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Article

Diet and isotopes of Late Pleistocene ground sloths: first results for *Lestodon* and *Glossotherium* (Xenarthra, Tardigrada)

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With 2 figures and 2 tables

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Abstract: Biogeochemical techniques have become most useful in the determination of the dietary niches of fossil mammalian species and in the reconstruction of past communities. Stable isotopes analyses (^{13}C and ^{15}N) were applied to study the diet of the mylodontids *Lestodon* and *Glossotherium* and other Late Pleistocene megamammals. Only the samples for these ground sloths yielded reliable values. The results of the $\delta^{15}N$ for *L. armatus* and *G. robustum* could be related to a non-ruminant herbivorous physiology or to the climate inferred for the Pampean region in the Late Pleistocene, colder and drier than present. The results of $\delta^{13}C$ for *L. armatus* and *G. robustum* indicate a preference for C_3 vegetation in open environments. This is also congruent with the trophic habits inferred for these ground sloths from morphological and biomechanical evidence that point them out as bulk feeders.

Key words: Late Pleistocene, megafauna, trophic habits, Pampean region.

1. Introduction

Biogeochemical techniques, such as the analysis of trace elements or of stable isotopes, have recently become most useful tools in the determination of the dietary niches of fossil mammalian species and in the detailed reconstruction of past communities (see Gröcke 1997; Bocherens & Drucker 2007; Koch 1998, 2007 and references therein), contributing to other approaches (i.e., morphofunctional studies of mastication, and locomotion and body size, among others) to palaeobiological reconstructions (Vizcaíno et al. 2004, 2008). For instance, Bargo & Vizcaíno (2008) mention that stable isotope analyses are growingly used in xenarthrans and that they will turn on of great importance for inferring diet in those animals, along with coprological and palynological evidence (Bargo & Vizcaíno 2008, and references therein).

The aim of this work is to study the diet of the mylodontids *Lestodon* and *Glossotherium* using stable isotopes (^{13}C and ^{15}N) in order to refine the knowledge on the palaeobiological features of these extinct xenarthrans, adding new dimensions to the already existing realm of interpretations about their unusual trophic ecology. Moreover, other accompanying megamammals, usually found along in South American Late Pleistocene sites, were also searched for their appropriate proportion of stable isotopes, although the quality of these results will be discussed below.

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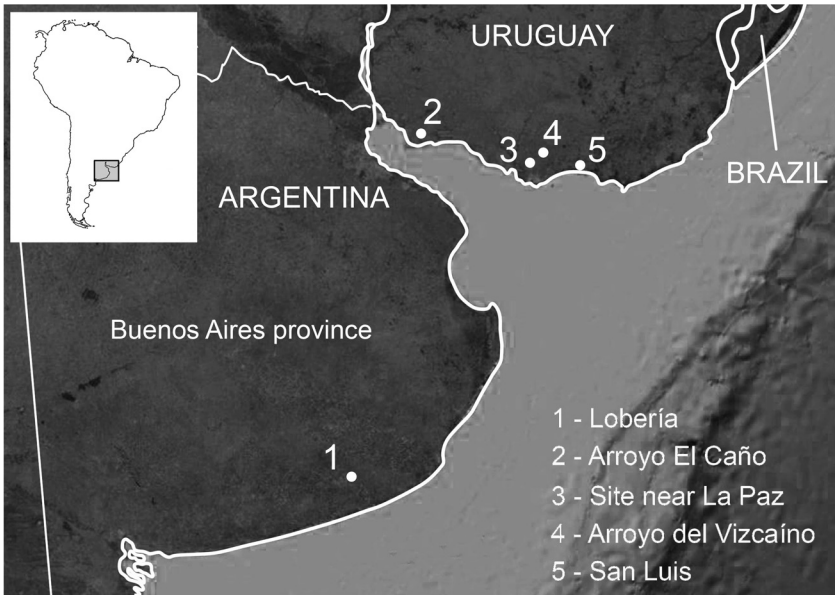

Fig. 1. Map of the localities where the samples come from.

