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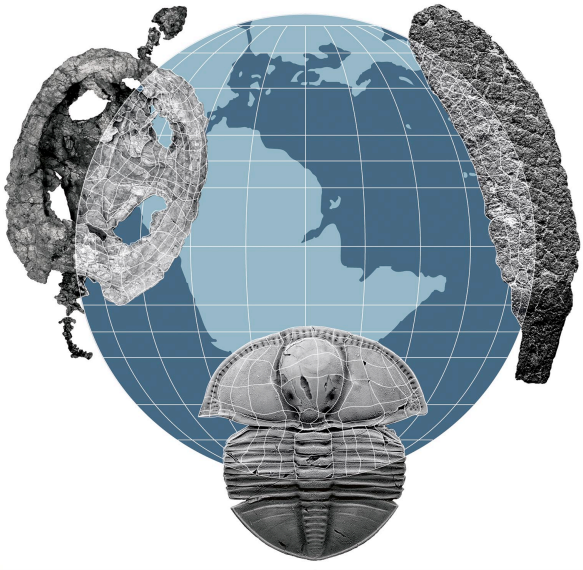

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FIRST AMS RADIOCARBON DIRECT DATES ON BONES FROM EXTINCT MEGAFAUNA IN CAMET NORTE (SANTA CLARA DEL MAR, BUENOS AIRES PROVINCE, ARGENTINA).

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23 Abstract

24 The first collagen AMS radiocarbon dates from the Camet Norte fossil site (Buenos Aires
25 Province, Argentina) range from ca. 24,730±110 years ^{14}C BP to 23,690±100 years ^{14}C BP, or
26 ca. 29,000 cal. BP to 27,600 cal. BP. The dates were obtained from specimens of the Level B
27 of this fossil site. The age of the site fits well with the results of previous studies, but the more
28 precise AMS radiocarbon dating allowed for a better determination of the site's chronology.
29 Models were used to better understand the chronological range of the investigated specimens.

30 Keywords:

31 AMS radiocarbon dates. Camet Norte. Argentina. Late Pleistocene. Pre-LGM. South America

32 Resumen:

33 Palabras clave:

34 edades radiocarbono. Camet Norte. Argentina. Pleistoceno tardío. Pre-LGM.

INTRODUCTION

South America is the continent that has been the most affected by the megafauna extinction around the Pleistocene/Holocene transition (Stuart, 2015). The Buenos Aires province, Argentina, has one of the most informative Pleistocene fossil records on the continent; it recorded the demise of large bodied mammals at the beginning of the Holocene when all mega mammals and about 80% of large mammals became extinct (e.g., Politis and Messineo, 2008; Cione *et al*., 2009; Steele and Politis, 2009; Prevosti and Schubert, 2013; Politis and Steele, 2014; Prado *et al*., 2015). Nearly all of them were adapted to the predominant open areas related to dry and cold climates of the late Quaternary (Cione *et al*., 2009).

However, understanding the relative impact that climate change and human colonization of South America had on extinction is difficult. A low number of reliable direct radiocarbon dates on megafaunal bones during the period corresponding to strong climatic oscillations and human immigrations, from around 30,000 to 10,000 years ago, pose a problem even if some progress has been made during the last decade (e.g. Barnosky and Lindsey, 2010; Prado *et al*., 2015). The number of taxon dates are very limited, even in comparison with other parts of South America like southern Patagonia (e.g., Metcalf *et al*. 2016). The low number of taxon dates limits the possibility to test competing or alternative hypotheses about the extinction of these mammals. Increasing the number of direct radiocarbon dates on late Quaternary megafauna fossils from South America is crucial to advance the debate of climate versus humans as main actors of the Pleistocene extinction (Barnosky *et al*., 2004; Cione *et al*., 2009).

