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Cerebellar encephalic vascularization of *Alouatta belzebul*: contributions to the comparative morphology of primates

[Vascularização encefálica do cerebelo de *Alouatta belzebul*: contribuições para a morfologia comparativa de primatas]

Tainá Kleinubing da Silva*[1], Luiza Rechi Macorin de Lima[2], Marcio Vinicius Mendoza Oliveira[2], Rafael Dutra Fiore[2], Kleber Fernando Pereira[2], Valcinir Aloisio Scalla Vulcani[3], Dayane Kelly Sabec-Pereira[1,3]

1 Faculdade Donaduzzi, Toledo, Paraná, Brazil
2 Universidade Federal do Paraná (UFPR), Toledo, Paraná, Brazil
3 Universidade Federal de Goiás (UFG), Goiânia, Goiás, Brazil
*corresponding author: kleinubingdasilva@gmail.com

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Abstract: We studied cerebellar vascularization in five specimens of the primate *Alouatta belzebul*, with the objective of describing the anatomy of the cerebellar arteries, their possible variations and comparing them with the pattern of other primates. The arterial system was injected with stained latex (Neoprene 650) through the thoracic aorta and fixed in a 10 % aqueous solution of formaldehyde. After dissection, we observed that the cerebellum of this primate species is irrigated exclusively by the vertebrobasilar system, formed by the vertebral and basilar arteries, which emit four main pairs of cerebellar arteries: caudal inferior cerebellar, rostral inferior cerebellar, anterior cerebellar, and superior cerebellar. These arteries originated from the vertebral and basilar arteries, and followed different paths to supply all portions of the cerebellum, meeting at intersecting and causing anastomoses between the different branches. The results obtained demonstrated similarities between the vascularization of *A. belzebul* and other human and non-human primates and reinforced the importance of this comparative analysis of brain vascularization between primates, as it contributes to the understanding of morphological variations and has applications in clinical, evolutionary, and conservationist studies.

Keywords: cerebellar arteries; comparative morphology; neotropical primates; vertebrobasilar system.

Resumo: Estudamos a vascularização cerebelar em cinco espécimes do primata *Alouatta belzebul* com o objetivo de descrever a anatomia das artérias cerebelares, suas possíveis variações e compará-las com o padrão de outros primatas. Os animais tiveram o sistema arterial injetado com látex corado (Neoprene 650) por meio da aorta torácica e fixado em solução aquosa de formaldeído a 10 %. Após dissecação, observou-se que o cerebelo desta espécie de primata é irrigado exclusivamente pelo sistema vértebro-basilar, formado pelas artérias vertebrais e basilar, o qual emite quatro pares principais de artérias cerebelares: artéria cerebelar inferior caudal, artéria cerebelar inferior rostral, artéria cerebelar anterior e artéria cerebelar superior. Essas artérias originaram-se das artérias vertebrais e da artéria basilar, e seguiram diferentes trajetos a fim de vascularizar todas as porções do cerebelo, encontrando-se em certos pontos e causando anastomoses entre os diferentes ramos. Os resultados obtidos demonstraram semelhanças entre a vascularização de *A. belzebul* e de demais primatas humanos e não humanos e reforçam a importância dessa análise comparativa da vascularização encefálica entre primatas, pois contribui para o entendimento de variações morfológicas e para aplicações em estudos clínicos, evolutivos e conservacionistas.

Palavras-chave: artérias cerebelares; morfologia comparativa; primatas neotropicais; sistema vértebro-basilar.

1. Introduction

Investigating the cerebellar vascular anatomy of primates is important for understanding the morphological adaptations associated with the functional evolution of the central nervous system. Due to its extensive participation in motor control, balance, and higher-order cognitive functions, the cerebellum depends on a highly specialized vascular architecture adapted to the specific metabolic demands of each species. In this context, the morphological characterization of cerebellar arteries of a Neotropical primate of relevant phylogenetic position, such as *Alouatta belzebul*, enables the identification of conserved patterns and interspecies anatomical variations, thereby contributing to the refinement of comparative neuroanatomical knowledge. In addition, unpublished data obtained from this species can elucidate aspects of encephalic vascular diversity among Platyrrhini and deepen the understanding of the relationships between morphology, function and evolution.

