

## ANTHROPOLOGY

# Campo Laborde: A Late Pleistocene giant ground sloth kill and butchering site in the Pampas

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The extinction of Pleistocene megafauna and the role played by humans have been subjects of constant debate in American archeology. Previous evidence from the Pampas region of Argentina suggested that this environment might have provided a refugium for the Holocene survival of several megamammals. However, recent excavations and more advanced accelerator mass spectrometry radiocarbon dating at Campo Laborde site in the Argentinian Pampas challenge the Holocene survival of Pleistocene megamammals and provide original and high-quality information documenting direct human impact on the Pleistocene fauna. The new data offer definitive evidence for hunting and butchering of *Megatherium americanum* (giant ground sloth) at 12,600 cal years BP and dispute previous interpretations that Pleistocene megamammals survived into the Holocene in the Pampas.

## INTRODUCTION

The late Quaternary megafaunal extinctions at the end of the Pleistocene, resulting in the loss of between 35 and 90% of large-bodied animal species on ice-free continents (excluding Africa), represented the most profound faunal transition that Earth's ecosystems experienced during the Cenozoic, but the cause or causes of this event remain hotly debated (1–4). In the New World, the causes and dynamics of the extinctions have proved especially challenging to elucidate because they coincided closely both with end-Pleistocene climate changes and with the invasion of a new predator—*Homo sapiens* (2, 3, 5, 6). Understanding this extinction in South America, both in archeology and paleontology, has suffered primarily from a lack of chronological control for the disappearance of megafauna taxa, as well as data on the extent of their temporal overlap and interactions with humans (3, 4, 7–9).

South America is particularly important for investigations of late Quaternary extinctions because the continent lost more megafauna taxa than any other continent during this event (2, 3, 9, 10). The answer to the questions of the underlying causes may reveal fundamental principles of ecology—are extinctions synchronous across species, are they abrupt or gradual, why do some taxa survive in refugia while the majority are lost, and can a single new species disrupt ecosystems in equilibrium for hundreds of millennia?

Previous studies of the late Quaternary extinctions in South America have found evidence for both human-driven (11) and climate-driven (12) megafauna loss, as well as synergistic impacts of these two factors (3, 13). However, direct evidence of human predation on extinct megafauna in South America is rare (8), despite thousands of years of apparent overlap in some regions (9) and evidence that some taxa could even have survived into the Holocene (10, 14, 15).

We use the Argentinian archeological site, Campo Laborde, to demonstrate that humans hunted and butchered a giant ground sloth, that advanced radiocarbon dating techniques do not support Holocene survival of megafauna in the area, and that these new

dates cast doubts on other published Holocene ages for Pleistocene fauna in the Pampas.