The chronological context of most megafaunal bones from the latest Pleistocene is established through indirect methods. Therefore the chronology of Quaternary paleontological sites in the Pampean region is mainly constructed through biostratigraphic studies; many of the available radiocarbon dates were obtained from organic matter and molluscan shells (Scanferla *et al*.,

2013). This is the case for the rich fossil mammal site of Camet Norte. It has been tentatively dated to the time around the Last Glacial Maximum (approximately 29,000-15,000 years ^{14}C BP; Denton *et al*., 1999; Clark *et al*., 2009) with Pardiñas *et al*. (1998) reporting a date of 24,550±600 years ^{14}C BP produced from herbivore dung and Alberdi & Prado (2008) giving an AMS date for the tooth enamel of a *Stegomastodon* of 17,880±60 years ^{14}C BP. Therefore the Camet Norte site is of special interest for the discussion about the possible impact of climate change on Megafauna in South America. Here, we present the first AMS dates on bone collagen from the late Pleistocene fossil site of Camet Norte.

MATERIALS

Twenty-two bones from the site Camet Norte were sampled. The bones are part of the collection of the Museo Municipal de Ciencias Naturales Pachamama (Santa Clara del Mar, Buenos Aires Province, Argentina). The fossil site of Camet Norte is located in the community of Santa Clara del Mar, approximately 20km north of Mar del Plata in the Buenos Aires Province of Argentina. Previous publications have described the stratigraphy and the fauna found in this site (e.g. Fasano *et al*., 1984; Pardiñas *et al*., 1998; Alberdi & Prado, 2008) including some dating attempts (Pardiñas *et al*., 1998; Alberdi & Prado, 2008). The lithostratigraphy and sedimentary structures of the site have been described in some detail by Fasano *et al*. (1984) and Pardiñas *et al*. (1998) and are associated with finely laminated, greenish sediments, which are exposed in the local sea cliffs. The site has been originally interpreted as a freshwater palaeo-lagoon near the coastline (Fasano *et al*., 1984). All bones come from the level B, according to Fasano *et al*. (1984), of the site, which is a clayey silt and clay deposit of greenish colour with a thin and convolute lamination (Fasano *et al*., 1984). All sampled specimen were eroded out of the cliff which makes a clear ascription to any position within level B beyond their association with that level impossible. The level B of Fasano *et al*.

(1984) corresponds to approximately the height between metres 0.25 to 1.45 in the profile (Fig. 1).

The collagen preservation was expected to be good as herbivore dung has been well preserved and was radiocarbon dated by Pardiñas *et al*. (1998) at 24,550 ± 600 BP. Of the 22 sampled bones, ten of them yielded collagen and were used in a palaeo-ecological investigation using stable isotope tracking (Bocherens *et al*., 2016), among which four were used for dating. The dated samples encompass various bones from taxa that became extinct at the end of the Pleistocene or beginning of the Holocene (Cione *et al*., 2009): they include a vertebra from the sabre-toothed cat *Smilodon populator*, two specimens from large bodied, endemic ungulates, specifically a *Macrauchenia* fibula and a *Toxodon* humerus and lastly a pelvic bone from the large, armoured armadillo relative *Glyptodon*.

METHODS

The collagen used for this study is the same previously used for the palaeo-ecological investigation of Bocherens et al. (2016). The sampling was conducted using a rotary tool with a circular diamond-coated blade. Small pieces of bone were sawn out, all being in the range of 0.4 to 1.9 grams. The bone fragments were treated using the protocol described in Bocherens *et al*. (1997a). To clean the pieces from remaining soil they were washed with acetone in an ultrasonic bath and then rinsed and washed with distilled water. The bones were dried at room temperature, then crushed and sieved using a 0.7 mm mesh (Bocherens *et al*., 1997a). To assess the degree of collagen preservation a subsample of each specimen was analysed for its nitrogen content (%N). The analysis was done using a Vario EL III elemental analyser with Sulfanilic acid from Merck as an internal standard. The mean standard errors for %N were better than 0.05%. The elemental analysis was performed at the Laboratory for Soil Science and Geoecology of the Department of Geosciences at the University of Tübingen (Germany).

