) obturator nerve (on) and (III) lumbosacral trunk (lst) in *Didelphis albiventris*. I: (A) L3-L4 – femoral and obturator nerves trunk (fot); (B) L3 and L4; (C) L4 and L5; (D) L3, L4 and L5. II: (A) L3-L4; (B) L3 and L4; (C) L4; (D) L4 and L5. III: (A) L5-L6-S1; (B) L4-L5-L6-S1.

its nerves were distributed along the side of the thigh. The most cranial nerve observed was the cranial gluteal nerve, which innervated the *gluteus medius*, *gluteus profundus piriformis* muscles (Figure 6).

The strongest nerve observed in the lumbosacral plexus was the sciatic nerve. It branched from the lumbosacral trunk, crossed the greater sciatic notch and at the height of the greater trochanter of the femur, curved ventrally

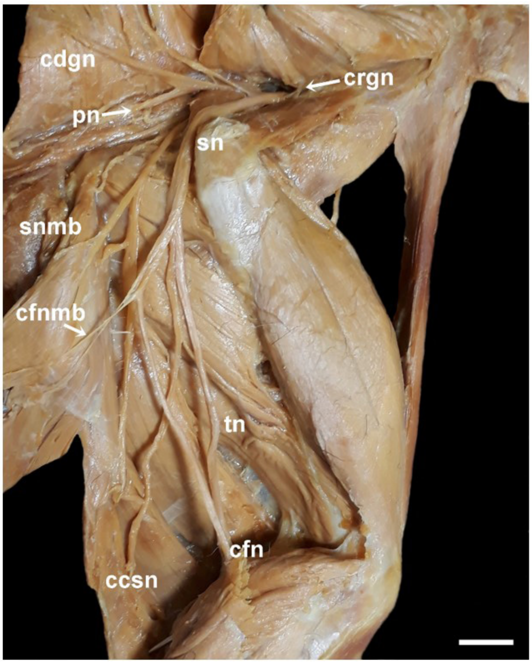

Figure 6. Digital photomacrography of the lateral face of the right pelvic limb of an adult male specimen of *Didelphis albiventris*. sn – sciatic nerve, crgn – cranial gluteal nerve, cdgn – caudal gluteal nerve, pn – pudendal nerve, snmb – sciatic nerve muscular branches, cfn – common fibular nerve, cfnmb – common fibular nerve muscular branch, tn – tibial nerve, ccsn – cutaneous caudal sural nerve. Scale bar = 10mm.

and continued distally through the thigh. The sciatic nerve gave off muscular branches, which followed more caudally under the *piriformis* muscle, which innervated the *biceps femoris*, *semitendinosus* and *semimembranosus* muscles. When bending at the level of the greater trochanter of the femur, it divided into the common fibular and tibial nerves.

As for the caudal gluteal nerve, which originated as a branch of the sciatic nerve in all antimeres, it was found that it supplied the *gluteus superficialis* muscle. The pudendal nerve, when crossing the greater sciatic notch, followed dorsolaterally, under the *piriformis* muscle, to innervate the perineal region and external genital organs.

4. Discussion

4.1. I. Origin

In the total of the 28 dissected plexuses of *Didelphis aurita* and of the 26 of *D. albiventris*, the nerves that supplied the pelvis and the thigh presented formations derived from the connections between the ventral branches of the last four lumbar spinal nerves (L3, L4, L5 and L6) and the first sacral (S1). This formation was similarly observed in *D. aurita* (Senos et al., 2016) and *D. marsupialis* Linnaeus, 1758 (Sepúlveda-Vásquez and Tamayo-Arango, 2023).