2. Stable isotopes and diet

Stable isotopes have different thermodynamic and kinetic properties due to their different atomic mass compared with the most abundant isotope (for example ^{12}C vs. ^{13}C, ^{14}N vs. ^{15}N, ^{16}O vs. ^{18}O), which yield measurable fractioning in physical and chemical processes that mark substances with recognisable isotopic signatures (Koch 1998), expressed as the deviation, noted δ, with respect to a standard in parts per thousand (‰).

Among terrestrial plants, there are three different metabolic pathways in the photosynthesis (Edwards & Walker 1983), and they are known as C_3 (which include all temperate shrubs, cool/moist climate and high altitude grasses), C_4 (which encompass basically herbaceous, tropical, arid-adapted grasses and warm/dry herbs), and CAM or succulent plants, which use an intermediate C_3/C_4 photosynthetic pathway and which are usually excluded in palaeoecological studies, due to their little importance as fodder (Bocherens et al. 1994 a, b; Cormie & Schwarcz 1994).

C_3 plants are those that discriminate most effectively against $^{13}CO_2$, with an average $\delta^{13}C$ value of –26 ± 2 ‰, ranging from –34 ‰ in dense forests to –21 ‰ in open areas exposed to water stress. C_4 plants, on the other hand, are less effective discriminating against $^{13}CO_2$. Hence, they have an average $\delta^{13}C$ value of –12 ± 1 ‰, with a more restricted isotopic range from –15 ‰ to –7‰ (Bocherens et al. 1996a; Gröcke 1997; Koch 1998; Zazzo et al. 2000). CAM plants show intermediate values (see review and references in Smith & Epstein 1971; Bocherens et al. 1996b; Gröcke 1997; Zazzo et al. 2000). In general those values are reflected through additional fractionation in the tissues of the herbivores that feed upon the plants. In each trophic level the enrichment of the $\delta^{13}C$ goes from +3 ‰ to +5 ‰.

Thus, a mammal that consumes C_3 vegetation (i.e., fruits, leaves and roots of trees and bushes) will show an average $\delta^{13}C_{collagen}$ value of –22 ‰ (range: –30 ‰ to –17 ‰), while those feeding on C_4 plants (i.e., tropical grass blades, seeds and roots) will exhibit a value around –8 ‰ (range: –11 ‰ to –3 ‰). In carnivores, a predator that feeds on a C_3-taking primary consumer will record an average $\delta^{13}C_{collagen}$ value of –18 ‰, while those that consume C_4-takers will show a value close to –4 ‰ (DeNiro & Epstein 1978; Van der Merwe 1982; Klepinger & Mintel 1986; Gröcke 1997a, b; Koch 1998). Similar values are obtained from the hydroxiapatite of bones and teeth (Bocherens et al. 1996a; Gröcke 1997a; Koch 1998).

The value of $\delta^{15}N$ in the collagen ($\delta^{15}N_{collagen}$) of mammals indicates its trophic level (primary or secondary consumers), following the enrichment in the $\delta^{15}N_{collagen}$ between +1 ‰ and +6 ‰ (average ~+3.4

‰, Robinson 2001) per trophic level, although this is affected by such factors as whether the animal consume nitrogen-fixing plants or not and its nitrogen metabolism (Koch 1998) or the acidity of the soil (higher in open grasslands than in closed environments, Rodière et al. 1996; Gröcke et al. 1997). Nitrogen-fixing plants have values of $\delta^{15}N$ close to that of atmospheric N_2 (or 0 ‰), but the results are more varied in those plants that use other nitrogen sources (Robinson 2001). Among primary consumers that feed on nitrogen-fixing plants show values of $\delta^{15}N_{collagen}$ between 0‰ and +4 ‰, while the values of those that consume other plants are between +2 ‰ and +8 ‰. Other enrichment is related to marine or salt-influenced areas, arid regions dwellers (due probably to adaptations for decreasing urine excretion, Gröcke 1997; Gröcke et al. 1997; Koch 1998), and to monogastric digestive physiology (Gröcke & Bocherens 1996), as well as age of the individuals (i.e., suckling juveniles, see Gröcke 1997 for review).