Since the emergence of the first primates, estimated between 30 and 50 million years ago, significant increases in encephalic complexity have been observed in this group, closely associated with the evolution of complex social behaviors, diurnality, and higher sensorimotor demands [(1)]. The increase in brain volume, particularly in the neocortex, is interpreted as an adaptive response to the cognitive demands imposed by group living and by the visual exploration of the environment.

Alouatta belzebul (Linnaeus, 1766) is a primate species belonging to the infraorder Platyrrhini (New World Primates), endemic to the Brazilian biomes of the Atlantic Forest and the Amazon Forest. This Neotropical species, commonly known as howler monkey or red-handed howler monkey, is part of the genus *Alouatta*, whose members are recognized for their characteristic vocal behavior and dietary specialization [(2)]. According to the Biodiversity Extinction Risk Assessment System - SALVE, developed by Chico Mendes Institute for Biodiversity Conservation (ICMBio) [(3)] and updated in 2024, *A. belzebul* is classified as a Vulnerable species, according to the criteria of the International Union for Conservation of Nature (IUCN) [(4)], listed in the *Red List of Threatened Species* due to habitat loss, forest fragmentation and increasing anthropogenic pressure in its distribution area. These data reinforce the importance of *A. belzebul* as a biological model for evolutionary, ecological, and conservation studies within the context of South American biodiversity.

The study of encephalic vascular architecture in non-human primates is essential for understanding the phylogenetic adaptations that shape the morphological and functional organization of the central nervous system. Cerebral vascularization, particularly of the cerebellum, represents a critical component in maintaining motor and sensory-integrative functions and is strongly influenced by evolutionary and ontogenetic processes that determine the organization and distribution of cerebral vessels [(5,6)]. Based on these references, authors such as Testut [(7)] an

Bugge [8] demonstrated that changes in brain blood supply accompany the increasing complexity of neural tissue, so that the structural evolution of the brain is intrinsically linked to the specialization of its vascular pattern.

A study by Bugge [8] highlights that the brain's morphological complexity is a key determinant in shaping vascular architecture, thereby directly influencing patterns of cerebral blood supply. Two evolutionary aspects warrant particular emphasis: the regression of the olfactory lobe, indicating a reduction in structures associated with primitive olfaction, and the addition of layers in the neocortex, reflecting enhanced cognitive capacity and sensory integration. These factors contribute to a redistribution of blood flow, which needs to accompany the functional reorganization of the brain. The morphological variation of approximately 20 characteristics of the vessels forming the arterial circle at the base of the brain has been extensively investigated across different mammalian species, encompassing the frequency of occurrence of this circuit, the direction of blood flow in specific functional regions, and the relationship between hemodynamic pressure and vascular diameter. In addition, the cerebral arterial circuit has been functionally subdivided into two main systems: the vertebrobasilar circuit and the carotid circuit, supporting the classifications adopted by De Vriese [6] and Testut [7] based on the architectural model originally proposed by Tandler [5].

In this context, modern anatomical and comparative studies have reinforced the relevance of these divisions by demonstrating that the organization of cerebral vasculature in primates varies in accordance with the metabolic and morphofunctional demands of different encephalic structures. In *Alouatta belzebul*, Sabec-Pereira et al. [9] described the anatomy of the cerebral arterial circle formed by the vertebrobasilar and carotid systems, with anastomoses that establish a closed irrigation pattern. Particular features, such as the presence of a double basilar artery (arterial island), the absence of the rostral communicating artery, and the segmental organization of the caudal cerebral artery, reveal a vascular arrangement with distinctive characteristics, although still preserving morphological features conserved among New and Old World primates.