The origin of the plexus in *D. aurita* and *D. albiventris* was more cranial than in other species also with six lumbar vertebrae, for example, the chinchilla (*Chinchilla lanigera* Bennett, 1829) (Martinez-Pereira and Rickes, 2011), which presented the most cranial origin of the plexus coming from the ventral spinal branch of L4. Animals with seven lumbar vertebrae, such as the Mongolian gerbil (*Meriones unguiculatus* Milne-Edwards, 1867) (Araújo-Júnior et al., 2016) presented more cranial formation in L3; the red squirrel (*Sciurus vulgaris* Linnaeus, 1758) (Aydin, 2010) and paca (*Cuniculus paca* Linnaeus, 1766) (Tonini et al., 2014) in L4; and the rock cavy (*Kerodon rupestris* Wied-Neuwied, 1820) (Lacerda et al., 2006), yellow-toothed cavy (*Galea spixii* Wagler, 1831) (Oliveira et al., 2014) and agouti (*Dasyprocta leporina* Linnaeus, 1758) (Oliveira et al., 2016) in L5. Other species of eutherian mammals showed more cranial formation than *Didelphis*, such as the xenarthran giant anteater (*Myrmecophaga tridactyla* Linnaeus, 1758) (Cruz et al., 2014) with origin in T16, lesser anteater (*Tamandua tetradactyla* Linnaeus, 1758) (Cardoso et al., 2013) in T18 and brown-throated three-toed sloth (*Bradypus variegatus* Schinz, 1825) (Silva, 2017) from L1. The same occurred to the crested porcupine (*Hystrix cristata* Linnaeus, 1758) (Aydin, 2009) in L2.

Voris (1928), Dyce et al. (2019) and Sepúlveda-Vásquez and Tamayo-Arango (2023) described that the lumbosacral plexus might be displaced cranially or caudally, which can be an anatomical variation in other species when considering the plexus cranial limit formation. This more cranial projection of the lumbosacral plexus origin was due to the greater range of abduction of didelphids with scansorial locomotor habits, emphasized by the shape of the femoral head, which is more hemispherical in proximal view (Argot, 2002). However, there is a correlation between animals with prehensile tail and the origin of the most cranial lumbosacral plexus, as in the *Didelphis* in this study and species of the superorder Xenarthra (Cardoso et al., 2013; Cruz et al., 2014; Silva, 2017). Despite having evolved independently of these eutherians, the similarities observed in this study may correspond to homoplasy.

The femoral nerve in *D. aurita* and *D. albiventris* was formed mainly by the union between the L3 and L4 branches, similarly observed in the Mongolian gerbil (Araújo-Júnior et al., 2016) and *D. marsupialis* (Sepúlveda-Vásquez and Tamayo-Arango, 2023). The double contribution of ventral spinal branches was also observed at L2 and L3 for crested porcupine (Aydin, 2009); L4 and L5 for red squirrel (Aydin, 2010) and chinchilla (Martinez-Pereira and Rickes, 2011); and by L5 and L6 in the rock cavy (Lacerda et al., 2006, Oliveira et al., 2011), yellow-toothed cavy (Oliveira et al., 2014) and paca (Tonini et al., 2014). In the giant anteater, the femoral nerve received three contributions from the ventral branches, L1, L2 and L3 (Souza, 2012) or from T16, L1 and L2 (Cruz et al., 2014); as well as in brown-throated three-toed sloth (Silva, 2017) from L1, L2 and L3; agouti (Oliveira et al., 2016) from L5, L6 and L7; and the lesser anteater (Cardoso et al., 2013) with four, T18, L1, L2 and L3.

The obturator nerve was formed exclusively from the ventral branch of L4 in *D. albiventris* in six antimeres (23%) and one antimere (3.57%) in *D. aurita*, in the ventral branch of L5. In paca (Tonini et al., 2014) the obturator nerve was formed exclusively from L6. However, the obturator nerve had the same origin as the femoral nerve in most cases, as described by König and Liebich (2021), Dyce et al. (2019), crested porcupine (Aydin, 2009), red squirrel (Aydin, 2010), chinchilla (Martinez-Pereira and Rickes, 2011), lesser anteater (Cardoso et al., 2013), giant anteater (Cruz et al., 2014), yellow-toothed cavy (Oliveira et al., 2014), coati (Silva, 2014), Mongolian gerbil (Araújo-Júnior et al., 2016), agouti (Oliveira et al., 2016) and brown-throated three-toed sloth (Silva, 2017). In the rock cavy (Lacerda et al., 2006), the obturator nerve originated from the L5, L6 and L7 or L6 and L7 branches.