3. Materials and methods

The samples were obtained from several collections, as listed below. The appropriate abbreviations are: CAV (Colección del Arroyo del Vizcaíno, Sauce, Canelones, Uruguay), CLRC (Colección Luis R. Castiglioni, Montevideo, Uruguay), FC-DPV (Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay) and MLP (Museo de La Plata, Argentina).

The fossil samples and their geographic and stratigraphic provenances (Fig. 1) are:

1. *Lestodon armatus* (Xenathra, Mylodontidae, Lestodontinae). CAV unnumbered rib; CAV 121, humerus. Arroyo del Vizcaíno (34º35'S – 56º03'W), Sauce, departamento de Canelones, Uruguay; Late Pleistocene. CLRC 491, tibia; near La Paz (34º44'S – 56º16'W), departamento de Montevideo, Uruguay; Late Pleistocene. FC-DPV, unnumbered molariform, San Luis (34º46'S – 55º36'W), departamento de Canelones, Uruguay; Late Pleistocene. FC-DPV 2311, radius; FC-DPV 2312, astragalus; Arroyo El Caño (34º25'S – 57º55'W), departamento de Colonia, Uruguay; Late Pleistocene.
2. *Glossotherium robustum* (Xenarthra, Mylodontinae). MLP 83-XI-5-1, osteoderms; partido de Lobería (38°10'S – 58°47'W), provincia de Buenos Aires, Argentina; La Postrera Formation (Late Pleistocene).

In addition to the samples of ground sloths, the samples of two species of glyptodonts and one of an extinct camelid were analysed for comparison purposes. These taxa are:

1. *Glyptodon* sp. (Xenarthra, Glyptodontidae, Glyptodontinae). CLRC 49, scute; near La Paz, departamento de Montevideo, Uruguay, Late Pleistocene. FC-DPV 2316, scute; Arroyo El Caño, departamento de Colonia, Uruguay; Late Pleistocene.
2. *Panochthus* sp. (Xenarthra, Glyptodontidae, Panochthinae). CLRC 491, scute; near La Paz, departamento de Montevideo, Uruguay, Late Pleistocene; FC-DPV 2317, scute; Arroyo El Caño, departamento de Colonia, Uruguay; Late Pleistocene.
3. *Hemiauchenia*? sp. (Artiodactyla, Camelidae, Lamini). FC-DPV 2313, metapodial; Arroyo El Caño, departamento de Colonia, Uruguay; Late Pleistocene.

There are various methods for identifying the alterations of the collagen, whose percentage in modern bones reaches only to about 18%, the rest consisting of some 80% of organic matter and 2.5% of other proteins and fats. The organic matter tends to decay in fossil bones, roughly as a function of the time elapsed since the death of the organism, but also influenced by weathering, preburial conditions, etc. (Gupta & Polach 1985). Hence, those processes are assessed by procedures such as the analysis of aminoacid composition and of C:N rates (DeNiro & Epstein 1978; DeNiro 1985; Gröcke 1997; Richards et al. 2000), with acceptable values between 2.8 and 3.6. This criterion allows the identification and exclusion of decayed or contaminated collagen, a material considered easier to purify. Longin (1971) extracted pure collagen from bone as gelatine using successive hydrolises at different concentrations of HCl.

The rib of *Lestodon* dated in Fariña & Castilla (2007) was sampled and processed again. Besides; a sample of this material was treated, purified and dated in the laboratory of ^{14}C, Cátedra de Radioquímica, Facultad of Química, Universidad de la República with the method described here.

The bone fragments were cleansed, both on the external surface and the trabecular tissue, by mechanical methods to eliminate the remaining sediment, washed with distilled water and dried 24 hours in the oven. Glyptodont scutes and osteoderms were washed with 50% acetic acid (Gupta & Polach 1985), ultrasounded, and then washed with distilled water and dried 24 hours in the oven.