Regarding cerebellar irrigation, it is ensured by branches of the vertebrobasilar system, notably the caudal inferior, rostral inferior, and superior cerebellar arteries. The superior cerebellar artery, a terminal branch of the basilar artery, emerges near the pontomesencephalic sulcus, runs transversely along the base of the midbrain, and is widely distributed along the superior surface of the cerebellum [10]. In *Sapajus libidinosus*, a neotropical primate phylogenetically close to *Alouatta belzebul*, this artery has been described as exhibiting a constant morphological pattern, with symmetrical origin in 84.21 % of cases and branching into four main arrangements: mesencephalic, lateroanterior, lateral, and medial. These branches supply the midbrain, the quadrangular lobe, the rostral semilunar lobe and the cerebellar vermis with frequent anastomoses involving the inferior cerebellar and pontine arteries. The presence of satellite superior cerebellar arteries, accompanying the main branches and contributing to both superficial and deep cerebellar vascularization, was also observed, indicating a complex and functionally integrated vascular pattern [11]. These findings highlight the importance of the superior cerebellar artery as a key component in primate brain irrigation and justify its detailed analysis in understudied species such as *Alouatta belzebul*.

The detailed analysis of in *A. belzebul* encephalic vascularization not only broadens the understanding of comparative primate morphology but also contributes to elucidating the evolutionary mechanisms of neurovascularization and its potencial clinical and conservation applications, particularly in experimental models of neurovascular disorders. Therefore, the present study aimed to describe the macroscopic anatomy of cerebellar vascularization in *Alouatta belzebul*, identifying the main cerebellar arteries and comparing them with vascular patterns observed in other primates, both human and non-human.

2. Material and methods

This study was approved by the Ethics Committee on the Use of Animals at the Federal University of Goiás (CEUA-UFG), under the protocol nº 083/17. The specimens were provided by the Project for the Rescue and Scientific Use of Fauna (PSACF) from Belo Monte Hydroelectric Power Plant, as documented in the official authorization letters nº 002/2015 – ARC/NAT, nº 009/2015 – ARC/NAT and nº 012/2015 – ARC/NAT, attached to the IBAMA process nº 02001.001848/2006-75.

For the anatomical observation of the cerebellum, five (5) specimens were dissected: four adult males weighing between 6.5 e 9.0 kg and an adult female weighing 4.5 kg. Initially, a median sagittal incision was made in the cephalic region, followed by reflection of the skin and temporal musculature. Using a band saw and osteotome, a rectangular opening was created in the cranial vault, encompassing the frontal, parietal, temporal, and occipital bones. After longitudinal sectioning of the encephalic meninges, the cerebellum was exposed while still in a fresh state. Subsequently, the brains were fixed by immersion in a 20 % aqueous formaldehyde solution. Photographic documentation was performed using a Sony α200 digital camera (10.2 megapixels). The data obtained were compared with anatomical descriptions of other primate species and, when relevant, with those of other mammals.

To perform the anatomical angiotechnical technique, Neoprene 650 latex pigmented with red dye was injected via the thoracic aorta as the access route. The specimens were then fixed in a 10 % aqueous formaldehyde solution through intramuscular, subcutaneous, and intracavitary injections, thereby complementing the fixation achieved through the vascular system. The specimens remained immersed in this solution for at least 72 hours. After this period, the brains were removed with the aid of an oscillating saw (Dremel® 3000) and then re-immersed in 10 % formaldehyde solution for an additional 72 hours. Subsequent descriptive analyses were documented photographically. All anatomical terminology used follows the Nomina Anatomica Veterinaria [12].

3. Results and discussion

The analysis of the cerebral vascularization of *Alouatta belzebul* allowed detailed identification of the arterial pattern responsible for cerebellar irrigation, with emphasis on the origin, course, and possible variations of the cerebellar arteries. The arterial supply to the cerebellum was observed to be provided by the vertebrobasilar system, consisting of right and left vertebral arteries, which unite at the bulbopontine junction to form the basilar artery (Figure 1).