Samples that promised good collagen preservation – those with a nitrogen content between 0.4% and 4% (Bocherens *et al*., 2005) – underwent collagen purification. The collagen was extracted from the samples following the protocol described in Bocherens *et al*. (1997a). The bone powder obtained from crushing the bones was decalcified in approximately 40 ml of 1 M HCl at room temperature (Bocherens *et al*., 1997a). Next the samples were filtered using a 5µm millipore filter and the residue was soaked in approximately 40 ml of 0.125 M NaOH at room temperature for 20 hours (Bocherens *et al*., 1997a). The samples were again filtered using a 5µm millipore filter. To gelatinise the remaining residue, it was collected in closed tubes with a 0.01 M HCl (pH 2) solution and cooked at 100°C for 17 h (Bocherens *et al*., 1997a). The sample solution was again filtered using a 5µm millipore filter and collected in closed glass vials. The filtrate was freeze-dried (Bocherens *et al*., 1997a). DeNiro (1985) and others (e.g. Ambrose, 1990; van Klinken, 1999) have shown in their studies that elemental carbon/nitrogen ratios (C/N) lower than 2.9 or higher than 3.6 are indicative of alteration and contamination in the collagen. Similarly, bone with a nitrogen content of less than 0.7% has been shown to not yield sufficient collagen for ^{14}C AMS dating (Brock *et al*., 2012). Thus the quality of the extracted collagen, and therefore the reliability of dates, was verified by using the bone powder's nitrogen (N_t) content as well as the collagen's carbon (%C) and nitrogen (%N) content and the resulting elemental ratios C/N. The ratios were calculated as atomic ratios. The measurements were conducted with an elemental analyzer NC 2500 at the Isotopic Geochemistry unit of the Department of Geosciences at the University of Tübingen (Germany).

Four of the ten samples that yielded collagen were dated using AMS radiocarbon dating. The radiocarbon dating was performed by the Labor für Ionenstrahlphysik at ETH in Zurich (Switzerland). The obtained radiocarbon ages were calibrated using the software OxCal v4.2.4 with the atmospheric curve ShCal13 (Hogg *et al*. 2013). The results were modelled in a

sequence model including existing dates for the site and a combination model was done for the new AMS dates to test the hypothesis of contemporaneousness.

RESULTS

The results for the elemental analysis, the radiocarbon dating as well as the calibration were summed up in Table 1. All radiocarbon dated collagen exhibited excellent preservation. The %C and %N as measured by elemental analysis ranged from 38.9% to 45.0% and from 13.5% to 14.9%, respectively (Tab. 1). All the C/N ratios are within the normal range and the amount of dated carbon is at least 1 mg for each collagen (Tab. 1). Therefore all radiocarbon ages can be considered reliable. The AMS radiocarbon dates obtained narrowly ranged from 23,690±100 BP to 24,730±110 ^{14}C BP. The calibration was performed with two standard-deviations. The calibrated ages ranged from 27,936 – 27,561 cal. BP to 28,988 – 28,440 cal. BP (Fig. 2).

The combination model for the four new AMS samples yielded a modelled age of 28,718 cal. BP to 28,391 cal. BP. The model detected an outlier though. The Toxodon sample ETH-57490 (27,936 cal. BP to 27,561 cal. BP) failed a X^2 test ($p<0.05$) for goodness of fit. This sample is unlikely to be contemporaneous with the remaining samples and was deposited later. A new model was run, excluding the outlier ETH-57490, to determine a modelled age of contemporaneousness for the remaining three samples. The newly modelled age ranges from 28,722 cal. BP to 28,410 cal. BP for the samples ETH-57488, ETH-57489 and ETH-57491 (Fig. 3). The modelled envelop is not markedly affected by the removal of the outlier suggesting that the modelled age of contemporaneousness is robust. The combined age shows a relatively high agreement index Acomb of 89.1 while the model exhibits an agreement index of 90.2 with an overall agreement index for the calibration of 96.6.

