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Topography and morphology of the *Eira barbara* diaphragm

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Abstract

Background The diaphragm, the main muscle involved in respiration and one of those responsible for maintaining life, is still little explored in terms of its morphology in wild animals. There are few studies on the anatomy of *Eira barbara*, a carnivorous mustelid that is a victim of the urbanization process. In order to contribute to the conservation of the species, we described the topography and morphology of the diaphragm, which may be involved in injuries caused by the impacts of human activities.

Results We studied five specimens of *Eira barbara*, whose diaphragmatic muscle had a dorsal insertion on the 14th thoracic vertebra, laterally between the 8th and 13th intercostal space (EIC) and ventrally on the 8th EIC, with attachment to the xiphoid process. Consisting of three muscle regions (lumbar, costal and sternal), the diaphragm in *Eira barbara* showed radially arranged bundles, with the right costal muscle being slimmer than the left; the left pillar wider than the right and between them were the aortic and esophageal hiatuses. The Y-shaped tendinous center housed the foramen of the vena cava bordering the right costal region. In the most dorsal portion of the diaphragm, between the costal regions and the diaphragmatic pillar, we found two triangular-shaped regions devoid of muscle.

Conclusions Our findings, when compared with the current literature, indicate that the location and positioning of the diaphragm are independent of the physical conformation of the species, and that the right costal region, as well as the triangular areas devoid of musculature, may be fragile points for herniation in cases of *Eira barbara* being run over.

Keywords Irara, Diaphragm, Diaphragmatic histology, Mustelids, Thorax

Background

Eira barbara, popularly known as the tayra or honeyeater, is a medium-sized mammal belonging to the order Carnivora, family Mustelidae, with a total length of between 55.9 cm and 71.2 cm, with the tail between 36.5 cm and 46.0 cm, and weighing between 2.7 kg and 7 kg. Adult males are more muscular and 30% larger than females. Young individuals are completely black, while older ones have light-tipped hairs with a matted appearance [1]. They can be found throughout Central America, in parts of South America, and in Brazil they are found in almost all biomes (Atlantic Forest, Amazon, Cerrado, Caatinga and Pantanal), but are more common in areas of dense vegetation.

Despite its wide distribution, little is known about the morphology of *Eira barbara*. In this regard, we would highlight the diaphragm, which is the main striated skeletal muscle involved in the respiratory process in

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mammals, marking the transition from intrauterine to extrauterine life. It has an important role in regurgitation, evacuation and the urination reflex, since it is positioned between the thoracic and abdominal cavities, leading to the expansion of the intrathoracic volume, reducing the pressure of this cavity [2–4].

Classical anatomical literature describes the diaphragm as divided into a central region, known as the tendinous center, which is mainly dinnervated by the phrenic nerve [5] and a peripheral region known as the muscular region, which in turn is divided into three different portions: sternal, costal and lumbar [6–8]. The shape, orientation and distribution of muscle and tendon fibers can vary depending on the species [9].

The diaphragm muscle has long been studied in terms of its evolution and functionality. In mammals it is considered an organ with two important roles: separation of the thoracic and abdominal cavities, which prevents the movement of abdominal organs into the thoracic space, especially during aspiration breathing; and provider of negative intrathoracic pressure and higher intra-abdominal pressure to promote venous return and favor expulsive behaviors [10].

As an important component of the respiratory aparattus, the diaphragm has been the focus of some studies, mainly in relation to innervation [4, 5, 11–21]. However, given its physiological, clinical and surgical importance, we believe that much remains to be investigated regarding the morphology of the diaphragm, especially with regard to wild mammals.

With this in mind, we aimed to evaluate the topography and morphology of the diaphragm of *Eira barbara*, in order to contribute to both the basic literature and conservation of the species, based on the data generated, which could support medical procedures in cases of surgical approaches for diaphragmatic herniations.

