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Isotopic insight on paleodiet of extinct Pleistocene megafaunal Xenarthrans from Argentina

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ABSTRACT

The diet of extinct giant Xenarthrans is a debated topic, especially for ground sloths, for which herbivory, insectivory, and carnivory through scavenging or active hunting have been suggested. In this study, stable carbon isotopic composition of collagen and carbonate fraction of well-preserved fossil bones was used as a tracer of trophic level. >200 modern mammal bones of species with a variety of diets were used as a reference dataset. The good preservation of the carbonate isotopic composition was checked using different diagenetic indicators and by using fossil carnivorous and herbivorous taxa from the same sites as test-taxa. The results on modern mammals indicate a clear distinction in the difference between the carbon isotopic composition of carbonate and collagen between faunivores (carnivores, insectivores, piscivores, omnivores) and herbivores. Using this framework, the results on the fossil megafaunal Xenarthrans indicate a herbivorous diet for both Glyptodontids and are also consistent with a herbivorous diet for giant ground sloths, including *Megatherium*. The hypothesis that *Megatherium* could be a cryptic meat-eater or an insectivore is not supported by the results obtained in the present study.

1. Introduction

Until around 10,000 years ago, South America was home of numerous species of giant Xenarthrans, giant ground sloths (relative to tree sloth) and glyptodontids (relative to tiny extant fairy armadillo), some of these giants even migrated into North America after the Great Biotic Interchange in the late Pliocene (e.g. Marshall et al., 1982; Webb, 2006). These megafauna taxa were the most diverse members of South American megafauna and are extinct today (e.g. Prevosti and Vizcaíno, 2006; Cione et al., 2009; Fariña et al., 2013; Scanferla et al., 2013; Cione et al., 2015), and they have no modern ecological analogue living today. They were very important components of the terrestrial ecosystems with various possible important ecological functions, (1) as megaherbivores potentially impacting the dispersal and growth of vegetation types (Janzen and Martin, 1982; Bakker et al., 2016), (2) as ecological engineers building large burrows that could be used by other species in an open and caveless environment (Vizcaíno et al., 2001; Dondas et al., 2009; Soibelzon et al., 2009), (3) as fertilizers of landscape through their dejections (e.g. Doughty et al., 2013) and (4) maybe as scavengers or predators (Fariña, 1996; Fariña and Blanco, 1996). Therefore, their extinction may still have an impact on modern ecosystems (Janzen and Martin, 1982; Doughty et al., 2013; Bakker et al., 2016).

Different hypotheses have been suggested about their possible diets. The dietary preferences of extinct mammals can usually be evaluated through their tooth morphology. In contrast to most other mammal groups, the teeth of Xenarthra are morphologically simple and do not provide unambiguous information about their diet (e.g. Bargo et al., 2006; Hautier et al., 2016). Fortunately, skull and jaw morphology differences among taxa yielded evidence for possible feeding mechanisms and therefore food composition (e.g. Bargo and Vizcaíno, 2008; Vizcaíno et al., 2011).

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Among the different species of late Pleistocene glyptodontids, there is a consensus about a herbivorous diet, but based on their morphology, some taxa, such as *Panochthus* and *Doedicurus*, are considered to have been bulk feeders in open environments, while other species especially *Glyptodon* was probably more selective feeder in closer habitats or had different food habits as other glyptodontids (Vizcaíno et al., 2011).

In contrast, there is more controversy about the palaoediet of ground sloths. Most palaeodietary investigations consider ground sloths to be essentially herbivores. By analogy with modern Xenarthra, extinct giant forms are considered to have had a low metabolic rate and a slow rate of passage of ingesta through the gut, which would have allowed them to eat plants of poor nutritional quality (Naples, 1989; McDonald, 2005). Dung attributed to Megatheriinae indicated a herbivore diet (Martínez et al., 2004). Moreover, palaeogenetic analyses of dung from South American ground sloth indicate a plant-based food for these large mammals (Hofreiter et al., 2003). Based on skull and jaw morphology differences among taxa, possible specialization on different plant types have been suggested, with narrow muzzle forms such as *Megatherium* considered as selective feeders (browsers) and broad muzzle forms such as *Lestodon* considered as bulk feeders (grazers) (Bargo and Vizcaíno, 2008). In contrast, some studies have concluded that ground sloths may have been insectivores and were digging large burrows to get access to underground colonial insects (Genise and Fariña, 2012). Finally, a feeding behaviour relying on meat consumption, either through active predation or scavenging, has also been suggested. For instance, based on the trophic relationships among Late Pleistocene mammals, Fariña (1996) proposed that there must have been one or more large-bodied species that ate carrion opportunistically, namely ground sloths, even if their diet also included plants. Moreover, based on ecomorphology and biomechanics, Bargo and Vizcaíno (2008) suggested that *Megatherium* might have included meat in addition to plant food in the diet, while Fariña and Blanco (1996) went even further suggesting that *Megatherium* may have been a hunter and a scavenger, based on his claws and mechanical characteristics of its front legs, and filling a non-analogue ecological niche of gigantic carnivore in the Pleistocene Pampas (Fariña, 1996; Fariña et al., 2013, 2014a). Therefore even if the majority of the evidence points to large amounts of plant consumption, one cannot totally rule out some animal food items in the diet of some ground sloth taxa, such as *Megatherium*.

