OSTEOHISTOLOGICAL ANALYSIS AND PRESERVATION STAGE OF
COENDOU MAGNUS LUND, 1839 (RODENTIA, ERETHIZONTIDAE)
FOSSIL RECOVERED AT TOCA DA BARRIGUDA
CAVE, NORTHEASTERN BRAZIL

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ABSTRACT – Studies on taxonomy, taphonomy, and paleoecology have been conducted on fossils from carbonate caves, however, surveys detailing the fossilization processes and bone microstructure of specimens from caves are still scarce. Our paper provides an osteohistological analysis and X-ray diffraction of a fossil rib from the extinct South American porcupine Coendou magnus Lund. Our results allowed us to interpret that there was microstructural preservation and low replacement of the organic part of the bone by minerals present in the surrounding sediments, such as apatite and abundant non-silicate minerals in sedimentary rocks, characterizing preservation in an environment with poor weathering.

Keywords: cave, X-ray diffraction, late Pleistocene, histology, porcupine.

INTRODUCTION

Pleistocene vertebrate fossils are commonly found in different types of fossiliferous deposits in northeastern Brazil. Amid these deposits, a large number of fossils found in carbonate caves in the Chapada Diamantina region, State of Bahia, Brazil, have drawn considerable attention (Lessa et al., 1998; Castro et al., 2014; Dantas et al., 2019, 2020; Silva et al., 2019; Eltink et al., 2020). Many of these fossils are representative of extant mammals, such as Tamandua tetradactyla Linnaeus, 1758, Panthera onca Linnaeus, 1758, Leopardus pardalis (Linnaeus, 1758), and Dicotyles tajacu Linnaeus, 1758 (Eltink et al., 2020; Alves-Silva et al., 2023), whereas others are from extinct animals, such as giant sloths Catonyx cuvieri (Lund, 1839), Nothrotherium maquinense (Lund, 1839), and Eremotherium laurillardi (Lund, 1839), and Eremotherium laurillardi Lund, 1839 (Vasconcelos et al., 2016; Dantas et al., 2019).

Recently, several studies have been conducted on fossils from carbonate caves, particularly regarding taphonomy and paleoecology (Berbert-Born & Karmann, 2002; Dantas et al.,
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This work presents a detailed microstructural description of a *Coendou magnus* rib fossil found in the Toca da Barriguda Cave (BA-250; 10º08’26” S, 40º51’08” W), located in the Municipality of Campo Formoso, Bahia, Brazil (Figure 1). This cave is situated in the semi-arid northeast region, a typical Caatinga environment comprising characteristic vegetation and great biodiversity. The Toca da Barriguda Cave has about 19.5 km of development and is the second-largest cave in Brazil. Together with nearby caves, such as Toca da Boa Vista, Toca do Calor de Cima, Toca do Pitú, and Toca do Morrinho, they form a geological complex of great importance for scientific studies (Auler & Smart, 2002).

*Coendou magnus* Lund, 1839 is an extinct species of the Erethizontidae family, known to be formed by South American porcupines, despite presenting systematic affinities with living species, which are still discussed today (Alves-Silva et al., 2023 and references therein). Erethizontidae have well-defined characteristics, mainly in terms of their nocturnal and arboreal habits, and feed mostly on fruits and seeds (Emmons, 1997). The identification was based on the nasal bones (Figure 2A), which were shorter and larger and more similar to *Coendou* than those of *Erethizon*, on the length of the cheek-teeth row, which was longer than that presented in extant species, and in the tetralophodont p4 (Figures 2B–C), which occurs only in *C. magnus* (Vezzosi & Kerber, 2018). The individual was dated at 33,171–33,765 cal yr BP (UGAMS 33269; \(^{14}C_{\text{biopatite}} = 23,890 \pm 70\) yr; converted to \(^{14}C_{\text{collagen}} = 28,990 \pm 70\) yr; Alves-Silva et al., 2023).

**MATERIAL AND METHODS**

**Study area and geology**

The fossil material was found in Toca da Barriguda Cave (BA-250; 10º08’26” S, 40º51’08” W), located in the Municipality of Campo Formoso, Bahia, Brazil (Figure 1). The fossil material was found in the Toca da Barriguda Cave (Campo Formoso, State of Bahia), mentioning data on osteohistology, and presenting aspects such as ontogenetic stages and developmental rhythms, growth patterns, and rates, as well as fossil-diagenetic processes identified by X-ray diffraction (XRD).