Materials and methods

A total of eight *Eira barbara* specimens, fatal victims of being run over by vehicles were evaluated, but only five died without diaphragm involvement, as a result of internal and/or external hemorrhage due to limb amputation or head trauma. The animals were found and collected by the mining company's authorized team in the area from the Paragominas Bauxite Mine - PA, under SEMA-PA authorization Nº 455/2009 and 522/2009. These were frozen and sent to the Animal Morphology Research Laboratory (LaPMA) of the Federal Rural University of Amazonia (UFRA), SISBIO No. 23401-8 (Biodiversity Authorization and Information System).

The animals were defrosted under running water and then, through an incision on the medial side of the left pelvic limb and dissection of the femoral artery, they were perfused with Neoprene 650 latex, stained with contrast red pigment, in order to fill the complete arterial system and subsidize future investigations related to the circulatory system.

After the latex infusion, the animals were fixed in a 10% aqueous formaldehyde solution by means of intramuscular and intracavitary infusion and the specimens were kept in the same solution for seven days.

To analyze the topography of the diaphragm and its insertion points, the animals were x-rayed in ventral and lateral recumbency, using dorsoventral and left laterolateral projections, respectively. Radiographs were taken using an Intecal® X-ray machine, model CR-7, with a power of 100 kV, a film focus distance of 100 cm, 100 mA, a time of 0.4 s, with exposure factors of 55 kVs and 2.5 m for lateral radiographs and 60 kVs for dorsoventral radiographs.

The animals were dissected by flapping the skin, thoracic muscles and incisions in the costochondral joints, allowing complete removal of the sternum bone and cranial and caudal visualization of the diaphragm.

The diaphragm was then completely removed through incisions on the lumbar, costal and sternal sides, followed by photodocumentation without and with direct illumination, using a spotlight to better visualize the tendon center and the distribution of muscle fibers.

For microscopic analysis, fragments were taken and preserved in 10% formaldehyde, following routine histological technique and basic staining with Hematoxylin-Eosin (HE) [22]. The slides were analyzed and photographed using a LEICA E-400 photomicroscope.

Finally, with the aim of making the material under study more realistic and interactive, a three-dimensional image of the diaphragm of *Eira barbara* was taken, so that it could be compared with the two-dimensional images and their structures using the Polycam Lidar 3D® app, directly from a smartphone.

All the nomenclature adopted was based on the Veterinary Anatomical Nomenclature [23] and the Veterinary Histological Nomenclature [24].

Results

In all the specimens, the lumbar insertion of the diaphragmatic muscle occurred at the 14th thoracic vertebra, laterally between the 8th and 13th intercostal spaces (EIC) and ventrally at the 8th EIC, with attachment to the xiphoid process of the sternum bone (Fig. 1A).

In all specimens evaluated, the peripheral muscular portion of the diaphragm was divided into three regions: lumbar, costal and sternal. The lumbar region was formed by two pillars, one right and one left. The latter was slightly thicker than the right (as shown in Fig. 1C). Still in the lumbar region, but more dorsally, between the pillars, there was the aortic hiatus. Slightly more ventrally