The application of stable isotopes on fossil bones has yielded very important information to solve debates about the diet of extinct large mammal groups, especially those with potentially flexible diets, such as bears. For instance, two large extinct bear species, cave bear *Ursus spelaeus* in Europe and giant short faced bear *Arctodus simus* in North America, have been determined to be herbivores and carnivores, respectively, by comparing the carbon and nitrogen isotopic composition of their bone collagen with those of coeval herbivorous and carnivorous taxa (e.g. Bocherens et al., 1994a, 1995; Matheus, 1995; Krajcarz et al., 2016). Although collagen preservation is more limited in South America middle latitude sites than in Europe and northern North America, some similar isotopic data have been obtained on some specimens of glyptodontids, ground sloth and coeval fauna in Argentina (Bocherens et al., 2016). In this case, the isotopic data yielded interesting information about habitat, but they are less informative about the dietary habits of some extinct species, due to very variable isotopic values, exhibiting some overlap between $\delta^{15}N$ values of herbivores (*Macrauchenia*), ground sloth (especially *Megatherium*) and carnivore (*Smilodon*), probably due to aridity, and this makes the interpretation of these data in terms of diet particularly difficult (Bocherens et al., 2016).

Another isotopic approach that can potentially yield information about herbivory or carnivory in an extinct species is the difference between the carbon isotopic abundances of the collagen and the carbonate fractions of skeletal tissues. Indeed, modern herbivorous mammals exhibit significantly larger differences than carnivores (e.g. Lee-Thorp et al., 1989; Bocherens and Mariotti, 1992; Bocherens, 2000; Clementz et al., 2009), even within individuals of one given species fed different

controlled diets (O'Connell and Hedges, 2017). The exact cause of this different is not yet fully understood, but it is possibly due to the use of different biochemical substrate for energy metabolism for carnivores (lipids with low $\delta^{13}C$ values) and for herbivores (carbohydrates with higher $\delta^{13}C$ values) (Krueger and Sullivan, 1984). In any case, this difference in carbon isotopic value exhibits little variation among herbivores despite variations in diet (browsers vs. grazers) and digestive physiology (foregut ruminants vs. hindgut fermenters) (Cerling and Harris, 1999). Another factor that seems to play a role is the expiration of varying amounts of methane with very low $\delta^{13}C$ values by fermenting herbivores (e.g. Hedges and van Klinken, 2000; Passey et al., 2005). This approach using two different carbon fractions (i.e. collagen and structurally bound carbonate in the bioapatite) that are prone to diagenetic alteration during fossilization can be useful in the case of large variation of $\delta^{15}N$ values in herbivore bone collagen that may overlap with those of carnivores, as it is the case for woolly mammoths (Bocherens et al., 1994b; Iacumin et al., 2000; Clementz et al., 2009) and it could be applied to ground sloths. However, so far, data for various types of faunivores, such as piscivores, insectivores, omnivores with high or low degree of meat consumption in their diet have not been systematically investigated in modern mammals, which could limit the interpretation of the data for ground sloth, since some palaeobiological hypotheses that we want to test involve omnivory and insectivory.

The goal of the study is to try to extract information about the paleodiet of the extinct giant Xenarthrans while using well-preserved fossil bones of extinct giant Xenarthrans and coeval mammals with well-known diets from the Pampas to measure $\delta^{13}C$ values in bone collagen and carbonate, and comparing these results to those obtained on a
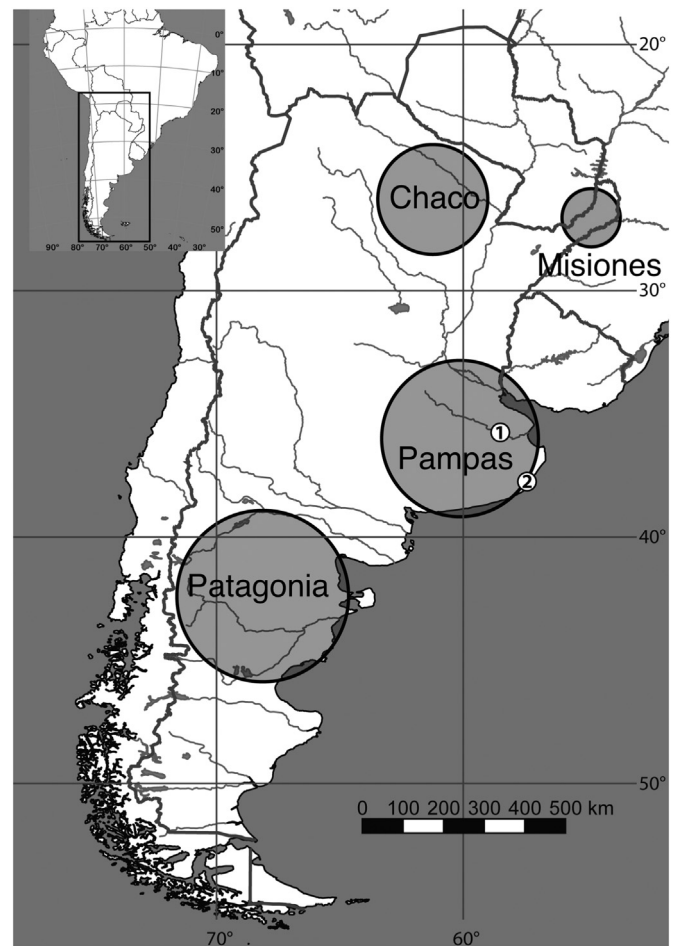

Fig. 1. Location map of areas where the modern mammal specimens and the fossil samples from Argentina have been collected. Site 1 is Rio Salado, site 2 is Camet Norte.

large suite of modern mammals with different well-known herbivorous to faunivorous diet and various habitats combined with relevant published data.