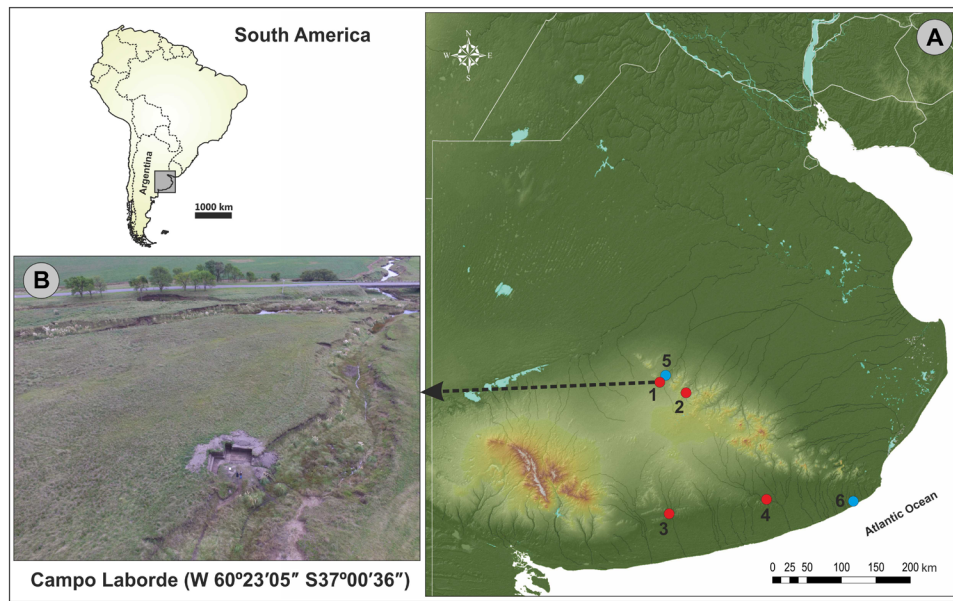
The essential data for assessing the interactions of fauna, climate, and humans are accurate chronologies for extinctions, climate changes, and the timing and rates of human dispersals. These data must derive from well-dated archeological sites having indisputable association with remains of extinct taxa (16–18). While most New World archeological sites with unquestionable extinct megafauna have Late Pleistocene dates [~14,500 to 13,000 cal years before the present (BP)], some sites in the South America Pampas (Fig. 1A) have been dated to the Early and Middle Holocene (10, 19–23), thereby causing archeologists and paleontologists to question the impact of humans on Late Pleistocene extinctions (3, 7, 15). Besides Campo Laborde, archeological sites that supported Holocene survivals of megafauna include La Moderna (19), Arroyo Seco 2 (18, 24), and Paso Otero 4 (22), as well as paleontological localities that yielded Early Holocene dates on extinct megamammals such as Arroyo Tapalqué (10) and Río Cuarto (25).

Of these sites, La Moderna has provided the greatest number of Early and Middle Holocene dates. It is an open-air site located on the banks of the Azul Creek (Fig. 1A), where a close stratigraphic association between lithic artifacts (expedient tools of crystalline quartz and some curated tools of orthoquartzite and chert) and extinct faunal remains [*Doedicurus clavicaudatus* (Glyptodontidae)] was confirmed (19). La Moderna is interpreted as a procurement site, where, during a single event, a *D. clavicaudatus* was butchered at the edge of an ancient swamp (19). The chronology of this event has always been problematic and controversial. For these reasons, multiple samples have been run on *D. clavicaudatus* bone collagen and organic sediment samples. The first dating result was a standard age processed by Beta Analytic and yielded an age considered to be too young:  $6550 \pm 160$  <sup>14</sup>C years BP (Beta-7824). A second bone sample yielded an accelerator mass spectrometry (AMS) age of  $12,350 \pm 370$  <sup>14</sup>C years BP (TO-1507) through the IsoTrace Lab. Two additional dates from the same bone from the same laboratory with a more appropriate pretreatment yielded ages of  $7010 \pm 100$  <sup>14</sup>C years BP (TO-1507-1) and  $7510 \pm 370$  <sup>14</sup>C years BP (TO-1507-1). Last, radiocarbon dating a third bone fragment gave a result close to these ages [ $7460 \pm 80$  <sup>14</sup>C years BP (TO-2610)]. Two organic samples were taken from lithostratigraphic unit (a'), which contained the archeological deposit produced ages of  $8356 \pm 65$  <sup>14</sup>C years BP (DRI-3012) and  $7448 \pm 109$  <sup>14</sup>C years BP [DRI-3012 (19, 26)]. As a consequence,

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**Fig. 1. Location of Campo Laborde site in South America.** (A) Map of the Pampas grassland of Argentina (province of Buenos Aires), showing main sites mentioned in the text. References: 1, Campo Laborde site; 2, La Moderna site; 3, Arroyo Seco 2 site; 4, Paso Otero 4 and 5 sites; 5, Arroyo Tapalqué; 6, Centinela del Mar; red circles, archeological sites; blue circles, paleontological sites. (B) Aerial view of excavation area, picture courtesy of C. F. Dubois. [Photo credit: Dr. Cristian Favier Dubois. INCUAPA-CONICET, Facultad de Ciencias Sociales (UNICEN)].

this series of dates suggested that the age of La Moderna was between 7000 and 7500  $^{14}\text{C}$  years BP. The  $6550 \pm 160$   $^{14}\text{C}$  years BP date was considered too young, while the  $12,350 \pm 370$   $^{14}\text{C}$  years BP age was discarded as too old; both dates were considered as outliers (26).