For the extraction of the organic fraction Longin's (1971) technique was heeded, with some modifications. The sample was manually grounded in a mortar with a fraction smaller than 50 µm (mesh Nº 35). After weighing, the sample was digested with HCl at 8% during 18 minutes. The acid was disposed using glass microfibre filter paper and residuals washed out with distilled water until neutralising. Then, a basic digestion was undertaken, using NaOH 0.125 M for 20 hours, to remove humic acids (DeNiro & Epstein 1978, 1981), and the residuals were washed with distilled water until neutralising. The non-soluble fraction so obtained was dried in the over for 24 hours. Once dried, the collagen in each sample was weighed and arranged in fractions of less than one gram to be packed and posted, and, when there was enough material, a subsample was further purified by refluxing in a buffer solution of H_3PO_4 and KH_2PO_4 at pH 3 for at least 17 hours at 80º to 90º C. The solution was then filtered with Whatman Nº1 cellulose paper, dried in the oven and packed for sending.

Samples were then analysed on a Costech Elemental Analyzer coupled to a Finnigan Delta IV Plus stable isotope ratio mass spectrometer under continuous flow using a CONFLO III interface in the Stable Isotope Biogeochemistry Laboratory at the Ohio State University. 5 of the samples were run in duplicate. Stable carbon and stable nitrogen measurements were made where the average standard deviation of repeated measurements of the

USGS24 and IAEA-N1 standards were ± 0.05 ‰ for $\delta^{13}C$ and ± 0.13 ‰ for $\delta^{15}N$.

4. Results

Table 1 shows the results of the pre-treatment. It is also indicated whether the sample was purified or not and whether it was divided. All samples yielded proportions between something less than 1% and close to 6%.

Table 2 summarises the results obtained in the analysis of ^{13}C and ^{15}N stable isotopes. The sample A2 was not included because no collagen was obtained. Of the 21 samples analysed (5 of which were duplicated), 11 showed a C : N ratio indicating that they had not undergone significant diagenetic alteration (DeNiro 1985; Ambrose 1990; Bocherens et al. 1996b, 1997; Drucker et al. 2001, 2003). Those samples whose C : N ratio did not fall within the range of 2.8 and 3.6 were not further considered; this applies to samples A3, A10, A11 and A13 (*L. armatus*), A4 (*Hemiauchenia*?), A6 and A8 (*Glyptodon*), A7 and A9 (*Panochthus*). Only the samples of two taxa yielded valid results: A1, A12 and A14 for *L. armatus* and A5 for *G. robustum* (Table 2). The average for the ground sloth *Lestodon armatus* was –18.8 ‰ for the $\delta^{13}C$ and +9.5 ‰ for the $\delta^{15}N$, while the results for the ground sloth *Glossotherium robustum* averaged at –20.5 ‰ for the $\delta^{13}C$ and +10.2 ‰ for the $\delta^{15}N$ (Fig. 2).

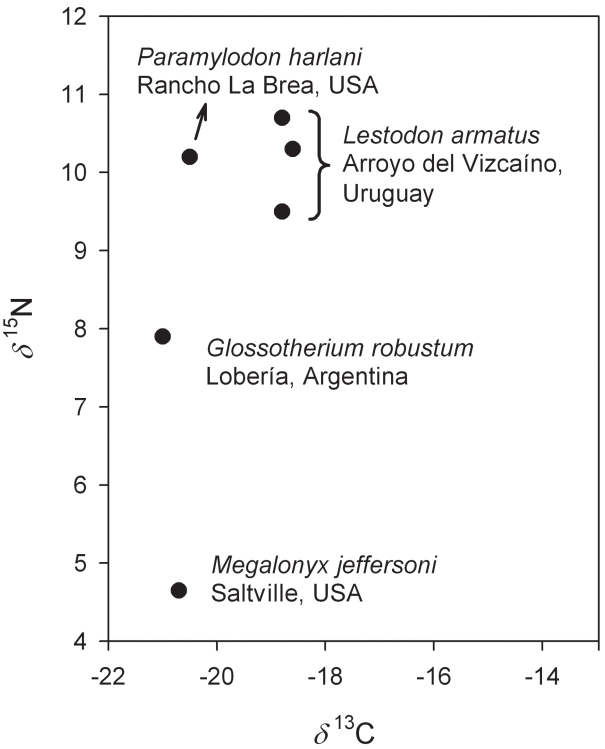

Fig. 2. Graph showing the values of δ13C vs. δ15N from the samples discussed in the text.