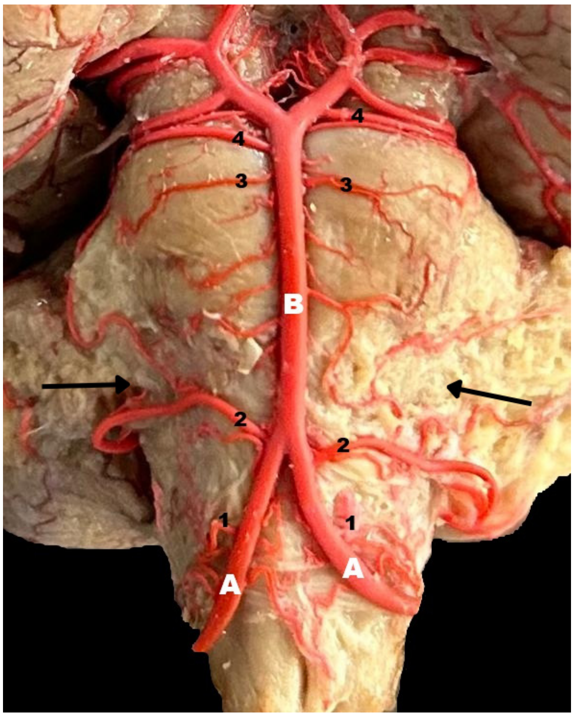

Figure 1. Ventral view of the vertebrobasilar system of *A. belzebul*, showing the vertebral **(A)** and basilar **(B)** arteries, and the origins of the cerebellar arteries: CICA (1), RICA (2), ACA (3) e SCA (4).

This system is responsible not only for the irrigation of the cerebellum but also for other brain structures, including the spinal cord, medulla oblongata, pons, midbrain, diencephalon, and the occipital and temporal lobes, corroborating the findings of Bugge [8] and Tandler [5].

In the analyzed specimens, four main pairs of cerebellar arteries were identified: the caudal inferior cerebellar artery (CICA), the rostral inferior cerebellar artery (RICA), the anterior cerebellar artery (ACA), and the superior cerebellar artery (SCA), as illustrated in Figure 1. These arteries, observed on the ventral surface of the bulbopontine junction, branch extensively to supply all regions of the cerebellum.

The caudal inferior cerebellar artery (CICA) arises from the distal portion of the vertebral artery on both the right and left sides. After originating, it follows a descending course, posteriorly along the inferior surface of the cerebellum, through the lateral medulla oblongata, to the biventral lobule, which is located on the inferior surface of the cerebellum, within the lateral hemispheres (Figure 1). From the biventral lobule, the artery courses toward the cerebellar tonsil within the intermediate hemisphere and then gives rise to small branches that supply the remaining inferior portion of the cerebellum. This finding differs from those reported by Silva e Ferreira [13] in specimens of *S. libidinosus*, who described the origin of the CICA in the rostral portion of the vertebral artery, near the junction forming the basilar artery, or arising from the initial segment of the basilar artery, as observed in *M. fascicularis* by Tsuji et al. [14]. In contrast, Lake et al. [15], identified in *P. ursinus*, *C. pygerythrus* e *G. senegalensis* a single cerebellar artery, termed the common inferior cerebellar artery, originating from the basilar artery and subsequently branching into the CICA and RICA. According to the literature, the species whose arterial origin and course are most similar to those observed in *A. belzebul* is *Homo sapiens* [16].

The rostral inferior cerebellar artery (RICA) also originates from the vertebral arteries near their junction, where the basilar artery is formed. The right RICA branch follows an ascending course from its origin, whereas the left branch follows a descending course. Although it contributes to the vascularization of the medulla oblongata and pons, this artery also gives rise to branches that supply the middle cerebellar peduncle and the lateral cerebellar hemispheres [16]. The CICA and RICA predominantly supply the inferior and lateral surfaces of the cerebellar hemispheres, the inferior vermis, and the cerebellar tonsil. Both arteries give rise to ascending branches that connect with the SCA, suggesting functional anastomoses (Figure 2). Notably, one branch of the RICA follows an upward course until it meets the SCA, forming an anastomosis. It was also observed that the RICA has a larger caliber than the CICA, indicating a more significant contribution to the vascularization of this region. This differs from the observations of Silva e Ferreira [13] in *S. libidinosus* and Tsuji *et al* [14] in *M. fascicularis*, where RICA originates from the basilar artery and is smaller in caliber than CICA, once again showing greater similarity to the pattern seen in the genus *Homo*.

