

Could *Eremotherium laurillardi* (Lund, 1842) (Megatheriidae, Xenarthra) be an omnivore species?

Poderia Eremotherium laurillardi (Lund, 1842) (Megatheriidae, Xenarthra) ser uma espécie onívora?

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Resumo

Apresentamos valores isotópicos de carbono ($\delta^{13}\text{C}_{\text{collagen}} = -10.1 \text{ ‰}$) e nitrogênio ($\delta^{15}\text{N} = 6.8 \text{ ‰}$) para *Eremotherium laurillardi* (Megatheriidae, Xenarthra) do Pleistoceno final de Brejo da Madre de Deus, Pernambuco (Brasil) e discutimos a possibilidade de uma dieta onívora para esta espécie. Apesar de pontual, nosso dado reforça a inferência de uma dieta herbívora para esta espécie.

Palavras-chave: Quaternário; Isótopos de carbono e nitrogênio; Megatheriidae

Abstract

We present carbon ($\delta^{13}\text{C}_{\text{collagen}} = -10.1 \text{ ‰}$) and nitrogen ($\delta^{15}\text{N} = 6.8 \text{ ‰}$) stable isotopes data for *Eremotherium laurillardi* (Megatheriidae, Xenarthra) from the late Pleistocene of Brejo da Madre de Deus, Pernambuco (Brazil) and discuss the possibility of an omnivore diet for the species. Our data, although punctual, reinforce the inferences that *E. laurillardi* was a herbivore species.

Keywords: Quaternary; Carbon and nitrogen isotopes; Megatheriidae

1 Introduction

Based on carbon ($\delta^{13}\text{C}_{\text{bioapatite}}$) and oxygen ($\delta^{18}\text{O}$) stable isotope data, and stereomicrowear analysis of fossils from the late Pleistocene of Brazilian Intertropical Region - BIR, *Eremotherium laurillardi* (Lund, 1842) is recognized as a generalist species, with a wide niche breadth ($B_A = 0.77 \pm 0.25$), being able to live in several habitats, since low-density forests to open savanna habitats, feeding on fruits, leaves and C_4 grasses (Dantas *et al.*, 2017, 2020; Oliveira *et al.*, 2020).

Megatherium americanum Cuvier, 1796 is also considered a herbivore species (e.g. Bocherens *et al.* (2016; 2017). Nevertheless, Fariña (1996) suggested that

M. americanum could be an omnivore species, and as *E. laurillardi* is a sister taxon (Gaudin, 2004), this doubt could be extended to it as well.

Bocherens *et al.* (2016; 2017) presented carbon ($\delta^{13}\text{C}_{\text{collagen}}$) and nitrogen isotopic data for *M. americanum*, which suggest that it indeed was a herbivore species, however, in fact, the combined use of both carbon and nitrogen isotopes that had never been analyzed for *E. laurillardi* ground sloth.

Thus, the main objective of this study is present carbon ($\delta^{13}\text{C}_{\text{collagen}}$) and nitrogen ($\delta^{15}\text{N}$) isotopic data for an adult individual of *E. laurillardi* from Brejo da Madre de Deus, Pernambuco and discuss if it had a herbivore or an omnivore diet.

2 Materials and Methods

2.1 Isotopic Analysis

The sample 6219, a tooth (dentine) from an adult individual of *Eremotherium laurillardii* from Brejo da Madre de Deus, Pernambuco was used to isotopic analysis. The stable isotope analyzes were performed at NEG-LABISE in “Centro de Tecnologia e Geociências” of Universidade Federal de Pernambuco (Recife, Pernambuco, Brazil).

For the analysis of $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{15}\text{N}$, the samples were subjected to pre-treatment for collagen extraction. The pulverized samples were demineralized through the reaction with hydrochloric acid (HCl) at a concentration of 1M for 20 minutes at room temperature. The residue was washed with deionized water and centrifuged five times until the acid residue was completely removed. Then, the residue was treated with NaOH at a concentration of 0.125M for twenty hours at room temperature, to eliminate other organic residues and humic acids, and posteriorly, the sample was washed with deionized water and centrifuged five times until they were neutralized (Bocherens *et al.*, 1994; Koch & Fogel, 1997; Sealy *et al.*, 2014).

After pre-treatment, approximately 700 μg of each sample was weighed and packed in specific capsules for analysis of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}_{\text{organic}}$ in COSTECH (elemental combustion furnace) coupled to a Delta V Advantage mass spectrometer.