**Study material**

The analyzed material is a rib, part of the scientific collection of the Laboratório de Ecologia & Geociências (LEG) of the Universidade Federal da Bahia, under the number LEG 790. Alves-Silva et al. (2023) described the nasal bones (LEG 790), left premaxilla (LEG 817), upper molars (LEG 813 to LEG 815), dentary (LEG 816), and several postcranial materials attributed to *Coendou magnus*. The identification was based on the nasal bones (Figure 2A), which were shorter and larger and more similar to those of *Coendou* than those of *Erethizon*, on the length of the cheek-teeth row, which was longer than that presented in extant species, and in the tetralophodont p4 (Figures 2B–C), which occurs only in *C. magnus* (Vezzosi & Kerber, 2018). The individual was dated at 33,171–33,765 cal yr BP (UGAMS 33269; \(^{14}C_{\text{biopatite}} = 23,890 \pm 70\) yr; converted to \(^{14}C_{\text{collagen}} = 28,990 \pm 70\) yr; Alves-Silva et al., 2023).

**Figure 1.** Map of the Toca da Barriguda Cave map showing the entrance and the Caatinga saloon, where the fossils were found.
This is a partial skeleton from an adult specimen, composed of 103 bones (45% of the skeleton), and was found unbound above the ground near each other. No duplicated elements were found, and all bones were well preserved without weathering or trampling marks. A few bones exhibited breakage and abrasion marks. This individual probably died in the cave, and the bones were slightly transported to where it was found.

Osteohistological analysis of *Coendou magnus* was performed using a single-rib sample LEG 790. Rib samples are more numerous and morphologically less diagnostic than long bones, such as humeri and femurs. This facilitates the assignment of these bones by curators of the scientific collection. Ribs have the potential to preserve much more complete growth record than long bones as they grow from proximal to distal, which causes observable changes when analyzing the cross-section during the different stages of the ontogeny of the individual (Waskow & Martin-Sander, 2014).

**Osteohistology**

Bone microstructure analysis followed the literature, with most studies referring to histological slides of pterosaur and dinosaur bones (e.g., Sayão, 2003). Few studies have investigated the osteohistology of Pleistocene mammals (e.g., Costa et al., 2012; Mayer et al., 2020; Sousa et al., 2020).

The rib was mounted in acrylic resin, cut, and passed through the first polishing step on one side of the sample, which was then glued to a glass slide. Subsequently, it was glued to a histological slide and thinned once more using sandpaper of different grain sizes, always starting with coarser sandpaper of lower numbering and replacing it with increasingly finer sandpaper of higher numbering. This practice aims to obtain a smooth and polished surface without imperfections, such as deep scratches, which may hinder the visualization of bone microstructures, as observed under an optical microscope.

**X-Ray Diffractometry**

X-ray diffraction analysis was performed using the methodology for obtaining the powder from the rib material, with Bruker D2 PHASER equipment operating at a voltage of 30 kV and a current of 10 mA (P = 300 W), Cu-Kα radiation = 1.5184 Å, and using the Bruker-AXS Lynxeye detector. The scanning range (2θ) was from 4° to 80°, with a goniometer step of 0.02019°, count time per step of 1.0 s, and a primary slit with an opening of 0.4 mm. A 3 mm shield and a sample constant rotation of 10 rpm were used. We used the HighScore Plus software to identify the elements and possible secondary phase formation based on XRD to identify a single phase in the analyzed materials.

**RESULTS AND DISCUSSION**

**Bone histology**

From the microstructural evaluation of the analyzed bone elements, it was possible to observe the primary and secondary osteons, cementum, Havers, and Volkmann canals (Figure 3). There is no evidence of replacement and incrustation of the medullary cavity or radial fractures, which indicates diagenetic processes of crystallization and recrystallization within the bone matrix. Chinsamy & Raath (1992) observed that preparing the histological slides resulted in a slight change in the bone morphology (Figure 3). The compact bone portion, both endosteal and periosteal, is well preserved, with no volumetric or morphological alterations.

Analysis of the endosteal cortex portion allows the identification of a bone matrix formed by the fibrolamellar bone, composed of fibrous tissue, with rounded gaps and osteocytes randomly positioned in the endosteal region. The presence of fibrolamellar bone forming the cortical matrix indicates that the specimen had high growth rates, characterized by a vascularized network in the bone matrix.

Another feature that defines this tissue is the disorganization of fibrous tissue bundles intercepted by primary osteons in various directions (Chinsamy, 2005). The disorganization of the vascular bundles of the fibrous tissue reflects the rapid deposition rates of the fibrous bone tissue, indicating that during the first stage of this specimen’s life, the bone tissue was deposited more quickly, a fact that can be visualized by the presence of this pattern in the medial portion of the cortex (Huttenlocker et al., 2013).
The simple vascular channels and primary osteons found in the endosteal portion of the cortex illustrate a clear vascularization of the longitudinal type, in which the irrigation of the bone tissue was limited because of the reduced number of vascular channels along the cortex. From the mid-periosteal portion of the cortex, the fibrolamellar bone is formed by parallel fibrous tissue and elliptical osteocyte gaps with marked organization (Huttenlocker et al., 2013).