In 26 antimeres (92.86%) of *D. aurita* and one antimere (3.5%) of *D. albiventris*, there was a formation of a common trunk between the femoral and obturator nerves at L3 and L4, as observed by Senos et al. (2016) in *D. aurita* and Sepúlveda-Vásquez and Tamayo-Arango (2023) in *D. marsupialis*. The trunk has also been observed in red squirrels (Aydin, 2010), originating in L4 and L5. The lumbosacral trunk was observed in *D. aurita* and *D. albiventris*, with a contribution mainly from the ventral branches of the spinal nerves of L5, L6 and S1, and continued out of the pelvis as the sciatic nerve, the largest nerve in the plexus. The lumbosacral trunk also emitted branches to the cranial gluteal, caudal gluteal and pudendal nerves. A similar formation was observed in red squirrels (Aydin, 2010) and chinchillas (Martinez-Pereira and Rickes, 2011), with the unification of the last two lumbar segments branches and the first two sacral ones.

The concept of the lumbosacral trunk in which there is a contribution of lumbar and sacral branches is supported by Dyce et al. (2019). The authors considered it being formed mainly by the last two lumbar nerves and the first two sacral nerves, and it leaves the pelvis through the greater sciatic notch and emits three branches, the cranial gluteal, caudal gluteal and sciatic nerves. Unlike König and Liebich (2021), who described the plexus being originated also in the caudal femoral and caudal rectal cutaneous nerves. Nomenclature differences can be found as Martinez-Pereira and Rickes (2011) who named the trunk as the sciatic plexus. The Nomina Anatomica Veterinaria (ICVGAN, 2017) adopted the terminology “lumbosacral trunk” being formed by the cranial gluteal, caudal gluteal, caudal femoral cutaneous and sciatic nerves. Dyce et al. (2019) do not consider the pudendal nerve as part of the lumbosacral plexus, as it has its more caudal origin in domestic animal species. On the other hand, Schaller (1999) mentioned that the lumbosacral trunk is represented by the branch originating from the lumbar plexus that reinforces the sacral plexus, that is, the author does not consider the participation of sacral roots in its formation, which was observed by Cardoso et al. (2013) in the lesser anteater.

The sciatic nerve had its origin in the formation of the lumbosacral trunk in the two species studied, originated mainly from L5, L6 and S1, that is, the last two lumbar ventral branches and the first sacral, similar to that reported in chinchilla (Martinez-Pereira and Rickes, 2011), yellow-toothed cavy (Oliveira et al., 2014) and coati (Silva, 2014). Pellenz et al. (2020) reported for *D. albiventris* and Senos et al. (2016) for *D. aurita* the sciatic nerve origin in L4, L5 and L6; and Sepúlveda-Vásquez and Tamayo-Arango (2023) reported origin for *D. marsupialis* in L5 and L6. In eutherian species, this nerve was formed of the lumbosacral trunk in the agouti (Oliveira et al., 2016); last lumbar branch and the last three sacral in the giant anteater (Ribeiro, 2012; Cruz et al., 2014) and lesser anteater (Cardoso et al., 2013); by the last two lumbar and first two sacral branches in crested porcupine (Aydin, 2009), red squirrel (Aydin, 2010), paca (Tonini et al., 2014), brown-throated three-toed sloth (Silva, 2017). No contribution from the sacral branches to the formation of the sciatic nerve was observed in the Mongolian gerbil (Araújo-Júnior et al., 2016).

The origin of the cranial gluteal nerve was from the lumbosacral trunk (L5, L6 and S1) in *D. aurita* and *D. albiventris*, similar to what was reported by Senos et al. (2016) for *D. aurita* and in red squirrels (Aydin, 2010), chinchilla (Martinez-Pereira and Rickes, 2011), lesser anteater (Cardoso et al., 2013), and *D. marsupialis* (Sepúlveda-Vásquez and Tamayo-Arango, 2023). The cranial gluteal nerve emerges independently from the ventral branch of L2, S1 and S2 in the giant anteater (Cruz et al., 2014); L3 in the female crested porcupine (Aydin, 2009); L3 and L4 in the male crested porcupine (Aydin, 2009); L3, L4 and S1 in the brown-throated three-toed sloth (Silva, 2017); L5 and L6 in the Mongolian gerbil (Araújo-Júnior et al., 2016); L6 and L7 in paca (Tonini et al., 2014); L7 in rock cavy (Lacerda et al., 2006), yellow-toothed cavy (Oliveira et al., 2014) and agouti (Oliveira et al., 2016).