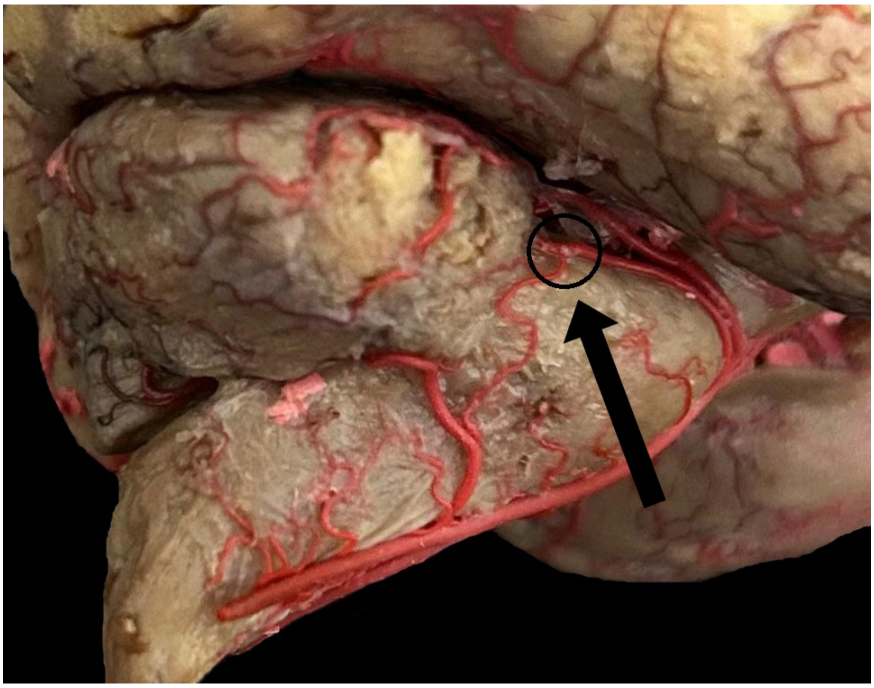

Figure 2. Right lateral view of the cerebellum of *A. belzebul*, showing the anastomosis between a branch of the rostral inferior cerebellar artery (RICA) and the superior cerebellar artery (SCA).

The anterior cerebellar artery (ACA) follows a linear course from its origin in the basilar artery and is the smallest cerebellar artery observed in *A. belzebul*. Regarding its vascularization, this artery contributes alongside the SCA in supplying the superior portion of the cerebellum, including the superior and lateral regions of the hemispheres. As a result of this collaboration, multiple anastomoses are present between the two arteries (Figure 3). This finding is similar to that reported in *S. libidinosus* in terms of origin and caliber. However, in that species, the ACA contributes to the vascularization of the inferior portion of the cerebellum, together with CICA and RICA [13].

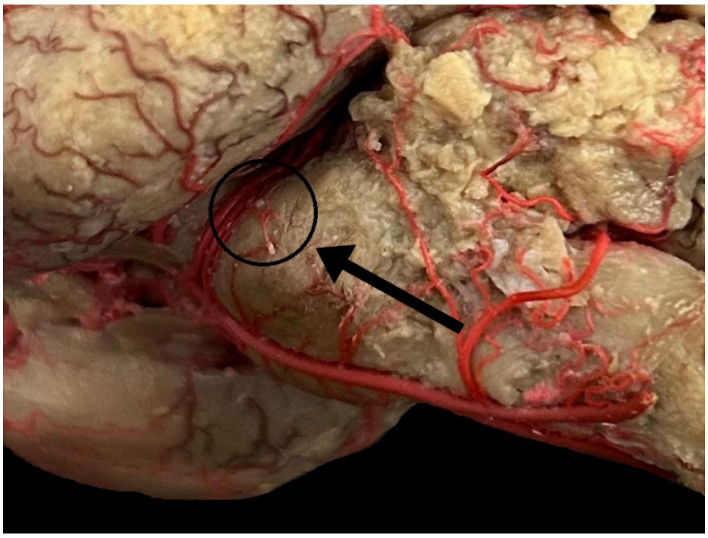

Figure 3. Left lateral view of the cerebellum of *A. belzebul*, showing the anastomosis between a branch of the anterior cerebellar artery (ACA) and the superior cerebellar artery (SCA).