5. Discussion

The results obtained in the analyses of the samples A1 and A1P and A12 and A12P (Table 2) shed similar values for the samples of purified collagen and those in which the obtained collagen was not purified, either for the $\delta^{13}C$ or for the $\delta^{15}N$.

These results confirm that the post-mortem alteration of the collagen ratios are not associated to the age of the sample (DeNiro 1985; Bocherens et al. 2008): the samples of *L. armatus* of Arroyo del Vizcaíno (A1 and A1P, Table 2) exhibit a ratio C : N between 3.31 and 3.33 and that rib was dated as 28200 ± 230 radiocarbon years BP (Fariña & Castilla 2007). Furthermore, the samples analysed with an appropriate C : N ratio are older and show a higher yield (4.14% for A1P and 6.34% for A1, Table 1). The other samples from Arroyo del Vizcaíno, A12 and A12P, also show an adequate relation C : N (between 3.25 and 3.33) and also a high yield for fossil samples (near 5%). Previous studies in fossils report collagen yields between ~1 and 21% (Ambrose 1990; Bocherens et al. 1991, 1994a). Ambrose (1990) identified the most reliable percentages in samples from tropical Africa, with yields of 1% for collagen, 13% for C and 4.8% for N, although with variations according to different regions. Another interesting aspect is that the results of $\delta^{13}C$ and $\delta^{15}N$ found in the rib of *L. armatus* dated in Fariña & Castilla (2007; sample Arroyo del Vizcaíno 1, $\delta^{13}C$ –18.6 and $\delta^{15}N$ +10.3, Beta 204256), as well as the results of $\delta^{13}C$ and $\delta^{15}N$ for the clavicle analysed in that paper (sample Arroyo del Vizcaíno 2, $\delta^{13}C$ –18.8 and $\delta^{15}N$ +10.7, Beta 206660), are perfectly congruent with those obtained here, not only in the re-analyses of the same material (rib, samples A1 and A1P, Table 2) but also in the case of the humerus of *L. armatus* from the same locality (sample A12 and A12P, Table 2).

Despite its younger age 17620 ±100 radiocarbon years BP (Gutiérrez et al. 2005), the samples from the site close to La Paz show a ratio C:N that suggests significant diagenetic alterations and were not taken into account for the conclusions. On the other hand, the sample of *G. robustum* from provincia de Buenos Aires (A5), whose age was set at 10710 ± 90

Table 1. Detail of samples analysed with indication of those that were purified, collagen mass obtained in the pre-treatment and yielding in percentage.

Sample	Description	Mass of collagen (g) obtained (without purification)	Yield in% (without purification)
A1P	CAV unnumbered: rib de *L. armatus* Arroyo del Vizcaíno (purified)	1.88	4.15
A1	CAV unnumbered: rib *L. armatus* Arroyo del Vizcaíno (wihout purification)	2.10	6.34
A2	FC-DPV 2314: rib *L. armatus* Arroyo El Caño	0.82	0.97
A3	FC-DPV 2311: radius *L. armatus* Arroyo El Caño	0.10	0.44
A4	FC-DPV 2313: *Hemiauchenia*? Arroyo El Caño	0.19	1.59
A5	MLP 83-XI-5-1: osteoderms G. *robustum* Bs. As.	0.27	0.94
A6	FC-DPV 2316: *Glyptodon* Arroyo El Caño	sample invalid	------
A7	FC-DPV 2317: *Panochthus* Arroyo El Caño	2.60	3.33
A8	CLRC 491: *Glyptodon* (cantera)	0.4	0.42
A9	CLRC 491: *Panochthus* (cantera)	0.30	0.44
A10	CLRC 491: tibia *L. armatus* (cantera) (purified)	5.16	4.98
A11	FC-DPV 2312: astrágalo *L. armatus* Arroyo El Caño (purified)	1.54	1.74
A12	CAV 121: humerus *L. armatus* Arroyo del Vizcaíno (wihout purification)	~5	~5
A12P	CAV 121: humerus *L. armatus* Arroyo del Vizcaíno (purified)		
A13	FC-DPV unnumbered: molariform *L. armatus* San Luis	0.22	0.60

radiocarbon years BP (Figini et al. 1987), showed an adequate ratio C : N (see Table 2).