DISCUSSION

158 This study yielded the first AMS dates on bone collagen for the Camet Norte site. The dating
159 of dung (30,087 cal. BP to 27,580 cal. BP) has shown that organic material has been
160 preserved at the site and therefore collagen preservation was expected under the same
161 conditions (e.g. Bocherens *et al*. 1997b). Even in such a promising context, more than half of
162 the tested bones failed to yield good collagen, making it necessary to screen collagen
163 preservation beforehand using nitrogen bone analysis (Bocherens *et al*., 2016). The results
164 refine the chronological assignment of the site to the period before the Last Glacial Maximum
165 (pre-LGM time) at around 28,000 to 29,000 years cal. BP.

166 The dates measured in this study cover a relatively narrow time range of no more than 2400
167 years , even when the lowest probabilities are taken into account. This narrow time range
168 holds true even though the dates were obtained on various species and specimens including
169 the extinct megafauna taxa *Glyptodon*, *Macrauchenia*, *Smilodon* and *Toxodon*. Furthermore,
170 the four new dates are congruent with the conventional radiocarbon date of herbivore dung
171 reported by Pardiñas *et al*. (1998), which dates to 24,550±600 years ^{14}C BP, equivalent to
172 30,087 to 27,580 years cal. BP. The newly dated specimens are reported to come from the
173 level B of the site (as defined by Fasano, 1984), while the dung dated by Pardiñas *et al*.
174 (1998) corresponds to the level A of the site. This would suggest that the levels A and B,
175 although being lithostratigraphically different, were deposited within a very limited time
176 frame and that the four newly dated specimen likely originate from the base of level B.

177 The fact that three specimens of different orders yielded contemporaneous dates, and the
178 outlier ETH-57490 varies by only about one thousand years, suggests that most fossil remains
179 of this site date to the same period. In contrast, Alberdi & Prado (2008) produced an AMS
180 date for the tooth enamel of a *Stegomastodon* to 17,880±60 years ^{14}C BP, corresponding to
181 21,842 to 21,385 years cal. BP. This tooth, associated with the upper part of the level B (as
182 defined by Fasano, 1984), would thus be much younger than both organic materials -dung and

183 bone collagen- that were radiocarbon dated. Tooth enamel is considered to have higher
184 crystallinity and lower porosity than other bioapatites (e.g., Ayliffe *et al*., 1992; Vallet-Regí &
185 Navarrete, 2016). Nonetheless the reliability of the age obtained on the carbonate fraction of
186 enamel must be called into question since several studies have shown that radiocarbon dates
187 done on tooth enamel suffer from increased levels of contamination with increasing age (e.g.
188 Hedges *et al*., 1995; Zazzo, 2014). Considering the spatially narrow depositional context of
189 the Camet Norte site, the tooth might have originated from younger layers not associated with
190 the fossil bearing lens that produced the newly analysed specimen. The radiocarbon date of
191 the tooth enamel does however seem to confirm the chronological assessment of the site as
192 Late Pleistocene and is thus consistent with the general interpretation.

193 The tephra layers in the upper section of the sites' level C were dated by Bigazzi *et al*. (1995)
194 with the fission track method. This dating yielded an age of 21,000±7,000 years BP. This
195 would make the layers at most contemporaneous with our measurements, but likely younger.
196 Should the AMS date of Alberdi and Prado (2008) prove to be accurate it would mean that the
197 tephra layers are likely younger or at most 21,000 years old. Gentile and Ribot (2001) have
198 investigated tephra layers of the late Pleistocene to Holocene in the Buenos Aires Provinces.
199 They found that the Olavarría tephra layers of the Arroyo Tapalqué site in the Guerrero
200 member must have been deposited around 19,320 and 21,760 years cal. BP. The Camet Norte
201 site is similarly considered part of the Guerrero member (Prado and Alberdi, 1999) and
202 stratigraphically correlated with the Guerrero member and Luján Formation in the Arroyo
203 Tapalqué site. Yet, it could not be determined by Gentile and Ribot (2001) if the tephra layers
204 of Camet Norte were contemporaneous to the Olavarria tephra. Nevertheless the dates
205 correspond well to the fission track dating of Bigazzi *et al*. (1995) and could thus give a good
206 indication for framing the time range of deposition for the site.