The superior cerebellar artery (SCA), in turn, has a dual origin: one branch arises from the basilar artery, and the other from the posterior communicating artery. From this point, it follows a linear course and gives rise to branches that supply the superior and lateral surfaces of the cerebellar hemispheres, the lobules contained within them, the superior cerebellar peduncle, and the superior vermis. The origin and course of the SCA are consistent with those observed in other Neotropical primates, such as *Sapajus libidinosus*, and in Old World primates, including *Macaca fascicularis, Papio ursinus*, *Chlorocebus pygerythrus*, *Galago senegalensis* and *Homo sapiens* (14, 15, 16, 17).

From a phylogenetic perspective, the cerebellar arterial pattern observed in *A. belzebul* is more similar to that of *Homo sapiens* than to genera such as *Sapajus* and *Macaca*, supporting analyses of encephalization and neuroanatomical development in primates. Neuroevolutionary studies indicate that the regression of the olfactory bulb and the expansion of the neocortex in higher primates lead to structural and functional changes in cerebral vascular patterns (5,8), a phenomenon also observed in this study, as evidenced by the complexity and extensive distribution of cerebellar branches in *A. belzebul*. Table 1 summarizes the major arteries responsible for cerebellar vascularization in both Old World and New World primates.

Table 1. Comparative data on the cerebellar arterial pattern in different primates

	Taxon	Common Name	Vertebral Artery	Basilar Artery	Posterior/ Caudal Inferior	Anterior/ Rostral Inferior	Anterior Cerebellar Artery	Superior Cerebellar Artery	Common Inferior Cerebellar Artery
New World	*Alouatta belzebul*	Howler Monkey	X	X	X	X	X	X	Absent
New World	*Sapajus libidinosus*	Capuchin Monkey	X	X	X	X	X	X	Absent
Old World	*Macaca fascicularis*	Cynomolgus Monkey	X	X	X	X	Absent	X	Absent
Old World	*Papio ursinus*	Baboon	X	X	Absent	Absent	Absent	X	X
Old World	*Chlorocebus pygerythrus*	Vervet Monkey	X	X	Absent	Absent	Absent	X	X
Old World	*Galago*	Senegal Bushbaby	X	X	Absent	Absent	Absent	X	X
Old World	*Homo sapiens*	Human	X	X	X	X	Absent	X	Absent

Adapted from: Sabec-Pereira et al. [9].

4. Conclusion

The cerebellar vascularization of Alouatta belzebul exhibits a predominantly vertebrobasilar pattern, characterized by four main pairs of cerebellar arteries. Similarities to the vascular pattern observed in humans, together with the presence of anastomoses and potential anatomical variations, indicate a functionally adapted system with significant phylogenetic relevance. Such findings contribute not only to the anatomical knowledge of the species but also provide support for comparative, conservation, and biomedical studies. In addition to the anatomical and evolutionary implications, the data presented here provide strong support for the use of Neotropical primates as experimental models in neurovascular research. Detailed knowledge of arterial anatomy in species phylogenetically close to humans is fundamental for studying pathologies such as strokes, aneurysms, and arteriovenous malformations, as well as for planning experimental surgical and therapeutic interventions. Moreover, the standardization of anatomical knowledge in endangered species such as *A. belzebul* reinforces their value as biological heritage and promotes actions aimed at the conservation and ethical management of these animals.

Conflict of interest statement

The authors declare no conflicts of interest.

Data availability statement

The data will be provided upon request to the corresponding author.

Author contributions

Conceptualization: Silva, T. K., Sabec-Pereira, D. K.; Investigation: Silva, T. K., Lima, L. R. M., Oliveira, M. V. M., Fiore, R. D., Pereira, K. F.; Methodology: Silva, T. K., Pereira, K. F., Sabec-Pereira, D. K.; Project administration: Sabec-Pereira, D. K.; Resources: Pereira, K. F., Vulcani, V. A. S.; Supervision: Pereira, K. F., Sabec-Pereira, D. K.; Validation: Sabec-Pereira, D. K.; Writing – original draft: Silva, T. K.; Writing – review and editing: Pereira, K. F., Vulcani, V. A. S., Sabec-Pereira, D. K.

Generative AI use statement

During the preparation of this manuscript, the authors used the ChatGPT tool to assist in the development of the illustration of cerebellar vascularization included in the Graphical Abstract. The authors subsequently reviewed and edited the content and take full responsibility for the accuracy and integrity of the publication.

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