2. Material

One hundred and twenty-six bone specimens from modern mammals in Argentina and Bolivia, that lived in regions with different climatic regimes and ecosystems, including subtropical rainforest, dry forest, arid warm steppe, warm temperate Pampean grassland and cold Patagonian steppe, were analyzed for their $\delta^{13}C_{coll}$ and $\delta^{13}C_{apatite}$ (Fig. 1). The sampled bones cover a broad taxonomic diversity including Felidae, Canidae, Ursidae, Procyonidae, Mustelidae, marsupials, Xenarthrans, artiodactyles, perissodactyles, and rodents. Diets include large and small carnivores, omnivores with high or low degree of meat consumption in their diet, piscivores (fish-eaters), insectivores, and small and large herbivores (supplementary data). Additionally, published carbon isotopic composition of collagen and carbonate of modern mammal bones (Bocherens, 2000; Clementz et al., 2009; Melin et al., 2014) were used to complete the reference database.

The studied fossil material comes from two late Pleistocene sites in the Buenos Aires Province, Camet Norte and Rio Salado around General Belgrano (Fig. 1), dated between around 25,000 to 12,000 years ago, where numerous bones have yielded well-preserved collagen (Bocherens et al., 2016). The samples considered here include 15 specimens that belong to carnivores (sabre-tooth cat *Smilodon populator*), herbivores (Equid *Hippidion*, Meriungulata *Toxodon* and *Macrauchenia*) and representatives of giant ground sloths (*Megatherium*, *Glossotherium*) and glyptodontids (*Glyptodon*, *Doedicurus*) (Table 1).

3. Methods

For modern bones, cleaning with chloroform/methanol 2:1 was performed prior to further cleaning and crushing to remove possible fat (Münzel et al., 2014). Thereafter, all samples were treated in the same way than the fossil material. Bone fragments were ultrasonicated in acetone to remove any possible synthetic glue and rinsed in distilled water (Bocherens et al., 1997). Chemical and isotopic analysis of modern and ancient bone collagen was performed using an elemental analyzer connected to an IRMS Mass Spectrometer, as described in detail in Bocherens et al. (2016).

For carbonate isotopic analysis, organic matter was removed from bone powders using 2.5% NaOCl solution for 24 h or more if bubbling resumed, followed by a 0.1 M Ca-acetate acetic acid buffer solution for another 24 h (Bocherens et al., 1996). Samples were analyzed at 70 °C using a ThermoFinnigan Gasbench II coupled to a Finnigan Delta Plus XL CFIRMS at the University of Tübingen for $\delta^{13}C$ values of the carbonate fraction of bioapatite. Isotopic abundances are expressed as $\delta^{13}C$ values in parts per mil (‰), as follows: $\delta^{13}C = (^{13}C/^{12}C_{sample}/^{13}C/^{12}C_{standard} - 1) \times 1000$. The standard is V-PDB. For fossil bone samples the analytical error is 0.5% for percent carbonate and 0.2‰ for $\delta^{13}C$, based on multiple isotopic analysis of modern bone of camel and elk prepared and analyzed at the same time as the fossil samples.

Several chemical indicators are linked to diagenetic rather than biogenic factors. These are (1) the nitrogen content in bone (%N_{bone}), reflecting the intensity of collagen loss (Bocherens et al., 2005), (2) the percentage of non-collagenic carbon in bone (%C_{ncoll}), calculated as %C_{bone} − (2.7×%N_{bone}) and reflecting the amount of carbonaceous contamination (Bocherens et al., 2008), and (3) the carbonate yield (%CO_3), with low values reflecting possible recrystallization with loss of structural carbonate and high values indicating that some exogenous carbonate fractions remained present after pretreatment (Toyne et al., 2017). If the $\delta^{13}C$ values measured on bioapatite carbonate fraction were significantly affected by diagenesis, one would expect a correlation between the isotopic values and one or more of these chemical indicators.

Table 1

List of fossil samples, with site, sampled piece, bone chemical composition (%N and %C are nitrogen and carbon content in bone or dentine, respectively, %C_{ncoll} is the carbon content not related to collagen: %C_{ncoll} = %C_{bone} − (2.7×%N_{bone})); collagen yield, chemical composition of bone collagen, carbonate content, $\delta^{13}C$ values of carbonate, and difference between $\delta^{13}C$ values in carbonate and collagen.