6. ^{15}N results

Several papers have demonstrated a strong negative relationship between herbivore bone collagen $\delta^{15}N$ and water availability (Heaton et al. 1986; Sealy et al. 1987; Gröcke et al. 1997), although there is considerable debate about the relative roles of plant $\delta^{15}N$ values and animal metabolism responsible for this relationship (Ambrose & DeNiro 1986; Ambrose 1991). Recent studies in kangaroos by Murphy & Bowman (2006) show that dietary $\delta^{15}N$ is the main cause of the negative relationship between bone collagen $\delta^{15}N$ and water availability, whereas metabolic factors have little discernible effect.

The values obtained for the $\delta^{15}N$ of *G. robustum* from provincia de Buenos Aires (+10.5 on average) and of *L. armatus* (+9.5 on average) are very similar and comparable; although they represent different times: about 32000 years BP for *L. armatus* and about 12000 years BP for *G. robustum*. Those ages represent a span of time that, despite belonging to the end of the Oxygen Isotopic Stage 3 (OIS 3) in one case and to the end of the OIS 2 in the other, brackets the arid, colder OIS 2, which includes the Last Glacial Maximum at 18000 years BP.

The obtained values are rather high for a herbivore. Amundson et al. (2003) showed that $\delta^{15}N$ in soil and plants systematically decrease with increasing mean annual precipitation and with decreasing mean annual temperature. The climate inferred for the Pampean region in the Late Pleistocene for the times in which the sampled individuals lived (end of the OIS 3 for *L. armatus* and possibly Younger Dryas for *G. robustum*) was colder and drier than the present (Iriondo & García 1993; Iriondo 1999; Tonni & Cione 1997; Tonni et al. 1999, 2010). Iriondo & García (1993) set the end of the cold climatic phase about 8500 years BP, when it turned into humid subtropical conditions. This is also observed in the transition from the psammophytic scrubby steppe to a humid phase (Prieto 1996, 2000), with swamps in the NE of the Pampa (Cerro La China, Empalme Querandíes, and La Horqueta II) beginning to occur 11000 years BP. In turn, Tonni et al. (1999; see also Cione et al. 2009; Tonni 2009) proposed that the humid conditions may have corre-

Table 2. Detail of samples indicating the results of the analysis of ^{15}N and ^{13}C and the C : N ratio obtained in the samples; "dup" indicates duplicate sample processed. Valid results in bold.