The reliability of the isotopic signatures of the collagen extracts was addressed using their chemical composition. Only extracts with %C, %N, and C/N similar to those of collagen extracted from fresh bone should be considered reliable for isotopic measurements. Several studies have shown that collagen with atomic C/N ratios lower than 2.9 or higher than 3.6 are altered or contaminated, and should be discarded (DeNiro, 1985; Ambrose, 1990).

All results are reported using delta notation, $\delta = [(R_{\text{sample}}/R_{\text{standard}} - 1) * 1000]$ (Coplen, 1994). The reference for carbon ($R = 13\text{C}/12\text{C}$) isotope values are in V-PDB, while for nitrogen ($R = 15\text{N}/14\text{N}$) are in atmospheric nitrogen (AIR).

2.2 Isotopic Diet Interpretation Using $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ Values

Nitrogen isotopes could help us to differentiate between grazers and browsers, high values of nitrogen are indicative of a grazer diet, while lower values are indicative of a browser diet (Bocherens & Drucker, 2013).

The interpretation of carbon isotopic values for medium-to large-bodied herbivorous mammals is generally made based on the known average for C_3 plants ($\mu\delta^{13}\text{C} = -27 \pm 3 \text{‰}$), C_4 plants ($\mu\delta^{13}\text{C} = -13 \pm 2 \text{‰}$) and CAM plants (intermediate values between $\delta^{13}\text{C}$, C_3 and C_4 plants). C_3 photosynthetic pathways include arbustive and/or arboreal plants, as also grasses that occur more in high altitudes (>3,000 m) and high latitudes (>35°S) (MacFadden, 2005 and references therein). C_4 photosynthetic pathways in the intertropical region include mainly grasses and other herbaceous plants (e.g. Dantas *et al.*, 2017).

We use the typical isotopic enrichment of collagen in mammal tissues of +5 ‰ (Bocherens & Drucker, 2013). We adapted the expected $\delta^{13}\text{C}$ values in different habitats of South America from bioapatite (Domingo *et al.*, 2012) to collagen. Thus, values between -31 ‰ to -25 ‰ represents closed-canopy forest; -25 ‰ to -20 ‰ represents low-density forest; -20 ‰ to -17 ‰, arboreal savanna; -17 ‰ to -12 ‰, arboreal to open savanna; and, -12 ‰ to -4 ‰, open savanna (Figure 1).

Food resources proportion of *E. laurillardii* could be suggested using a single isotope mathematical mixing model (Phillips, 2012) for food types: C_3 and C_4 plants (-22 ‰ and -8 ‰, respectively), to be applied in equations (1) in Excel (Microsoft Corporation, Redmond, Washington) through Solver supplement (presuming non-negative values):

$$\begin{aligned} \delta^{13}\text{C}_{\text{mix}} &= \delta^{13}\text{C}_1 f_1 + \delta^{13}\text{C}_2 f_2 \\ 1 &= f_1 + f_2 \end{aligned} \quad (01)$$

2.3 Ecological Measurements

To estimate ecological measurements, we calculate isotope niche breadth (B) using Levins (1968) measure (2), where p_i is the relative proportion of individuals in isotope bin i . This measure was then standardized (B_A) from 0 to 1 following equation (3), where n is total number of isotope bins available. Values lower or equal to 0.5 suggests a specialist, and above 0.5, a generalist.