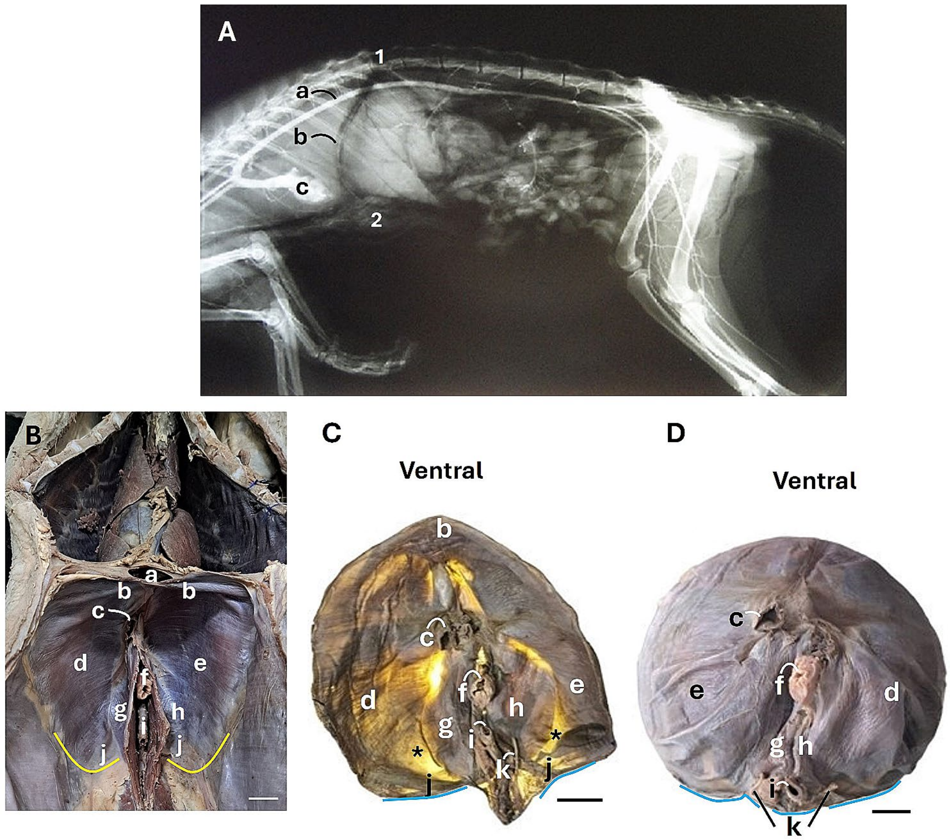

Fig. 1 Photomacrograph of the diaphragm of *Eira barbara*. (**A**). Radiographic image left laterolateral view. Note the diaphragm (b) attached dorsally to the 14th thoracic vertebra (1) and ventrally to the xiphoid region (2). a. thoracic aorta and c. heart. (**B**). Ventrocaudal view showing the exact point of separation of the thoracic and abdominal cavities. a- Central area of the mediastinodiaphragmatic recess, b- sternal region, c- vena cava (vena cava foramen), d- right costal region, e- left costal region, f- esophagus, i- aorta (both in the aortic hiatus), g- right pillar, h- left pillar, together forming the j- lumbar region juxtaposed to the lumbocostal arches (A - yellow, B and C - blue). (**C**). Cranial view. (**D**). Caudal view. In B and C we can see the same structures and regions of b - j, k - phrenic nerve. Note in C the pointed demarcation of the tendon center and the traced demarcation of the triangular area devoid of muscle (*). Scale bars: 1 cm

at the end of the pillars, we found the esophageal hiatus (Fig. 1B-D).

It was easy to identify that the right costal muscle is slimmer than the left and that the muscle bundles were positioned parallel to each other and arranged radially (Fig. 1D).

Between the lumbar, costal and sternal regions was the tendon center in a "Y" shape, and in this center, bordering the right costal region, we located the foramen of the vena cava (Fig. 1D).

Dorsally to the tendon center, between the costal regions and the diaphragmatic pillars, we found two triangular-shaped regions devoid of muscle (Fig. 1C).

By clicking on the link https://poly.cam/capture/1a3376af-0e07-48e8-9712-f926b79b9b3a it is possible to visualize the structures shown here in three dimensions, making it easier for the reader to understand them. However, we would like to point out that the retraction of the material was inevitable, given that the 3D images could only be taken with the diaphragm *ex situ*, so there was no anchoring of the diaphragm, just as there is in situ.

Microscopically, the diaphragm was involved by a layer of dense connective tissue, the epimysium, which invaginated towards the inside of the muscle (Fig. 2A), forming connective tissue septa, called the perimysium, and skeletal muscle fibers (Fig. 2B).

Vessels and nerves were also found in the perimysium (Fig. 2C). In the nerve, it was possible to distinguish the orientation of the transverse and longitudinal section, as well as identifying the epineurium involving the axon extensions. The muscle fibers showed characteristics typical of striated skeletal muscle tissue (Fig. 2D).