The other site with Holocene dates on extinct fauna is Arroyo Seco 2. The site presents abundant evidence related to the study of the Pleistocene megafauna (7, 18, 24). This multicomponent, open-air locality lies between a low ridge of loess and a small stream, Arroyo Seco Creek (Fig. 1A). The Arroyo Seco 2 site has an early component containing a lithic assemblage composed of unifacial, marginally re-touched tools associated with bone remains of nine extinct taxa: *Equus neogeus*, *Hippidion* sp., *Toxodon platensis*, *Megatherium americanum*, *Eutatus seguini*, *Glossotherium robustum*, *Macrauchenia* sp., *Glyptodon* sp., and Camelidae cf. *Hemiauchenia* (18). Three extinct species found in the early component show evidence of human exploitation: *E. neogeus*, *Hippidion* sp., and *M. americanum*. The lower component of Arroyo Seco 2 is interpreted to be the result of several, low-resolution, human occupations at the end of the Pleistocene. In the late 1980s and early 1990s, three radiocarbon ages from bone collagen of *E. (Amerhippus) neogeus* and *M. americanum* yielded Early Holocene ages:  $8890 \pm 90$   $^{14}\text{C}$  years BP (TO-1504),  $8470 \pm 240$   $^{14}\text{C}$  years BP (LP-53), and  $7320 \pm 50$   $^{14}\text{C}$  years BP [TO-1506 (7)]. However, 15 AMS ages on megafauna bones, from different taxa, from Arroyo Seco 2 run at different radiocarbon laboratories yielded ages between  $12,240 \pm 110$   $^{14}\text{C}$  years BP and  $10,500 \pm 90$   $^{14}\text{C}$  years BP (18). Among these, three new results were particularly substantial as they were obtained on the same *M. americanum* bone sample previously dated to  $7320 \pm 50$   $^{14}\text{C}$  years BP. These new results negate the Holocene age of the sample and place it at the end of the Pleistocene:  $12,200 \pm 170$   $^{14}\text{C}$  years BP (CAMS-58182),  $12,155 \pm 70$   $^{14}\text{C}$  years BP (OxA-10387), and  $11,770 \pm 120$   $^{14}\text{C}$  years BP (AA-62514). Therefore, the  $7320 \pm 50$   $^{14}\text{C}$  years BP and  $8470 \pm 240$   $^{14}\text{C}$  years BP measurements should both be rejected. The age of  $8890 \pm 90$   $^{14}\text{C}$  years BP from *E. neogeus* was also not replicated. Four ages, from separate

bone samples of *Equus*, gave Late Pleistocene ages [between  $11,320 \pm 110$   $^{14}\text{C}$  years BP (AA-39365) and  $11,000 \pm 100$   $^{14}\text{C}$  years BP (OxA-4590)]. As a consequence, the new group of radiocarbon dates from Arroyo Seco 2 does not appear to support a Holocene fauna survival at the site as previously proposed (7), with the exception of medium-sized *E. seguini* [dated in  $7388 \pm 74$   $^{14}\text{C}$  years BP (AA-90117)] (18).