Sample	Description	Nitrogen [%]	Carbon [%]	δ^{15}NAir (‰)	$\delta^{13}C_{VPDB}$ (‰)	C:N
A1P	CAV unnumbered: rib *L. armatus* Arroyo del Vizcaíno (purified)	12.0	34.0	**9.59**	**–18.73**	**3.31**
A1	CAV unnumbered: rib *L. armatus* Arroyo del Vizcaíno (without purification)	10.1	28.8	**9.25**	**–18.81**	**3.33**
A1–dup	CAV unnumbered: rib *L. armatus* Arroyo del Vizcaíno (without purification)	11.8	33.2	**9.49**	**–18.75**	**3.28**
A3	FC–DPV 2311: radius *L. armatus* near Arroyo El Caño	0.1	1.8	7.96	–28.79	21.00
A4	FC–DPV 2313: *Hemiauchenia*? Arroyo El Caño	0.1	1.7	7.71	–20.43	19.83
A5	MLP 83–XI–5–1: osteoderms G. *robustum* Buenos Aires.	13.8	40.1	**10.35**	**–20.55**	**3.39**
A5–dup	MLP 83–XI–5–1: osteoderms G. *robustum* Buenos Aires.	13.4	38.9	**10.06**	**–20.52**	**3.39**
A6	FC–DPV 2316: *Glyptodon* Arroyo El Caño	0.1	3.1	7.11	–26.57	36.17
A7	FC–DPV 2317: *Panochthus* Arroyo El Caño	0.0	0.4	8.20	–24.12	0.00
A8	CLRC 491: *Glyptodon* (near La Paz)	0.0	0.6	6.46	–28.12	0.00
A9	CLRC 491: *Panochthus* (near La Paz)	0.0	0.2	4.50	–26.24	0.00
A10	CLRC 491: tibia *L. armatus* (near La Paz) (purified)	0.0	0.2	5.91	–25.73	0.00
A11	FC–DPV 2312: astragalus *L. armatus* Arroyo El Caño (purified)	0.2	1.7	10.61	–26.19	9.92
A12	CAV 121: humerus *L. armatus* Arroyo del Vizcaíno (without purification)	13.4	38.3	**10.02**	**–19.13**	**3.33**
A12P	CAV 121: humerus *L. armatus* Arroyo del Vizcaíno (purified)	11.5	32.1	**9.43**	**–18.81**	**3.26**
A12P–dup	CAV 121: humerus *L. armatus* Arroyo del Vizcaíno (purified)	11.6	32.3	**9.59**	**–18.91**	**3.25**
A12P–dup	CAV 121: humerus *L. armatus* Arroyo del Vizcaíno (purified)	11.4	32.1	**9.44**	**–19.09**	**3.29**
A13	FC–DPV unnumbered: molariform *L. armatus* San Luis	0.2	2.9	7.64	–24.70	16.92
A13–dup	FC–DPV unnumbered: molariform *L. armatus* San Luis	0.2	2.6	7.71	–24.79	15.17
A14P	CAV unnumbered: rib *L. armatus* Arroyo del Vizcaíno processed by R. Bracco (purified)	12.5	34.9	9.44	–18.19	3.26
A14P–dup	CAV unnumbered: rib *L. armatus* Arroyo del Vizcaíno processed by R. Bracco (purified)	12.1	33.3	9.50	–18.39	3.21

sponded to local conditions, whereas the rest of the Pampas were predominated by aeolic sediments, and mammals dwelling arid areas are recorded from the La Postrera Formation.

Also, Coltrain et al. (2004) reported data on *Paramylodon harlani*, the most frequently found ground sloth in Rancho La Brea, Late Pleistocene of North America, with an average of $\delta^{15}N$ of +7.9, above the equids (or "true non-ruminants") but below the ruminants, which made those authors to conclude that those values correspond to their inferred digestive physiology, similar to that on their modern relatives (Cork 1994). Indeed, modern sloths lack active protozoa in the epigastric region, i.e., between the mouth and duodenum and hence they are not as efficient in degrading cellulose, which is compensated by retaining food in the intestine (Goffart 1971). Moreover, France et al. (2007) included among

the species studied in Saltville, Late Pleistocene of Virginia, USA, the megalonychid ground sloth *Megalonyx jeffersonii*, and obtained rather high values of $\delta^{15}N$. (+4.65 ± 0.11 ‰), which they interpreted also as a consequence of non-ruminant physiology. The study of the relatively small occlusal areas indicate that mylodontid ground sloths were not able of efficient oral processing of the food (Bargo & Vizcaíno 2008), which might have been compensated with a high fermentation chamber in the digestive tube, with a low metabolic rate or through a combination of both strategies (Vizcaíno et al. 2006). This might also have been the case for *P. harlani* (Coltrain et al. 2004).

However, Murphy & Bowman (2006) disagree on the importance of physiology and focus instead on water availability, congruently with the aforementioned $\delta^{15}N$ results in soil and plants (Amundson et al. 2003). Murphy & Bowman (2006) also warn about another influencing factor: herbivore $\delta^{15}N$ is affected by CO_2 concentration on ecosystem via its strong effect on the water use efficiency of plants (WUE, Morison 1993; Peñuelas & Estiarte 1997). Decreased CO_2 concentrations, such as that at the last glacial maximum, would lower plant WUE and hence net primary productivity (NPP), for a given level of water availability. A reduction in NPP would reduce the biological demand for nitrogen, increasing the openness of the nitrogen cycle and increasing plant $\delta^{15}N$. Both samples studied here belonging to the ground sloths *Lestodon* and *Glossotherium* do not come from the last glacial maximum times, but the drop in temperature, sea level and CO_2 concentration in their respective times in regard to present (and the consequential increase in plant $\delta^{15}N$) must have been equivalent, as in both cases the sea level must have been about 60 metres below today (Lambeck et al. 2002). This could have yielded an increase in the $\delta^{15}N$ values of those samples.