$$B = \frac{1}{\sum p_i^2} \quad (02)$$

$$B_A = \frac{B - 1}{N - 1} \quad (03)$$

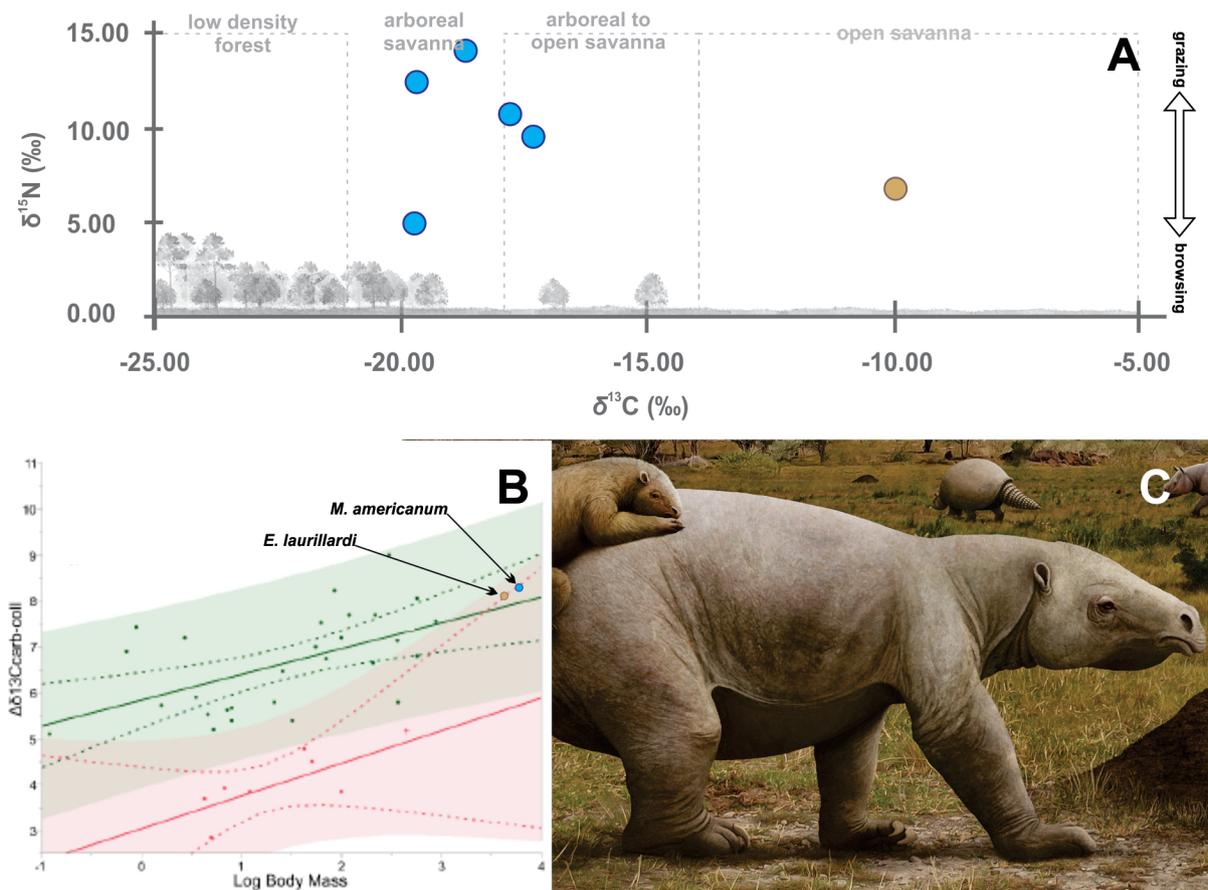


Figure 1 A. Carbon ($\delta^{13}\text{C}_{\text{collagen}}$) and Nitrogen ($\delta^{15}\text{N}$) isotopic values for *Megatherium americanum* (blue dots) and *Eremotherium laurillardii* (brown dot); B. Regressions of log body mass (modified from Fariña & Varela, 2018) and $\Delta\delta^{13}\text{C}_{\text{collagen}}$ for *M. americanum* (blue dot) and *E. laurillardii* (brown dot), Carnivores (light symbols), herbivores (dark symbols). Regression lines (solid) include confidence intervals (dotted) and prediction intervals (shaded area). C. *E. laurillardii* reconstitution (Image: Julio Lacerda, 2020).

3 Results and Discussion

The *Eremotherium laurillardii* (sample 6219) from Brejo da Madre de Deus, Pernambuco presented a C/N ratio of 3.2 and 3.4 %N, allowing us to suggest good preservation of collagen in organic carbon as mentioned in previous studies (e.g., DeNiro, 1985; Ambrose, 1990; Bocherens *et al.*, 1994; 2016).

The carbon isotopic value ($\delta^{13}\text{C}_{\text{collagen}}$) was -10.1 ‰ (Table 1), which represents a diet composed of 86 % of C_4 grasses, being a specialist adult individual ($B_A = 0.31$) that possibly could have lived in open savanna habitats (Figure 1A and C). Isotopic data in collagen from BIR fossils are rare, due to diagenetic process which promoted the loss of collagen. The only previous isotopic data for *E. laurillardii* made in collagen with association of radiocarbon dating

was presented by Drefahl (2010). The study showed that the individual would have lived at 18,730-19,280 Cal yr BP in Quinjunge, Bahia and had a generalist diet ($\delta^{13}\text{C}_{\text{collagen}} = -18.2$ ‰) composed mainly of C_3 plants ($p_1\text{C}_3 = 71$ %; $B_A = 0.70$) from arboreal savanna habitats.