Lab #	Museum #	Genus	Sampled	Site	Bone %N_{bone}	%C_{bone}	%C_{ncoll}	Collagen Coll yield (mg.g^{-1})	%C	%N	C/N	$\delta^{13}C_{coll}$	$\delta^{15}Nd$	Carbonate CO_3-content (% CaCO$_3$)	$\delta^{13}C_{carb}$	$\Delta\delta^{13}C_{carb}$-$\delta^{13}C_{coll}$
ARG-561	MHM-P85	*Smilodon*	Caninus	Rio Salado, General Belgrano	1.1	6.3	3.3	61.2	39.6	14.2	3.3	−17.7	15.4	4.9	−11.4	6.3
ARG-562	MHM-P53	*Smilodon*	Femur	Rio Salado, General Belgrano	4.2	13.2	1.8	192.5	43.8	16.0	3.2	−17.7	14.2	5.6	−13.9	3.8
ARG-565	MHM-P31	*Smilodon*	Cranium	Rio Salado, General Belgrano	4.3	14.3	2.8	198	42.9	15.5	3.2	−17.7	14.2	5.7	−12.2	5.4
ARG-566	Santa Clara	*Smilodon*	Vertebra	Camet Norte North, level B	3.7	11.9	1.8	132.8	45.0	14.9	3.5	−17.4	12.1	5.8	−12.4	5.0
ARG-567	Santa Clara	*Smilodon*	Tibia	Camet Norte North, level B	4.0	12.5	1.7	171.3	42.7	14.8	3.4	−17.6	11.2	5.9	−12.2	5.4
ARG-564	MHM-P57	*Hippidion*	Distal tibia	Rio Salado, General Belgrano	1.6	6.7	2.5	41.2	35.9	12.7	3.3	−21.2	11.2	6.0	−13.8	7.4
ARG-572	Santa Clara	*Toxodon*	Humerus	Camet Norte North, level B	2.3	8.5	2.3	57.7	38.9	13.5	3.4	−20.8	10.3	5.9	−10.8	9.9
ARG-573	Santa Clara	*Macrauchenia*	Fibula (+ tibia)	Camet Norte North, no context	1.5	7.4	3.2	nd	31.9	11.0	3.4	−17.0	10.2	7.0	−9.3	7.6
ARG-569	Santa Clara	*Macrauchenia*	Fibula (+ tibia)	Camet Norte North, level B	3.3	10.8	2.0	287.3	41.3	14.4	3.3	−17.1	10.3	6.2	−9.3	7.8
ARG-563	MHM-P52	*Megatherium*	Molar, dentine (juvenile)	Rio Salado, General Belgrano	0.9	5.4	3.0	46.6	27.1	9.7	3.3	−18.7	14.0	5.4	−11.4	7.3
ARG-568	Santa Clara	*Megatherium*	Mandible	Camet Norte North, level B	1.8	6.9	2.2	70.2	35.5	12.4	3.4	−17.3	9.5	6.4	−9.0	8.3
ARG-575	Santa Clara	*Megatherium*	Molariform dentine	Camet Norte North, level B	1.6	6.5	2.2	45.2	38.8	13.2	3.4	−17.8	10.7	5.3	−8.4	9.4
ARG-570	Santa Clara	*Glossotherium*	Maxilla	Camet Norte North, level B	1.9	7.0	1.9	46.6	34.1	11.8	3.4	−19.4	10.4	5.3	−9.8	9.6
ARG-571	Santa Clara	*Doedicurus*	Osteoderm plates	Camet Norte North, level B	1.5	7.8	3.9	34.9	35.9	12.2	3.4	−20.9	10.4	6.2	−10.5	10.4
ARG-574	Santa Clara	*Glyptodon*	Pelvis	Camet Norte North, level B	2.0	8.1	2.8	79.1	41.7	14.5	3.4	−19.4	7.7	8.2	−10.2	9.2

We used a non-parametric test to evaluate the statistical significance of the differences of carbon isotopic values since the distribution of these values per category is not always normal. Statistical tests were performed using JMP® version 11.1.1.

4. Results and discussion

4.1. Modern samples

The modern dataset includes all the newly measured specimens which are presented in Table S1 (Supplementary data). Once combined with those already published in Bocherens (2000), Clementz et al. (2009) and Melin et al. (2014), they provide a complete dataset of 223 modern specimens (Supplementary data, Table S2). Only $\delta^{13}C$ values measured on bone carbonate were taken into account, since several studies have reported significant differences, up to 3‰, between values measured on bone and enamel of the same modern individual in terrestrial mammals (Warinner and Tuross, 2009; Gehler et al., 2012; Loftus and Sealy, 2012; Melin et al., 2014; Fahy et al., 2015). This complicating factor could lead to misinterpretations when comparing the modern results with those measured on bone carbonate for the fossil samples.

Considering all the modern samples together, there is a clear distinction in $\Delta\delta^{13}C_{carb-coll}$ values according to their trophic ecology. Especially clear are the differences between faunivores (including large and small carnivores, omnivores with high meat, piscivores, omnivores with low meat, and insectivores) and herbivores (small and large) (Fig. 2). The average $\Delta\delta^{13}C_{carb-coll}$ values of faunivores (4.31 ± 0.97, n = 127) considered globally are lower than those of herbivores (6.82 ± 1.13, n = 96), and the difference is highly statistically significant (p < 0.0001 in a non-parametric Wilcoxon test). Moreover, the average $\Delta\delta^{13}C_{carb-coll}$ values of each category of faunivores, i.e. large carnivores, small carnivores, piscivores, high meat omnivores, bears and low meat omnivores are lower and statistically different from the $\Delta\delta^{13}C_{carb-coll}$ values of large herbivores and small herbivores considered separately (Table 2). This means that each category of faunivore is clearly distinguishable from each category of herbivore using this isotopic approach based on the $\Delta\delta^{13}C_{carb-coll}$ values of modern bones.