The caudal gluteal nerve appeared as a branch of the sciatic nerve in the species of *Didelphis* studied, in paca (Tonini et al., 2014) and *D. marsupialis* (Sepúlveda-Vásquez and Tamayo-Arango, 2023). In the red squirrel (Aydin, 2009) and *D. aurita* (Senos et al., 2016), the caudal gluteal nerve appeared as a branch of the lumbosacral trunk. However, this nerve appeared as an independent branch of L3, S1 and S2 in the giant anteater (Cruz et al., 2014); of L4 and S1 in the crested porcupine (Aydin, 2009); L7 in rock cavy (Lacerda et al., 2006); L7 and S1 in the yellow-toothed cavy (Oliveira et al., 2014); S1 in agouti (Oliveira et al., 2016); S1 and S2 in the Mongolian gerbil (Araújo-Júnior et al., 2016); and of S2 and S3 in the brown-throated three-toed sloth (Silva, 2017).

The pudendal nerve originated from the lumbosacral trunk in the two species studied, similar to what was reported by Sepúlveda-Vásquez and Tamayo-Arango (2023) for *D. marsupialis*. In *D. aurita* (Senos et al., 2016), this nerve originated as a single branch of S1. This divergence from that observed by Senos et al. (2016), because the concept of lumbosacral trunk is controversial. This discussion reinforces the importance of further studies on the lumbosacral trunk and branches that emerge from it. Variations in the origin of the pudendal nerve were observed in other mammals originated from L7 and S1 in the Mongolian gerbil (Araújo-Júnior et al., 2016); crested porcupine (Aydin, 2009); S1, S2 and S3 in the rock cavy (Lacerda et al., 2006), yellow-toothed cavy (Oliveira et al., 2014), paca (Tonini et al., 2014), agouti (Oliveira et al., 2016); and S4 and S5 giant anteater (Cruz et al., 2014). In the lesser anteater (Cardoso et al., 2013), it was derived from branches S3 and S4 or S4 and S5.

4.2. II. Distribution

The absence of the *tensor fasciae latae* muscle proposed by Diogo et al. (2016) in *Didelphis* and also reported by Senos et al. (2106) in *Didelphis aurita*, may reflect the different distribution of nerves in the lumbosacral plexus of *Didelphis*, especially when compared to rodents, due to their scansorial locomotion.

Regarding the distribution of the femoral nerve, it was found that it emitted branches to the *psoas major*, *quadratus lumborum*, *sartorius*, *quadriceps femoris* and *gracilis* muscles in the two species of opossum studied. Furthermore, in *D. aurita*, the femoral nerve also distributed branches to the *iliacus* muscle and in 14.29% of the plexuses to the *pectineus* muscle. In *D. albiventris*, a branch to the *pectineus* muscle was observed in all plexuses. In both species the terminal branch of the femoral nerve was the saphenous nerve. In the study of the lumbosacral plexus of *D. aurita*, Senos et al. (2016) observed branches to the *iliopsoas*, *sartorius* and *quadriceps femoris* muscles. Oliveira et al. (2011) mentioned that in the rocky cavy, the femoral nerve is responsible for serving the *iliacus*, *pectineus*, *sartorius* and *quadriceps femoris* muscles. In the giant anteater (Souza, 2012) and lesser anteater (Paula et al., 2016), the femoral nerve sent branches to the *psoas major* and *psoas minor*, lateral and medial *iliacus*, *pectineus*, *adductor magnus*, *sartorius* and *quadriceps femoris* muscles.

The distribution of the femoral nerve to the *quadriceps femoris* muscle corroborated the reports of domestic and wild animals, which denotes the importance of this muscle for animal locomotion, being the main knee extensor and auxiliary hip flexor (Dyce et al., 2019). The branches to the *pectineus* muscle reinforce the "cross-couplet" or "paired-cross" walking pattern, where the right forelimb moves simultaneously with the left pelvic limb (Reilly and White, 2003; Reilly et al., 2010). In marsupials, the *pectineus* muscle originates from both ventral and dorsal sides of the lateral tubercle of the epipubic bone and inserting on the femoral shaft. The *pyramidal*is muscle originates from the *linea alba* and attaches to the dorsal surface of the shaft of the epipubic bone, from the crest to the medial articular process, in the pyramidal line (Guilhon et al., 2021). Both act in an antagonistic way (Reilly et al., 2010). An electromyographic study of these muscles during the locomotion of the didelphids *Monodelphis domestica*, *Didelphis virginiana* and *Philander opossum* showed that the *pectineus* muscle contracts, projecting the femur forward while the *pyramidalis* muscle relaxes, bringing the epipubic bone medially, providing balance to the axial skeleton during locomotion (Reilly and White, 2003; Reilly et al., 2010).