The third site with a purported Holocene age is Paso Otero 4 (Fig. 1A). There are no direct dates on extinct faunal bones despite several failed attempts. The only dates for Paso Otero 4 are Early Holocene  $^{14}\text{C}$  dates on humates [between  $8913 \pm 49$   $^{14}\text{C}$  years BP (AA-87938) and  $7729 \pm 48$   $^{14}\text{C}$  years BP (AA-85157)] from the lower unit 2 containing *E. seguini* bones (22). Other  $^{14}\text{C}$  Holocene dates from extinct fauna in the Pampas (Fig. 1A) include two dates on *Scelidotherium leptocephalum*, one of  $7615 \pm 85$   $^{14}\text{C}$  years BP (GrA-48388) from Arroyo Tapalqué (10) and another of  $7550 \pm 60$   $^{14}\text{C}$  years BP (LP-1407) from Río Cuarto (25). Last, an age of  $9890 \pm 50$   $^{14}\text{C}$  years BP (GrA-49321) was reported for *Macrauchenia patachonica* from Centinela del Mar (10).

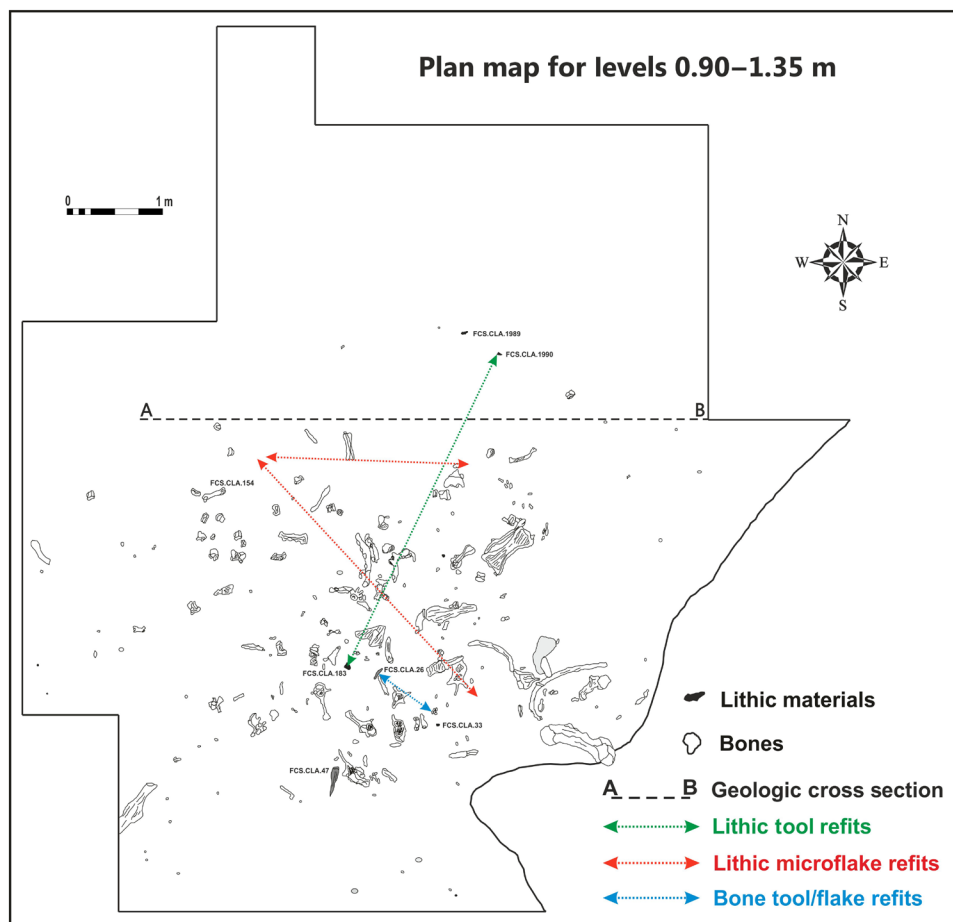
The Campo Laborde site can provide high-quality data for discussing the human impact on the Pleistocene fauna and the timing of the megamammal extinctions in the Pampas and in South America due to its good stratigraphic resolution and high-accuracy radiocarbon dates. The site is located in the upper basin of Tapalqué Creek, ~15 km north-northeast of Olavarría city (Pampas region of Argentina; Fig. 1, A and B). In 2000, the landowner discovered in situ a complete femur and vertebrae fragments from a giant ground sloth, *M. americanum*. The original test pits were started here, and the site was expanded with subsequent excavations during two field programs. The first was between 2001 and 2003, when an area of  $29 \text{ m}^2$  was excavated (20, 21). The second field session was in 2016 and 2017, when a new area of  $21 \text{ m}^2$  was excavated. This report presents and integrates the findings of the 2016–2017 field seasons and includes the new radiocarbon dates made with more advanced and accurate methods for radiocarbon dating bone.