When looking in more detail at the trophic categories among the faunivores, some further distinctions are visible (Table 2). For instance, the $\Delta\delta^{13}C_{carb-coll}$ values of small carnivores (3.69 ± 0.68, n = 17), piscivores (3.58 ± 0.54, n = 3), high meat omnivores (4.09 ± 0.86, n = 29), and bears (3.97 ± 0.83, n = 10) appear significantly lower than those of large carnivores ($\Delta\delta^{13}C_{carb-coll}$ = 4.59 ± 0.63, n =

36). In contrast, the $\Delta\delta^{13}C_{carb-coll}$ values of insectivores (4.39 ± 1.15, n = 13) and low meat omnivores (4.87 ± 1.38, n = 19) are not significantly different from those of large carnivores. It is worth noting that all insectivores considered in this comparison are Xenarthrans, either Myrmecophaga (giant anteater and tamandua) or armadillos. This means that, if ground sloths were insectivores or low meat omnivores, they should exhibit $\Delta\delta^{13}C_{carb-coll}$ values in a similar range as large carnivores. Interestingly, the $\Delta\delta^{13}C_{carb-coll}$ values of bears used in this study, which can be considered as large body size low meat omnivores, were lower and statistically different from those of large carnivores, suggesting that if large animals such as ground sloth have been low meat omnivores, they would not have had higher $\Delta\delta^{13}C_{carb-coll}$ values than large carnivores. The freshwater piscivores exhibit the lowest average $\Delta\delta^{13}C_{carb-coll}$ values of all analyzed faunivores, showing a similar trend as marine mammal predators, such as seals (Lee-Thorp et al., 1989). Among the analyzed faunivores, with varying amounts of animal food in their diet and diverse origin for their animal food, and from vertebrates or invertebrates, no category exhibit $\Delta\delta^{13}C_{carb-coll}$ values that may overlap with those of herbivores, either large or small. Therefore, this approach seems particularly suited to test the possible input of animal food of any kind in the diet of extinct giant Xenarthrans such as ground sloths.

Among the herbivores, $\Delta\delta^{13}C_{carb-coll}$ values appear significantly lower for small herbivores (including rodents, lagomorphs, monkeys and sloths: 5.85 ± 0.9‰, n = 29) than for large herbivores (including bovids, suids, large rodents, camelids, cervids, giraffes: 7.24 ± 0.95, n = 67), even if the ranges of both categories of herbivores overlap greatly (Fig. 1). This suggests that very large herbivores, such as extinct giant Xenarthrans, with larger body masses than the ones analyzed here, may have even larger $\Delta\delta^{13}C_{carb-coll}$ values than the ones presented here. However, woolly mammoth from Siberia do not present significantly higher $\Delta\delta^{13}C_{carb-coll}$ values than coeval horses, indicating that further research would be necessary to test the role of size in the $\Delta\delta^{13}C_{carb-coll}$ values of herbivores.

4.2. Fossil samples from the Pampean region

4.2.1. Reliability indicators

The first test for reliability of the carbon isotopic data measured on the carbonate fraction of the fossil bones was to look for possible statistically significant correlations between diagenetic indicators, such as nitrogen content of bone (%N_{bone}), amount of non-collagenous carbon (%C_{ncoll}), and percent of carbonate (%CO_3), and the difference between

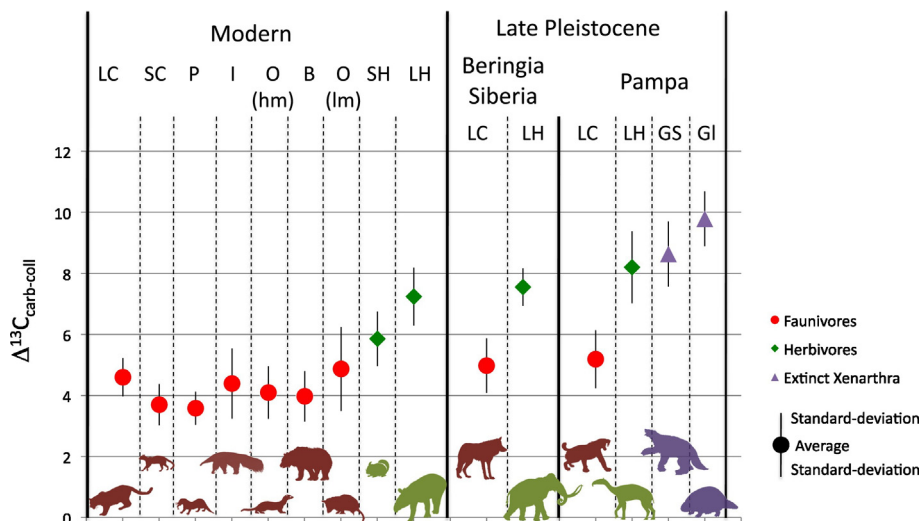Fig. 2. Summary of the $\Delta\delta^{13}C_{carb-coll}$ values for modern bones from species of different trophic categories, as well as fossil bones from Siberia and Beringia and fossil bones from the Pampean region.