Discussion

Evolutionarily, the diaphragm migrated from the condition of a membranous physical partition (peritoneal folds), which separated a single coelomic cavity, to an organ that separates this cavity into two (thoracic and abdominal), which is one of its functions [25]. In this

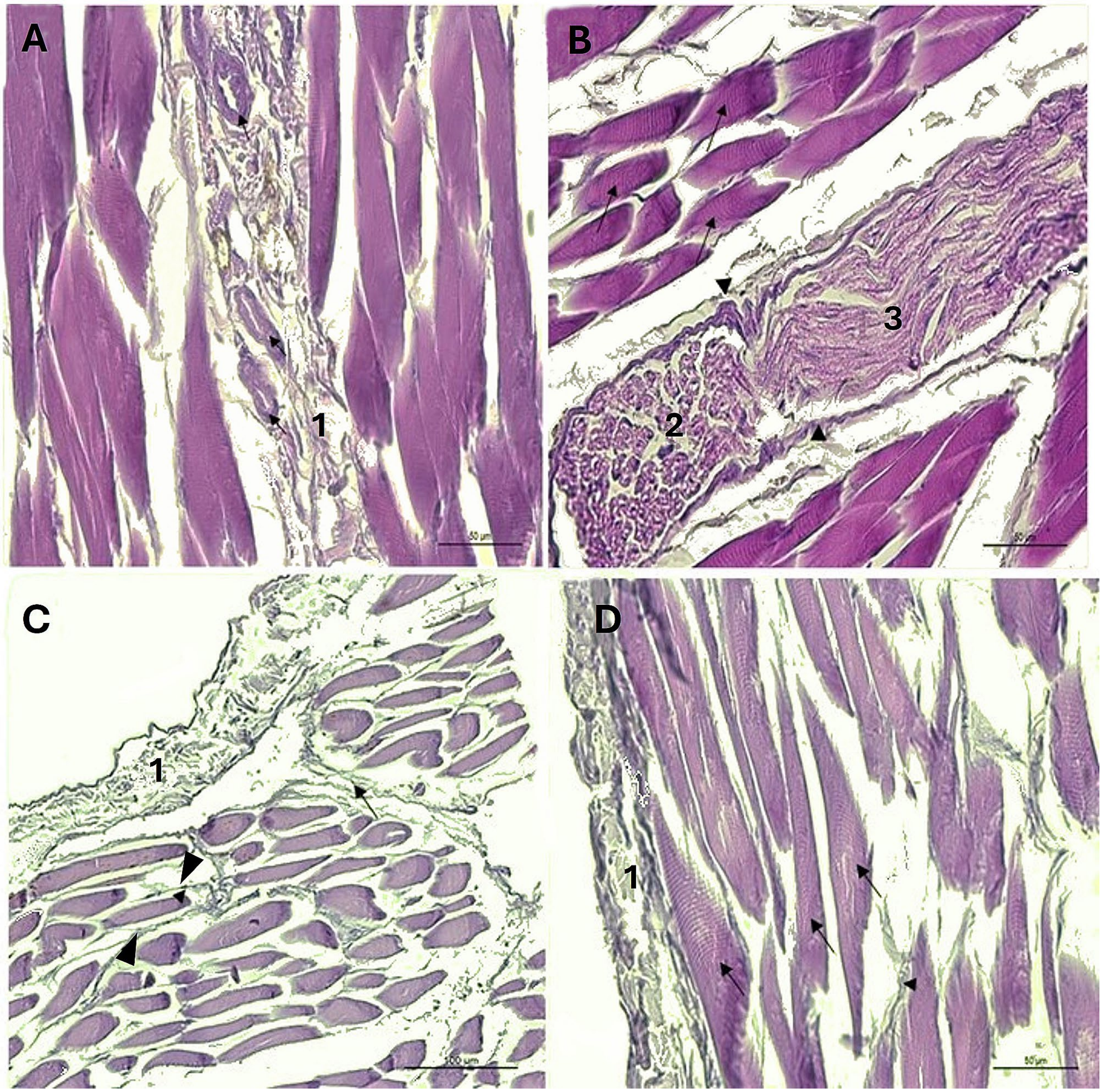

Fig. 2 Photomicrograph of the diaphragm of *Eira barbara*. (**A**). Presence of blood vessels (arrows) in the perimysium (1). (**B**). Presence of nerves inserted between the skeletal muscle fibers (arrows), where it is possible to see the epineurium (arrowhead) surrounding the nerve, in transverse (2) and longitudinal (3) sections. (**C**). Layer of dense connective tissue surrounding the diaphragm called the epimysium (1), invaginating and forming septa called the perimysium (arrow), from where thinner bundles of connective tissue surrounding the muscle fibers, the endomysium (arrowhead). (**D**). Epimysium (1), endomysium (arrowhead) and muscle fibers (arrows). HE staining. Scale bars: A, B and D 50 µm, C 100 µm