## RESULTS

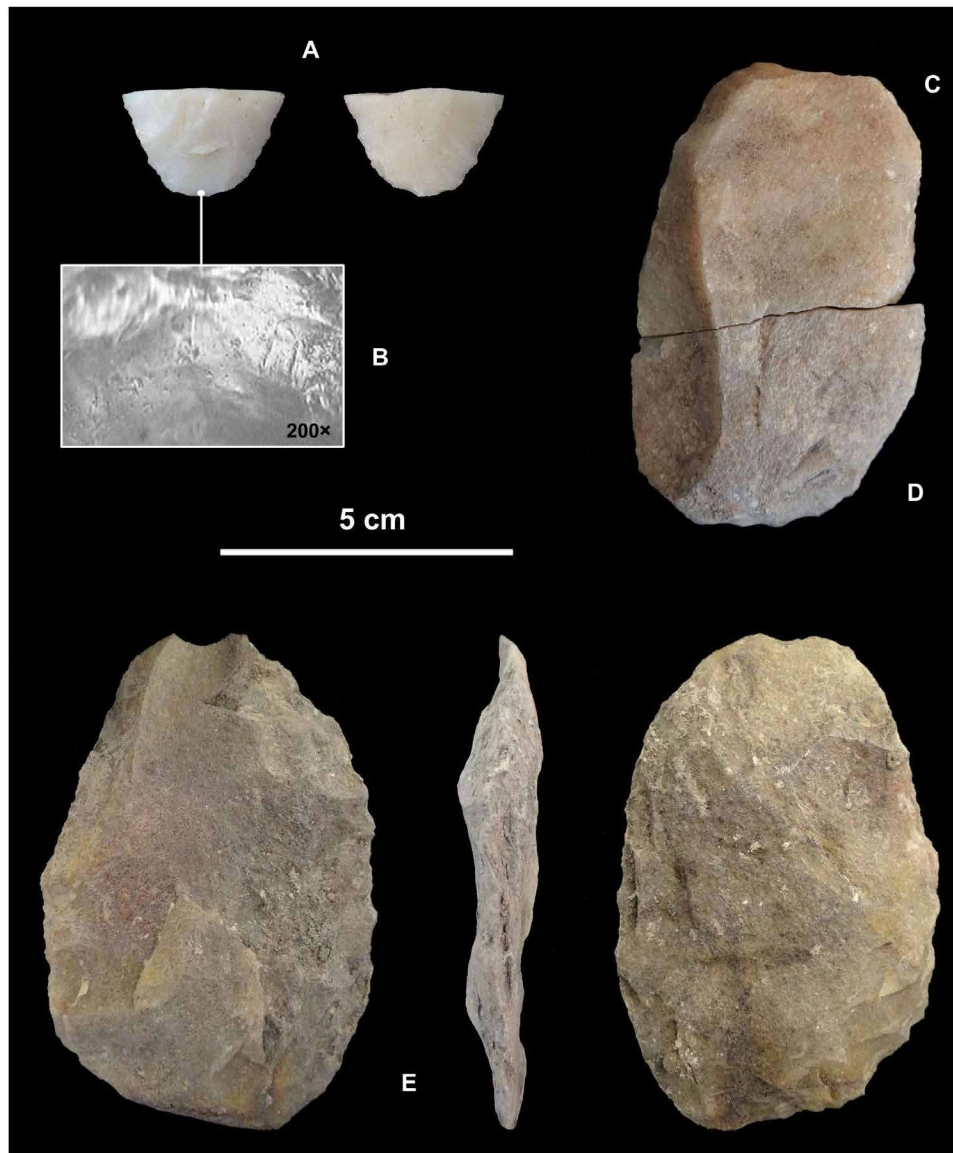
The 2001–2003 excavations uncovered a great amount of giant ground sloth bones associated with lithics and only two glyptodont bones (Fig. 2 and fig. S1) in swamp sediments between 1.00 and 1.30 m below ground level (BGL). Approximately 70% of the lithic artifacts were found among the megamammal bone concentration, while the remaining percentage was recovered around the concentration but in sectors very close to the bones (fig. S2). We made the statistical test correlation between the frequency of spatial distribution of lithics and bones. As a result, we find a positive and significant correlation (Spearman's  $\rho = 0.454$ ,  $P = 0.00072$ ) supporting an intimate spatial association between bones and cultural remains. Lithic material included a lanceolate bifacial projectile point stem, a broken side scraper, one orthoquartzite flake, and 128 orthoquartzite, silicified dolomite, and chert microflakes ranging from 2 to 9 mm long (27). The artifact interpreted as the base of a broken lanceolate bifacial projectile point (FCS.CLA.33) has a convex bottom, a transverse distal fracture (Fig. 3A), and edges with no intentional abrasion (28). Use-wear analysis indicates that both edges have postdepositional alterations (e.g., soil sheen), and for this reason, they do not show diagnostic characters associated with the tool's use. One face of this point has laminar pressure-flaked scars along the base, and the opposite face has a single, small, basal thinning flake. The latter has