Table 2
Non-parametric comparisons for each pair of trophic categories using the Wilcoxon test (threshold at p = 0.05 for statistical significance). Statistically significant results are shown in bold.

	n	avD13C ± sd	Large carnivore	Small carnivore	Piscivore	Insectivore	High meat omnivore	Bear	Low meat omnivore	Small herbivore	Large herbivore	Large carnivore (B)	Large herbivore (B)	Large carnivore (P)	Large herbivore (P)	Ground sloth (P)
n			36	17	3	13	29	10	19	29	67	5	64	5	4	4
avD13C ± sd			4.59 ± 0.63	3.69 ± 0.68	3.58 ± 0.54	4.39 ± 1.15	4.09 ± 0.86	3.97 ± 0.83	4.87 ± 1.38	5.85 ± 0.90	7.24 ± 0.95	4.98 ± 0.36	7.55 ± 0.61	5.19 ± 0.89	8.20 ± 1.18	8.64 ± 1.07
Small carnivore	17	3.69 ± 0.68	**<0.0001**													
Piscivore	3	3.58 ± 0.54	**0.0201**	0.6318												
Insectivore	13	4.39 ± 1.15	0.2916	0.0973	0.2006											
High meat omnivore	29	4.09 ± 0.86	**0.0008**	0.1473	0.2185	0.4538										
Bear	10	3.97 ± 0.83	**0.0122**	0.5956	0.3519	0.4019	0.5618									
Low meat omnivore	19	4.87 ± 1.38	0.2718	**0.0066**	0.1375	0.2909	**0.0197**	0.0662								
Small herbivore	29	5.85 ± 0.90	**<0.0001**	**<0.0001**	**0.0054**	**0.0003**	**<0.0001**	**<0.0001**	**0.0120**							
Large herbivore	67	7.24 ± 0.95	**<0.0001**	**<0.0001**	**0.0037**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**						
Large carnivore (B)	5	4.98 ± 0.36	0.1562	**0.0009**	**0.0358**	0.1819	**0.0027**	**0.0167**	0.9432	**0.0130**	**0.0003**					
Large herbivore (B)	64	7.55 ± 0.61	**<0.0001**	**<0.0001**	**0.0037**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0751	**0.0002**				
Large carnivore (P)	5	5.19 ± 0.89	0.0824	**0.0094**	0.0719	0.1384	**0.0194**	0.0572	0.6183	0.2228	**0.0008**	0.4606	**0.0002**			
Large herbivore (P)	4	8.20 ± 1.18	**0.0012**	**0.0026**	0.0518	**0.0039**	**0.0016**	**0.0058**	**0.0023**	**0.0026**	0.1341	**0.0195**	0.2614	**0.0195**		
Ground sloth (P)	4	8.64 ± 1.07	**0.0012**	**0.0026**	0.0518	**0.0039**	**0.0016**	**0.0058**	**0.0023**	**0.0022**	**0.0239**	**0.0195**	**0.0302**	**0.0195**	0.8852	
Glyptodontid (P)	2	9.79 ± 0.90	**0.0200**	**0.0274**	0.1489	**0.0336**	**0.0217**	**0.0409**	**0.0265**	**0.0218**	**0.0173**	0.0786	**0.0173**	0.0786	0.2472	0.4875

$\Delta\delta^{13}C_{carb\text{-}coll}$ values and the average value for the trophic or taxonomic categories combined (supplementary data). For each trophic (carnivore, herbivore) or taxonomic (large Xenarthrans) category, the average value for the difference between the $\delta^{13}C$ value of collagen and carbonate of the same sample was calculated and the difference between the measured value for each specimen and this average value for its trophic or taxonomic category was calculated ($\Delta\delta^{13}C_{carb\text{-}coll}$). This way, it is possible to use these values for the 15 fossil specimens as one standardized dataset.

The difference between $\Delta\delta^{13}C_{carb\text{-}coll}$ values and the average value for the trophic or taxonomic category appeared to be not correlated to any of the diagenetic indicators, i.e. either nitrogen content of bone (%N_{bone}), amount of non-collagenous carbon (%C_{ncoll}), or percent of carbonate (%CO_3) (Supplementary data, Fig. S1). Especially, the fossil bone specimens with the highest percent of carbonate, slightly higher than the percent of carbonate measured on modern bones, did not exhibit any isotopic trend compared to the others with lower carbonate content (Fig. S1). For all observed correlations, the Pearson r coefficient is well below the critical value for 15 samples for a level of significance of 0.05. Therefore there is no indication that changes related to post-mortem processes are responsible for the observed pattern of isotopic values.

Moreover, the expected general patterns for $\Delta\delta^{13}C_{carb\text{-}coll}$ values according to diet are similar to those observed in modern bones for the fossil specimens with unambiguous trophic ecology. Indeed, fossil large carnivores (*Smilodon*) exhibit lower $\Delta\delta^{13}C_{carb\text{-}coll}$ values (5.19 ± 0.89, n = 5) than fossil large herbivores (equid and meriungulata: 7.55 ± 0.61, n = 4) with no overlap and the difference is statistically significant (Table 2). Moreover, the $\Delta\delta^{13}C_{carb\text{-}coll}$ values of *Smilodon* are not statistically different from those of modern large carnivores (p = 0.0824), as it was the case for late Pleistocene large carnivores from Beringia (Clementz et al., 2009), and those of the fossil large herbivores are not significantly different from those of modern large herbivores (p = 0.1341), confirming that the carbonate $\delta^{13}C$ values of the fossil specimens have retained biogenic signatures that can be used for the diet reconstruction of the extinct species. This good preservation of the $\delta^{13}C$ values of the carbonate fraction of fossil bones in both sites used in the present study is probably linked to the fact that the analyzed bones have retained at least 25% and up to all of their collagen (based on the %N_{bone} ranging from ~1% to ~4%), which could have limited the risk of isotopic exchanges between the bone carbonate fraction and the circulating diagenetic pore fluids (e.g. Person et al., 1996; Sillen and Parkington, 1996; Trueman et al., 2004; Tütken et al., 2008).

The $\Delta\delta^{13}C_{carb\text{-}coll}$ values of the extinct giant Xenarthrans can therefore be discussed in palaeodietary terms in the next section.