The obturator nerve emitted branches to the *obturator externus*, *pectineus*, *adductor* and *gracilis* muscles in the two species of *Didelphis* studied. Senos et al. (2016) reported, for *D. aurita*, innervation to the *obturator internus* muscle instead of the *obturator externus*, as observed in this study. Through that, in *D. albiventris*, the obturator nerve was also observed innervating the *obturator internus* muscle. A similar distribution was observed in the crested porcupine (Aydin, 2009) and chinchilla (Martinez-Pereira and Rickes, 2011). In the lesser anteater (Paula et al., 2016) the obturator nerve innervated the *obturator externus*, *gracilis* and adductor muscles. In the brown-throated three-toed sloth (Silva, 2017), it emitted branches to the *gracilis*, *obturator externus*, adductor, *pectineus* and *semimembranosus* muscles.

The sciatic nerve developed muscular branches to the *biceps femoris* and *semitendinosus* muscles in both opossum species and to the *semimembranosus* in *D. albiventris*. Pellenz et al. (2020) reported a similar distribution for *D. albiventris*. The innervation of the *biceps femoris*, *semitendinosus* and *semimembranosus* muscles by muscular branches of the sciatic nerve has been reported in the rock cavy (Lacerda et al., 2006) and crested porcupines (Aydin, 2009). In the brown-throated three-toed sloth (Silva, 2017), it emitted branches to the *gluteus*, *biceps femoris*, *semitendinosus* and *semimembranosus* muscles. In the lesser anteater (Paula et al., 2016), the sciatic nerve emitted branches to the *biceps femoris*, *semitendinosus*, *semimembranosus* muscles and a branch muscle proximal to the *gemelli* and *quadratus femoris* muscles.

The cranial gluteal nerve innervated the *gluteus medius* and *gluteus profundus* muscles in the species of *Didelphis* studied and in *D. albiventris* it also emitted branches to the *piriformis* muscle, as described in the lesser anteater (Paula et al., 2016). In brown-throated three-toed sloth (Silva, 2017), the innervation of the *gluteus medius* and *gluteus profundus* muscles was observed. In the crested porcupine (Aydin, 2009), it innervated all the three muscles from the gluteal group (superficial, medius and profundus). A branch to the *tensor fasciae latae* muscle was observed in lesser anteater (Paula et al., 2016) and in the brown-throated three-toed sloth (Silva, 2017).

The caudal gluteal nerve in all studied antimeres innervated the *gluteus superficialis* muscle, similar to what was observed in the lesser anteater (Paula et al., 2016) and in the brown-throated three-toed sloth (Silva, 2017). In the crested porcupine (Aydin, 2009), the caudal gluteal nerve discharged branches to the *gluteus superficialis*, *biceps femoris* and *semitendinosus*.

The pudendal nerve emitted branches to the perineal region in all specimens studied. This innervation was similar with the one described for the crested porcupine (Aydin, 2009).

The origin and antimeric distribution of the nerves of the lumbosacral plexus in *D. albiventris* and *D. aurita* are partially similar to those observed in rodents, since *Didelphis* is anatomically more plesiomorphic than rodents, being a good model for a common ancestor of extant species of subclass Theria, as observed by Diogo et al. (2016).

5. Conclusion

The lumbosacral plexuses in *Didelphis albiventris* and *D. aurita* are formed by contributions from the last four lumbar ventral branch of the spinal nerves (L3, L4, L5 and L6) and the first sacral (S1). In these specimens, the nerves received contribution mainly from the ventral rami of L4 and L5.

The findings reported in this study may contribute to applied research, serving as a basis for clinical and surgical procedures in these species and provide information for the comparative anatomy, being also helpful for muscle identification during dissections.

Despite that, comparative anatomical studies with species of the infraclass Metatheria are necessary and will allow a better understanding of the anatomical characteristics of the lumbosacral plexus in marsupials.

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