Based on this assumption it seems that the entire depositional sequence of the Camet Norte site was deposited in a narrow timeframe roughly between after 30,000 and before 19,000 to 14,000 years ago. The onset of the deposits formation would therefore correspond to a period of falling global sea levels in response to the global glaciation reaching the extent of the LGM between 33,000 and 26,500 years ago (Clark *et al*., 2009). Similarly the youngest possible age for this deposit would correspond to a marked global increase in sea level at around 14,500 years ago (Clark *et al*., 2009).

The Argentinian shelf has been shown to experience sea level variations of up to 100m during the late Pleistocene (e.g. Guilderson *et al*., 2000; Rostami *et al*., 2000; Ponce *et al*., 2011). If we consider either the site's deposition as a whole or the much tighter constrained time range of the collagen AMS dates, sea levels were 70 to 100m lower than today (Guilderson *et al*., 2000). Such a shift would push the palaeo-coastline approximately to the shelf break line about 200km further eastward from its current setting. If no other factors are taken into account, such as uplift or subsidence, the fossil site of Camet Norte could potentially be an inland palaeo-environment rather than a freshwater lagoon close to the shoreline, as originally suggested by Fasano *et al*. (1984). However, this hypothesis would need further proof and necessitate an investigation of more direct sea level proxies.

As the site is attributed to the Guerrero member it would also correspond to colder and dryer conditions than today (Prado and Alberdi, 1999). Colder and dryer conditions would likewise be indicated by climatic curves produced for the southern hemisphere during the dated time range (Pahnke *et al*., 2003). Similarly the presence of some extant taxa (i.e., *Lyncodon patagonicus*, *Microcavia* sp.) in this site that in the present time are limited to southern and western areas, was interpreted as evidence of the presence of drier conditions during the deposition of this assemblage (Pardiñas *et al*., 1998; Prevosti and Pardiñas, 2001). The high

231 nitrogen isotopic values measured on the bone collagen from mammals from Camet Norte are
232 also consistent with a dry context (Bocherens *et al*., 2016).

233 **CONCLUSIONS**

234 The bones of four different individuals belonging to different species from the Camet Norte
235 fossil site in the Buenos Aires Province of Argentina were dated. They yielded ages shortly
236 predating the onset of the Last Glacial Maximum. Three of the specimens seemed to be
237 contemporaneous and allowed the modelling of a combined age ranging from 28,722 cal. BP
238 to 28,410 cal. BP. The remaining sample yielded a slightly younger date at 27,936 cal. BP to
239 27,561 cal. BP. The new dates fit into the frame set by previous datings and allow for a more
240 precise determination of the site's chronology. The narrow time interval between the
241 previously dated Level A and the dates of Level B presented in this study, indicate a fast
242 deposition of the respective levels.. The new age attribution in conjunction with models of
243 eustatic sea level change allows the hypothesis of an inland depositional environment for the
244 site. However this would be in contrast to the current understanding of the sight's deposition
245 as a coastal fresh water lagoon. With the site modelled not as close to the shoreline as
246 previously indicated, a renewed evaluation of the site's depositional environment might be
247 necessary.

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376 Figure captions:

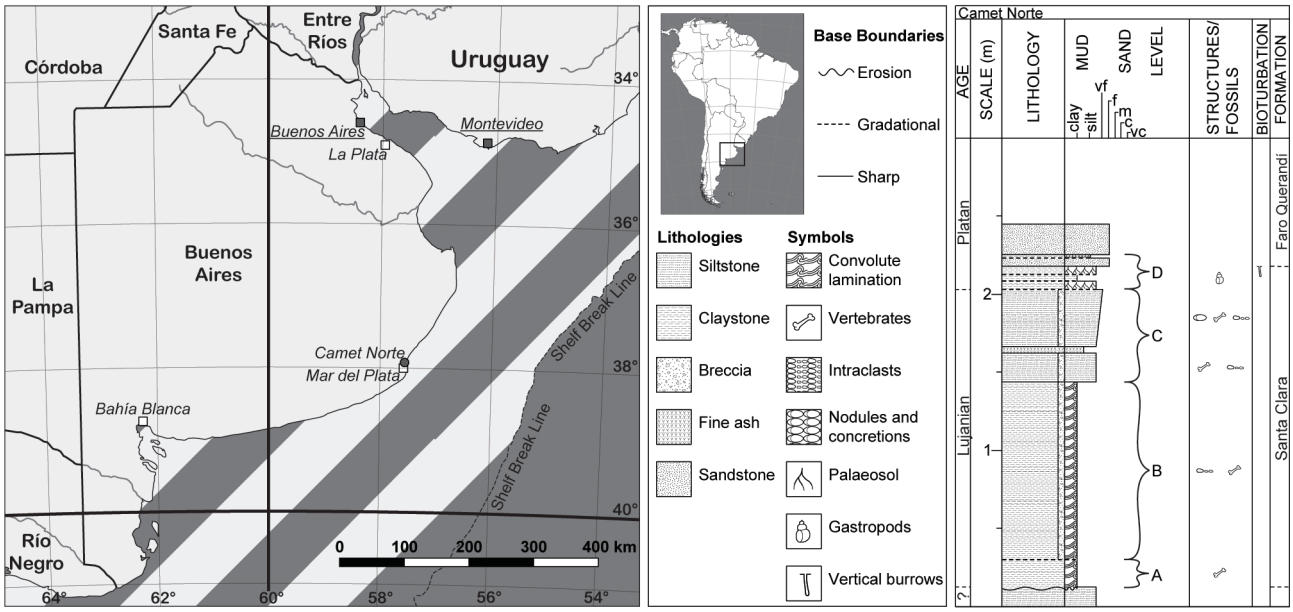
377 Figure 1: Location map showing the Camet Norte locality and a stratigraphic profile of the
378 coastal cliffs at the site.

379

380 Figure 2: Calibration curve with calibrated dates (cal BP) of Collagen samples from Camet
381 Norte.

382

383 Figure 3: Model comparing previous dating attempts on dung (LP-760) and carbonate from
384 tooth enamel (Stegomastodon) with new AMS dates on collagen from Camet Norte.
385 Samples ETH-57491, ETH-57488 and ETH-57489 have been tested for concurrence
386 and a hypothetical age of coeval existence was determined.


Santa Fe
Entre Ríos
Córdoba
Uruguay
34°
Buenos Aires
La Plata
Montevideo
36°
Buenos Aires
La Pampa
Camet Norte
Mar del Plata
38°
Shelf Break Line
Bahía Blanca
Shelf Break Line
40°
Río Negro
0 100 200 300 400 km
64°
62°
60°
58°
56°
54°
Base Boundaries
Erosion
Gradational
Sharp
Lithologies
Siltstone
Claystone
Breccia
Fine ash
Sandstone
Symbols
Convolute lamination
Vertebrates
Intraclasts
Nodules and concretions
Palaeosol
Gastropods
Vertical burrows
Camet Norte
AGE
SCALE (m)
LITHOLOGY
MUD
SAND
LEVEL
clay
silt
vf
f
m
c
vc
STRUCTURES/ FOSSILS
BIOTURBATION
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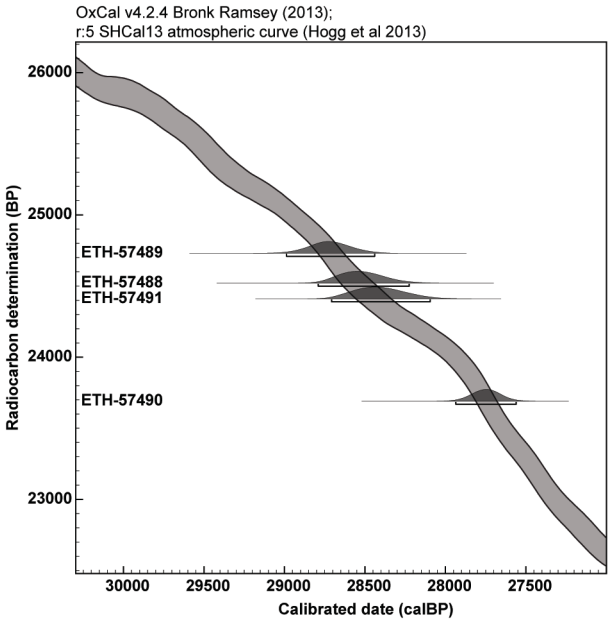
Faro Querandí
Santa Clara