context, the diaphragm became a large tendinous muscle, now covered cranially by the pleura and caudally by the peritoneum. Its costal face is then fixed to the last ribs, and the central portion extends cranially into the thoracic cavity [26].

The second function to diaphragm is to operate as a pressure pump, which is negative in the thoracic cavity and positive in the abdominal cavity, ensuring that the lungs do not collapse due to efficient ventilation, as well as compromising the abdominal viscera [10].

Topographically, the diaphragm has a direct relationship with other organs, arranged immediately caudal to the lungs and heart; and cranially to the liver, spleen and stomach, organs that make up the front line of the abdominal cavity, in the cranial-caudal view [6, 8]. This description agrees with our findings in *Eira barbara*, as well as in several other mammals (3, 5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 27, 28].

As described in domestic animals [7, 8], the diaphragm of *Eira barbara* has three attachment points (lumbar, costal and sternal). Its dorsal (lumbar) insertion was observed between the 13th and 14th thoracic vertebrae. In other carnivore species such as *Felis catus* (domestic cat) [26], omnivores such as *Nasua nasua* (ring-tailed coati) [27] and *Didelphis albiventris* (white-eared opossum) [17], we observe a certain similarity in the lumbar insertion point varying between the 10th and 13th thoracic vertebrae. In *Tamandua tetratactyla* (anteater) and *Myrmecophaga tridactyla* (white-lipped anteater), the dorsal insertion point occurs being slightly caudally, at the level of the 12th thoracic vertebra [29, 30].

As for the diaphragm's costal attachment, the regions are more varied. While in *Eira barbara* it occurs between the 8th and 13th EIC, in *Tamandua tetradactyla* it occurs between the 11th and 12th EIC [29]. However, we noticed a flaw or omission in the perception of some authors, who only recorded the most cranial point of the intercostal space, not recording the caudal point, as is the case in *Didelphis albiventris* and *Saimiri collinsi* (8th EIC), *Nasua nasua* and *Myrmecophaga tridactyla* (10th EIC) [17, 20, 27, 30].

Despite these small disagreements, but still with a certain similarity, there is a common point of sternal attachment in all the species compared here with *Eira barbara*, which is the xiphoid process, on the sternum.

Plana et al. [31] describe the diaphragm in *Canis familiaris* (domestic dogs) as being composed of two muscular pillars that make up the lumbar region, two costal regions and a sternal region, which communicates at the xiphoid process. We found a similar arrangement in the *Eira barbara*. However, in this species, we found that the musculature of the left costal region and part of the sternal region is thicker than the right costal region. Even though we did not measure this difference, when we used a spotlight against the diaphragm of *Eira barbara*, the inequality in thickness between the two costal muscles of the diaphragm was evidente.

Although there is no record of this difference in thickness between the right and left diaphragmatic costal muscles in other species, there is an important finding in the literature, which reveals that there is greater impairment and rupture of the right costal muscle in domestic dogs and cats with diaphragmatic trauma caused by motor vehicles [32, 33–36].

This condition was also found by Mehrjerdin and colleagues [37], who carried out a retrospective study on diaphragmatic hernia in domestic cats that had been hit by cars. Of the 14 animals assessed, 11 had ruptured right costal muscles, two of the left costal muscles and one of the lumbar muscles. In this study, there was no indication of which side of the animals had been hit by the vehicles, so it was not possible to correlate the side of the trauma with the herniation, as suggested by Fossum [38].