Based on radiocarbon datings and carbon isotopic data acquired from hydroxyapatite we know that *E. laurillardii* lived in BIR, at least, between 34 to 10 Cal yr BP (Dantas *et al.*, 2017; Oliveira *et al.*, 2020), and it was a generalist species, with a wide niche breadth ($B_A = 0.77 \pm 0.25$). *E. laurillardii* possibly could have lived in several habitats, as low-density forests to open savanna habitats, feeding on fruits, leaves ($p_1\text{C}_3 = 52$ %) and C_4 grasses ($p_1\text{C}_4 = 48$ %) (Dantas *et al.*, 2017). Thus, these two discordant values found in collagen, is in fact expected for this species.

The $\delta^{15}\text{N}$ value of *E. laurillardii* was 6.8 ‰, which is expected for a grazer, since C_4 plants can have higher values of $\delta^{15}\text{N}$ (e.g. Bocherens & Drucker, 2013). Whether *E. laurillardii* was an omnivore species, it could feed prey taxa with $\delta^{15}\text{N}$ values of 3.8 ‰ or 1.8 ‰, considering the most common enrichments of trophic niche +3 ‰ and +5 ‰, respectively (Bocherens & Drucker, 2013). However,

these $\delta^{15}\text{N}$ values could represent browser taxa, that would have lived in closed habitats, as low-density forests or arboreal savanna, thus in a different habitat expected for this *E. laurillardii* individual according to its carbon isotope value. Therefore, the proposition made by Fariña (1996) for *Megatherium americanum* suggesting that it could be an omnivore species, could not be attributed for *E. laurillardii*.

Table 1 Carbon and nitrogen isotopic data, proportion of food resources, and niche breadth for *E. laurillardii* and *M. americanum*. Labels. ⁽¹⁾ Our data; ⁽²⁾ Drefahl (2010); ⁽³⁾ Bocherens *et al.* (2016, and references therein)

Taxa	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	P_i		B_A
			C_3	C_4	
<i>E. laurillardii</i>	-10.1 ⁽¹⁾	6.8 ⁽¹⁾	0.14	0.86	0.31
	-18.2 ⁽²⁾	-	0.71	0.29	0.70
<i>M. americanum</i>	-18.7 ⁽³⁾	14.0 ⁽³⁾	0.76	0.24	0.57
	-19.7 ⁽³⁾	12.4 ⁽³⁾	0.84	0.16	0.37
	-17.3 ⁽³⁾	9.5 ⁽³⁾	0.66	0.34	0.81
	-17.8 ⁽³⁾	10.7 ⁽³⁾	0.70	0.30	0.72
	-19.7 ⁽³⁾	5.2 ⁽³⁾	0.84	0.16	0.37

Bocherens *et al.* (2016, and references therein) presented five isotopic data of carbon ($\delta^{13}\text{C}_{\text{collagen}}$) and nitrogen ($\delta^{15}\text{N}$) for *M. americanum*, and inferred that this species could feed almost ~76 % of C_3 plants, whose grasses would be the most important resources foraged, as the nitrogen values were high ($\mu\delta^{15}\text{N} = 10.4 \pm 3.3$ ‰). Bocherens *et al.* (2016) also showed that the $\delta^{15}\text{N}$ from the saber-tooth *Smilodon populator* ($\mu\delta^{15}\text{N} = 13.4 \pm 1.4$ ‰) was higher and statistically different from those of *M. americanum*, reinforcing the inferences about a herbivorous diet for this ground sloth

Nevertheless, Fariña & Varella (2018) point that Bocherens *et al.* (2017) did not take into account the body mass of *M. americanum*, and using a log of body mass proposed by Smith *et al.* (2004) and carbon isotopic data (acquired in collagen) for several mammals reinforce that *M. americanum* could be an omnivore, as it appears in the limit of carnivorans (Figure 1B).

Using the two available carbon isotopic data for *Eremotherium laurillardii* ($\Delta\delta^{13}\text{C}_{\text{collagen}} = 8.1$ ‰) and the body mass (3,400 kg) proposed by Dantas (2019), we include it in the regression (Figure 1B). We noted that *E. laurillardii* falls in the carnivorans limit, as *M. americanum* (Figure 1B), which shows that use only carbon isotopic data is not the best tool, only nitrogen isotopes could help to infer if these species presented a herbivorous or an omnivorous diet.

4 Final Remarks

In the present manuscript, although with a punctual carbon ($\delta^{13}\text{C}_{\text{collagen}} = -10.1$ ‰) and nitrogen ($\delta^{15}\text{N} = 6.8$ ‰) isotopic data, we suggest that *E. laurillardii* was a herbivore species and that the proposition of an omnivore diet for *M. americanum* and, consequently, for *E. laurillardii* could be mistaken.

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