OxCal v4.2.4 Bronk Ramsey (2013);
r:5 SHCal13 atmospheric curve (Hogg et al 2013)
26000
25000
24000
23000
Radiocarbon determination (BP)
ETH-57489
ETH-57488
ETH-57491
ETH-57490
30000
29500
29000
28500
28000
27500
Calibrated date (calBP)

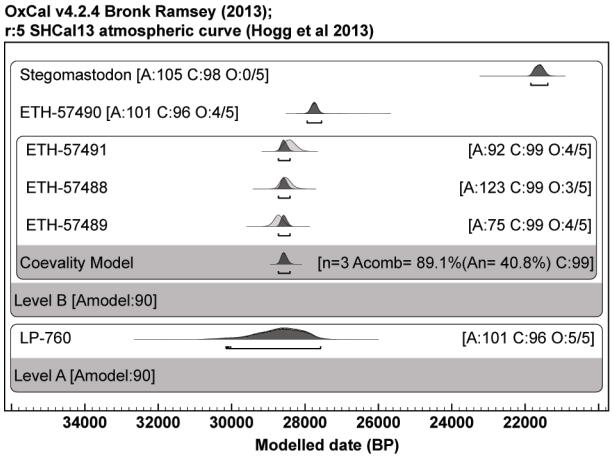

OxCal v4.2.4 Bronk Ramsey (2013);
r:5 SHCal13 atmospheric curve (Hogg et al 2013)
Stegomastodon [A:105 C:98 O:0/5]
ETH-57490 [A:101 C:96 O:4/5]
ETH-57491 [A:92 C:99 O:4/5]
ETH-57488 [A:123 C:99 O:3/5]
ETH-57489 [A:75 C:99 O:4/5]
Coevality Model [n=3 Acomb= 89.1%(An= 40.8%) C:99]
Level B [Amodel:90]
LP-760 [A:101 C:96 O:5/5]
Level A [Amodel:90]
34000
32000
30000
28000
26000
24000
22000
Modelled date (BP)

TABLE 1 – Previous and new radiocarbon dates from Camet Norte

Lab #	*Sample #*	*Taxon*	*Source*	*bone*	*collagen*			*C^{14}*	*±1σ*	*mg C*	*from*	*to*
				N_t [%]	*%C*	*%N*	*C/N*	*(BP)*			*cal. BP (-95.4%)*	
ETH-57488	*ARGC-44*	**Smilodon**	*vertebrae*	*3.75*	*45.0*	*14.9*	*3.5*	*24,520*	*110*	*1.00*	*28,790*	*28,225*
ETH-57489	*ARGC-48*	**Macrauchenia**	*fibula*	*3.27*	*41.3*	*14.4*	*3.3*	*24,730*	*110*	*0.98*	*28,988*	*28,440*
ETH-57490	*ARGC-61*	**Toxodon**	*humerus*	*2.28*	*38.9*	*13.5*	*3.4*	*23,690*	*100*	*1.00*	*27,936*	*27,561*
ETH-57491	*ARGC-63*	**Glyptodon**	*pelvis*	*1.96*	*41.7*	*14.5*	*3.4*	*24,410*	*110*	*1.00*	*28,707*	*28,095*
-	*LP-760[1]*	*"Herbivore"*	*dung*	*-*	*-*	*-*	*-*	*24,550*	*600*	*-*	*30,087*	*27,580*
-	*Stegomastodon[2]*	**Stegomastodon**	*m3*	*-*	*-*	*-*	*-*	*17,880*	*60*	*-*	*21,842*	*21,385*

The radiocarbon ages were calibrated using the software OxCal v4.2.4 with the atmospheric curve ShCal13 by Hogg et al. (2013).
1) Pardiñas et al. (1998)
2) Alberdi and Prado (2008)