Finally, a more diverse diet than strict herbivory, and possibly including animal matter, has been proposed for ground sloths (Fariña 1996), particularly *Megatherium americanum*. Although a more general view on this topic is still lacking, this could be another factor to take into account at interpreting the high values obtained.

7. ^{13}C results

Values of ^{13}C in animal tissues, as said above, are influenced by the relative proportions of C_3 and C_4 plants consumed, but climate can also affect the isotopic composition up to a 3 ‰ (see Iacumin et al. 2000 and references therein). The averages of the results obtained for *L. armatus* (–18.8 ‰) and *G. robustum* (–20.5 ‰) are compatible with those obtained in modern primary consumers of rather open environments (DeNiro & Epstein 1978). The sample of *Glossotherium* comes from Lobería in the southern region of the provincia de Buenos Aires, in which is found today a dominance of C_3 vegetation (Powell & Still 2009). On the other hand, the present day territory of Uruguay, where the sample of *Lestodon* comes from, is dominated by C_4 plants. However, and according to palaeoclimatic reconstructions (Iriondo & García 1993) and pollen findings in the Argentinian pampas (Prieto 1996, 2000; Tonello & Prieto 2008), the prevalent conditions in both places at their appropriate times must have been those of today's northern Patagonia, where C_3 plants are dominant (see Powell & Still 2009, fig. 3).

The results presented here are comparable to the $\delta^{13}C$ obtained in the North American Pleistocene site of Rancho La Brea for *P. harlani* (average –21.0 ‰, Coltrain et al. 2004), and for *M. jeffersoni* of Saltville (–20.66, France et al. 2007). In any case, the values of $\delta^{13}C$ for *L. armatus* are the highest (i.e., the less negative) of all mylodontids. DeNiro & Epstein (1981) mention that higher (less negative) values of $\delta^{13}C$, as those observed, could indicate a shift in the diet towards the utilisation of C_4 and/or CAM plants.

Finally, in the reconstruction of habitats in central Amazonia, Rosetti et al. (2004, and references therein) state that before the interpretation of the results of ^{13}C, the distance between collagen and the diet must be considered. In other words, the absorption of carbon by the collagen might vary according to metabolic rates, alimentary preferences, body size and to a certain extent to phylogenetic distances. For that reason, the relation collagen-diet in the fossil megafauna should be seen as an approximation. On the other hand, since there is no modern analogues for ground sloths, the efforts about ecological reconstructions should be based on geographic distribution, faunistic associations and inferences from biomechanical and ecomorphological information (Rosetti et al. 2004). Thus, the results of biogeochemical analyses obtained here intend to add new information to the already available evidence in order to refine our knowledge of the peculiar palaeoautecology of the extinct ground sloths.

8. Conclusions

The results of the $\delta^{15}N$ obtained for *Lestodon armatus* are consistent with the values obtained previously (Fariña & Castilla 2007). Those results, as well as those obtained for *Glossotherium robustum* could be related to a non-ruminant herbivorous physiology

or to the dryer and colder climate inferred for their respective ages. The results of $\delta^{15}N$ obtained for *L. armatus* and *G. robustum* are congruent with the climate inferred for the Pampean region in the Late Pleistocene during the OIS 2, colder and drier than present (Iriondo & García 1993; Iriondo 1999; Tonni & Cione 1997; Tonni et al. 1999).

The results of $\delta^{13}C$ for *L. armatus* and *G. robustum* (–18.8 and –20.5 ‰ on average, respectively), indicate a preference for C_3 vegetation in open environments similar to those in northern Patagonia, which is congruent with those inferred for the North American ground sloth *P. harlani* in Rancho La Brea (Coltrain et al. 2004) This is also consistent with the trophic habits inferred for these ground sloths from morphological and biomechanical evidence that point them out as bulk feeders (Bargo et al. 2006; Bargo & Vizcaíno 2008).

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