4.2.2. Palaeodietary implications for extinct Xenarthrans

As expected, due to their morphological features indicating a herbivorous diet, the Glyptodontids exhibit high $\Delta\delta^{13}C_{carb\text{-}coll}$ values, even statistically higher than those of modern or Pleistocene herbivores from Beringia and Siberia (Iacumin et al., 2000; Clementz et al., 2009), but not statistically different from those of the large herbivores from the same context of the late Pleistocene Pampean region (Fig. 2, Table 2). This larger difference could be due to physiological differences between large herbivorous Xenarthrans and other mammals. Not much is known about the digestive physiology of Glyptodontids. Their closest relatives are the armadillos (e.g. Fernicola et al., 2008; Gaudin and McDonald, 2008; Delsuc et al., 2016; Mitchell et al., 2016), and no pure herbivorous diet is represented within the extant representatives of this group (Redford, 1985). Based on analogy with other extant herbivorous Xenarthrans, i.e. tree sloths that have a chambered stomach, it is supposed that extinct ground sloths also had chambered stomachs, while Glyptodontids, by analogy with modern armadillos, probably had simple sac-like stomachs rather than complex chambered stomachs (McDonald, 2005; Vizcaíno et al., 2006). Also, based on indirect evidence, it is usually considered that the metabolic rate of Glyptodontids

was probably low (McNab, 1985). This lower metabolic rate may have allowed Glyptodontids to eat nutritionally poor plants or consume plants with high levels of secondary metabolites (McDonald, 2005). A high fermentation rate for Glyptodontids could be a reason for the high $\Delta\delta^{13}C_{carb-coll}$ values exhibited by this group, but another hypothesis could be revived by this feature: Glyptodontids have been sometimes considered to be "aquatic grazers" rather than terrestrial (Gillette and Ray, 1981, but this suggestion is not widely accepted, see Fariña, 1995) and aquatic herbivores such as sea cows exhibit slightly higher $\Delta\delta^{13}C_{carb-coll}$ values than terrestrial herbivores (Clementz et al., 2009). Even if more isotopic data on Glyptodontids would be necessary to confirm the particularity of this group in terms of $\Delta\delta^{13}C_{carb-coll}$ values, it would be interesting to explore further the possibility of a special form of herbivory for this group, either in terms of plant consumed (including aquatic ones?) or digestive physiology (high fermentation rate?).

The analyzed ground sloths exhibit $\Delta\delta^{13}C_{carb-coll}$ values (8.64 ± 1.07, n = 4) that are similar and not statistically different from those of herbivorous mammals from the same sites, including the equid *Hippidion*, the notoungulate *Toxodon* and the liptoptern *Macrauchenia* (8.20 ± 1.18, n = 4), but slightly lower than those of Glyptodontids (9.79 ± 0.90, n = 2), even if the difference is not statistically significant (p = 0.4875) (Table 2). Interestingly, the $\Delta\delta^{13}C_{carb-coll}$ value measured on a *Megatherium* specimen from western Argentina was 7.6‰ (Praderio et al., 2012), very close to the values measured in the Pampean region. The $\Delta\delta^{13}C_{carb-coll}$ values of the ground sloths from the Pampean region are higher than those of modern large herbivores and fossil large herbivores form Siberia and Beringia with statistical significance, but they are much higher than all $\Delta\delta^{13}C_{carb-coll}$ values of fossil large carnivores from the same sites, as well as all modern faunivores and fossil carnivores from Siberia and Beringia (Table 2). Their $\Delta\delta^{13}C_{carb-coll}$ values are especially much higher than those of modern insectivorous Xenarthrans, low meat omnivores bears, and other low meat omnivores, with statistical significance (Table 2, Fig. 3). This strongly suggests that the studied ground sloths were essentially or even purely herbivorous and contradicts the hypothesis of ground sloths being purely insectivorous or carnivorous, as suggested by some researchers (Fariña, 1996; Fariña and Blanco, 1996; Genise and Fariña, 2012), or even occasional scavenger (Bargo and Vizcaíno, 2008). These results suggest that the high $\delta^{15}N$ values measured on bone collagen from some giant ground sloth individuals, especially *Megatherium*, were probably due to the consumption of plants with high $\delta^{15}N$ values, possibly due to arid conditions (e.g. Amundson et al., 2003; Murphy and Bowman, 2009) (Fig. 4). To test this hypothesis further, it would be necessary to perform $\delta^{15}N$ measurements on individual amino acids from collagen, as it was done to solve the riddle of high $\delta^{15}N$ values of mammoths and of some cave bears
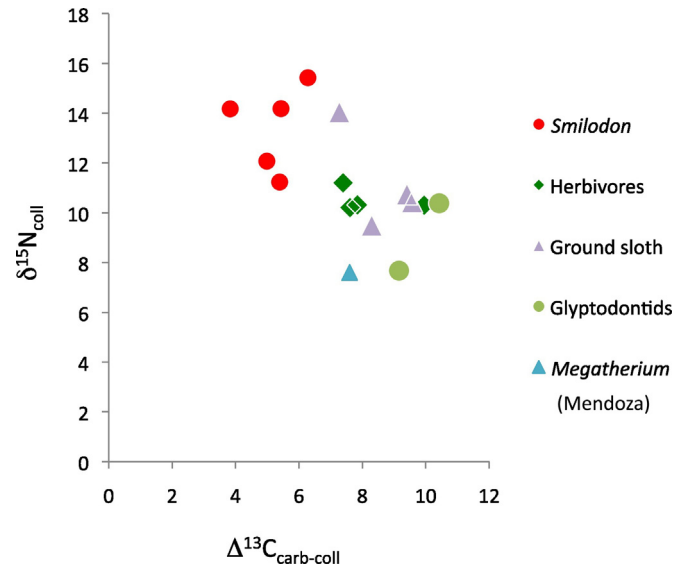

Fig. 4. Scatter-plot of collagen $\delta^{15}N$ values versus $\Delta\delta^{13}C_{carb-coll}$ values.