Vascular channels and primary osteons are not present in the parallel-fibrous tissue; however, the formation of Lines of Arrested Growth (LAGs), which extend throughout the periosteal region, is visible in this second phase of bone

Figure 3. Osteohistology of *Coendou magnus* rib LEG 790. A, transversal section showing the fibrolamellar bone; B, simple and double Lines of Arrested Growth (LAGs); C, periostal portion showing fibrous parallel tissue; D, endosteal portion formed by fibrous bone tissue. No scale.
deposition. The reconstitution of this specimen’s development indicates the transition from a fibrolamellar bone with fibrous tissue to a bone composed of parallel fibrous tissue with less accelerated deposition. This tissue, with closely compacted collagen fibers arranged in parallel and in the same direction, requires a longer time for deposition, reflecting periods of slow bone growth (Francillon-Vieillot et al., 1990).

In addition to the bone apposition suffering a decline in rhythm with the transition of deposited tissues, a reduction in the number of vascular channels and their dimensions occurred during the second half of the animal’s life. Only a few simple channels were found in the parallel-fibrous tissue, indicating an intense decrease in vascularization in this bone tissue.

Among the parallel-fibrous tissues, five growth pause lines, three simple LAGs, and two double LAGs were formed, characterizing a specimen that was probably going through its sixth or seventh growth cycle at the time of death. Animal studies have shown that zones that represent periods of continuous growth are deposited during favorable growth seasons, whereas LAGs, which represent periods of total growth arrest, occur seasonally during unfavorable development periods throughout the vertebrate’s life (Chinsamy, 2005; Padian & Lamm, 2013).

Based on this premise, a sharp decline in growth rate was identified in this specimen because of the accentuated amount of growth pauses, represented by LAGs in the periosteal portion. Furthermore, the quantity and proximity between these interruptions in bone development (LAGs) in the midst of avascular tissue indicate a deposition rhythm common to individuals in adulthood who are approaching the end of asymptotic growth (Huttenlocker et al., 2013).

The description of the fibrolamellar bone identified here proves the interlinked pattern of two types of tissues that form this bone, demonstrating different growth strategies throughout the animal’s life: (i) in the endosteal portion of the cortex, the fibrous bone tissue, representing the individual’s initial growth in a faster way, while juveniles; (ii) from the medial portion of the cortex, deposition of parallel-fibrous tissue, representing growth deceleration with the arrival of sexual maturity, and indicating a slower deposition pattern, with several moments of growth pauses during adulthood.

X-ray diffractometry

X-ray diffraction data analysis (Figure 4) demonstrated the presence of calcium silicate and the absence of calcite. The enrichment of chemical elements in fossils is related to the weathering of the surrounding rocks (Corecco et al., 2021). The data suggest that the Caating saloon, where the fossils were preserved, had low physical weathering (Benhresmeyer, 1978) due to it being ~700 m distant from the cave entrance and chemical weathering (Pokines et al., 2018), probably due to the low humidity rate in this saloon. The geochemical changes were relatively small compared to the environment outside the cave.

The appearance of some apatite was expected, as it is one of the most abundant non-silicate minerals on Earth. In the sample, there was probably crystallization of fluorapatite from the combination with existing structures such as hydroxyapatite. These combinations are common, as fluorapatite is an accessory element in rocks rich in calcium or may even have a diagenetic origin in sedimentary rocks (Reichel et al., 2005).

X-ray diffraction analysis revealed that the sample contained calcium silicate (CaSiO₃). The presence of silicate elements in fossils is expected, given that silicate elements constitute a large mass of terrestrial rocks (Dana, 1981). This class of mineral functions combines several common elements, such as calcium and sodium, from various types of existing silicates. These substances are a type of durable mineral that helps preserve skeletal remains after an individual’s death (Pickrell, 2018).

Furthermore, calcite (CaCO₃) was absent in the DR-X analysis. When found in the rock matrix, calcite is the most common mineral for filling bone cavities (Wings, 2004), facilitating fossilization. Its absence from the sample suggests that, despite its age (33,171-33,765 cal years BP; Alves-Silva et al., 2023), the skeleton has undergone little replacement of its original organic matter. The presence of calcite can lead to bone deformation caused by the growth of calcite crystals, which destroy the microscopic structures of the fossilized specimens (Holz & Schultz, 1998). The lack of this in the Coendou magnus rib sample suggests that the osteohistological aspects were well preserved over time.

**FINAL REMARKS**

The analyses demonstrated good preservation of the osteohistological microstructure in the Coendou magnus, although this individual lived at ~33 cal kyr BP. Both osteohistological analysis and X-ray diffraction showed low replacement of the organic parts of the bone by minerals.
present in the surrounding environment inside the cave. Furthermore, the data obtained by X-ray diffractometry indicated that the fossilization environment to which C. magnus was submitted was poor in geochemical exchange owing to its low weathering. However, further analyses are required. Therefore, it was possible to identify that the C. magnus specimen endured its adult stage at the time of its death and that it was already close to the end of asymptotic growth after five growth cycles.

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