Histologically, the diaphragm of *Eira barbara*, made up essentially of striated skeletal muscle, has short, conical muscle fibers arranged in rows perpendicular to the longitudinal axis of the fascicles and discontinuity in the architecture of the fibers, being covered by the epimysium, thus enabling muscle contraction, as described in other species of animals, both domestic and wild [3, 20, 39–41].

As an organ with continuous mobility throughout the life of the individual, it is reasonable to question how the muscle fibers of the diaphragm do not become completely fatigued over the years. According to Leme et al. [42], this may be due to the increased vascular pattern when compared to other striated skeletal muscles. In *Eira barbara*, we did not observe intense vascularization, but we believe that this evaluation requires special and appropriate investigation, comparatively using steriology, but mainly an ultrastructural analysis in terms of mitochondrial density and oxidative capacity [43].

The muscle fibers of the diaphragmatic pillars radiate and converge in a central fibrous area called the tendinous center. As described in *Nasua nasua*, *Saimiri sciureus* and the domestic dog, the tendon center is Y-shaped [20, 27, 32], unlike that described for *Didelphis albiventris*, *Procyon cancrivorus*, *Tamandua tetradactyla*, *Callithrix Jacchus*, *Callithrix geoffroyi* and the domestic cat, which have the same V-shaped tendon center [3, 17, 26, 28, 29].

The literature cited here unanimously reveals that the tendinous center is the common location of the aortic hiatus, for the passage of the aorta; thoracic duct and azygos vein; esophageal hiatus, for the passage of the esophagus and vena cava foramen, which as the name implies, allows the passage of this vessel.

Differently from *Callithrix jacchus*, *Callithrix geoffroyi*, *Procyon cancrivorus*, *Tamandua tetradactyla*, *Saimiri sciureus* and domestic dogs and cats [3, 17, 26, 29, 32] in *Eira barbara* it was possible to observe two triangular areas in the lumbar region, close to the two most dorsal portions of the tendon center, as described in *Didelphis albiventris* [28].

The Veterinary Anatomical Nomenclature [25] makes no reference to these triangular areas, nor does it provide a specific name for each portion of the tendon center. Curiously, Miglino et al. [15], Almeida et al. [18] and Helrigle et al. [28] use a different anatomical nomenclature to name these portions in *Mazama americana*, *Mazama simplicicornis* and *Blastoceros bezoarticus*, *Ovis aries* (sheep) and *Procyon cancrivorus* (crab-eating racoon), respectively, calling the triangular areas intercostal trigones and the parts of the tendon center follicles.

We believe that these names do not apply, since veterinary anatomy has its own nomenclature. When we consulted older anatomical nomenclatures [43, 44], which covered the works published at the time, they also did not refer to these structures, as mentioned by these authors.

Conclusion

In view of the comparison made with the literature consulted, we concluded that the location and positioning of the *Eira barbara* diaphragm between the thoracic and abdominal cavities suffers little variation when compared to other species of mammals.

The diaphragm of *Eira barbara* is very similar to that described in the literature for other species of wild and domestic animals, the only differences being that the right costal muscle region is slimmer than the left and and the presence of two triangular areas devoid of muscle between the costal regions and pillars.

This condition makes us think that in view of the inevitable process of urbanization, and animals bordering roads, it is possible that both the right costal region, which is thinner, and the triangular areas identified, are fragile points for herniation in cases of *Eira barbara* that are run over.

Author contributions

AMMS Writing, data curation (equal); research (equal); RPS Writing, data curation (equal); research (equal), RARR Data curation (equal); research (equal); methodology (equal); EGG Data curation (equal); research (equal); methodology (equal); ARL Supervision (equal); validation (equal); EB Conceptualization (equal), data curation (equal); research (equal); methodology (equal); writing - review and editing.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Conflict of interest

We wish to confrm that there are no conficts of interest associated with this publication and there has been no signifcant fnancial support for this work that could have infuenced its outcome. We confrm that the manuscript has been read and approved by all named authors and there are no other people who meet the criteria for authorship that are no listed. We further confrm that the order of authors listed in the manuscript has been approved by all of us.

Ethics approval and consent to participate

Animals from the collection of the Animal Morphology Research Laboratory (UFRA). No new specimens were collected for this study so no special permissions were required.

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