(Schwartz-Narbonne et al., 2015; Naito et al., 2016a, 2016b). In the present study, the $\Delta\delta^{13}C_{carb-coll}$ values of the three specimens of *Megatherium*, the genus for which carnivory was suggested (Fariña, 1996; Fariña and Blanco, 1996), clearly fall in the same range as the large herbivores such as the equid *Hippidion*, the notoungulate *Toxodon* and the liptoptern *Macrauchenia*, for which there is no doubt about their herbivorous diet. The same applies to a *Megatherium* specimen from Mendoza published by Praderio et al. (2012). Therefore *Megatherium* is not the cryptic flesh-eater suggested by some authors that could have accounted for the supposed imbalance of carnivores in the South American megafauna (Fariña, 1996; Fariña et al., 2014a). The one specimen of *Glossotherium* analyzed here also falls clearly within the herbivore $\Delta\delta^{13}C_{carb-coll}$ values. Further research on more genera of extinct giant ground sloth should allow us to determine if all members of this group were indeed herbivores, or if some exception occurred. Several species of this group survived in the late Pleistocene in South and North America and many bone specimens have yielded well-preserved collagen in South America (e.g. Czerwonogora et al., 2011; Prevosti and Martin, 2013; Prado et al., 2015) and North America (e.g. Bocherens et al., 1994b; Coltrain et al., 2004; France et al., 2007; Fariña et al., 2014b; Gilmour et al., 2015; McDonald et al., 2015), therefore being potential candidates for the same kind of isotopic investigation, provided that
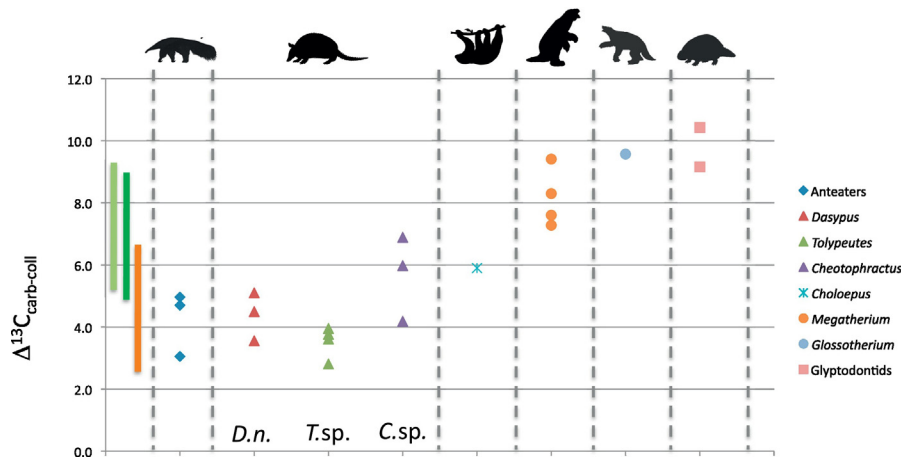

Fig. 3. Comparison of the $\Delta\delta^{13}C_{carb-coll}$ values of extant and extinct Xenarthrans. Keys for abbreviations: *D.n.* = *Dasypus novemcinctus* (nine-banded armadillo), *T.*sp. = *Tolypeutes* sp. (three-banded armadillo), *C.*sp. = *Choetophractus* sp. (greater hairy armadillo). The light green, dark green and orange lines represent the range for modern large herbivores, small herbivores and low meat omnivores, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the carbonate fraction of their bone is as well preserved in the case of the sites of the Pampean region studied here. To perform valid tests of reliability, one needs contexts where coeval herbivores and carnivores have been found together with the ground sloth, to verify that the $\Delta\delta^{13}C_{carb-coll}$ values according to the respective torphic category are still preserved.

5. Conclusion

Using the difference in carbon isotopic composition of the bone collagen and carbonate fraction of well-preserved fossil megamammal remains from the Pampean region, it was possible to decipher the trophic ecology of extinct giant Xenarthrans, i.e. Glyptodontids and giant ground sloths. It appears that none of the tested specimens exhibited $\Delta\delta^{13}C_{carb-coll}$ values that would indicate the inclusion of animal food in their diet. Therefore, the hypotheses of insectivory or carnivory for these extinct mammals are not supported by the isotopic data presented here. To use this approach further and test for possible dietary diversity among taxa and through time and space, the reliability of the carbon isotopic values of fossil bone must be checked carefully with two approaches, the first is to verify the absence of correlation between the carbon isotopic compositions and the values of some diagenetic indicators such as bone nitrogen content, extraneous carbon and carbonate content, the second is to check that fossil specimens from the same sites with predictable diets exhibit the expected isotopic values (i.e. trophic spacing between herbivores and faunivores). This isotopic approach could provide very valuable insights on the structure of terrestrial ecosystems (given that collagen is still preserved) including giant extinct Xenarthrans in North and South America before the end Pleistocene extinction.

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