modifications in the fracture of the quartz crystals, and micropolishing in the first stages of formation associated with striations and small pits suggests that this projectile point was hafted (Fig. 3B). The orthoquartzite side scraper fragment (FCS.CLA.183) is made from a large flake without cortex. It has two working edges with unifacial and marginal retouches (Fig. 3C). This tool is completely modified by sedimentary abrasion and soil sheen that render any polishing unrecognizable (28).

Excluding micromammals, 282 faunal bones were recovered. One individual each of three extinct megafauna taxa was identified: a giant ground sloth (*M. americanum*) and two glyptodonts (*Neosclerocalyptus* sp. and *Doedicurus* sp.). Giant ground sloth fossils were, by far, the most abundant [79 elements, number of identified specimens (NISP) = 108], and all the anatomical elements of the body were present, including 102 dermal bones assigned to *M. americanum* (table S1). One *Neosclerocalyptus* sp. humerus and one partial *Doedicurus* sp. femur were also recovered (table S1). Modern species are represented by a few remains of Patagonian hare (*Dolichotis patagonum*), vizcacha (*Lagostomus maximus*), dwarf armadillo (*Zaedyus pichiy*), peccary (*Tayassu* sp.), fox (*Lycalopex* sp.), bird (Rheidae), and artiodactyls (Camelidae) (table S2). A significant number of smaller vertebrates were also recovered: *Reithrodon auritus*, *Ctenomys* sp., *Akodon* cf. *Akodon azarae*, and *Galea leucoblephara* (20, 29). With the exception



**Fig. 2. Plan map of the Campo Laborde excavation site.** Distribution of complete and fragmentary bones and lithic tools. Shaded drawings (black) indicate lithic materials (FCS.CLA.33, FCS.CLA.183, FCS.CLA.1989, and FCS.CLA.1990). Red and green dashed lines show lithic refits. Sample dated in this paper (FCS.CLA.154). A-B black line indicates geologic cross section (see Fig. 4 and fig. S5).



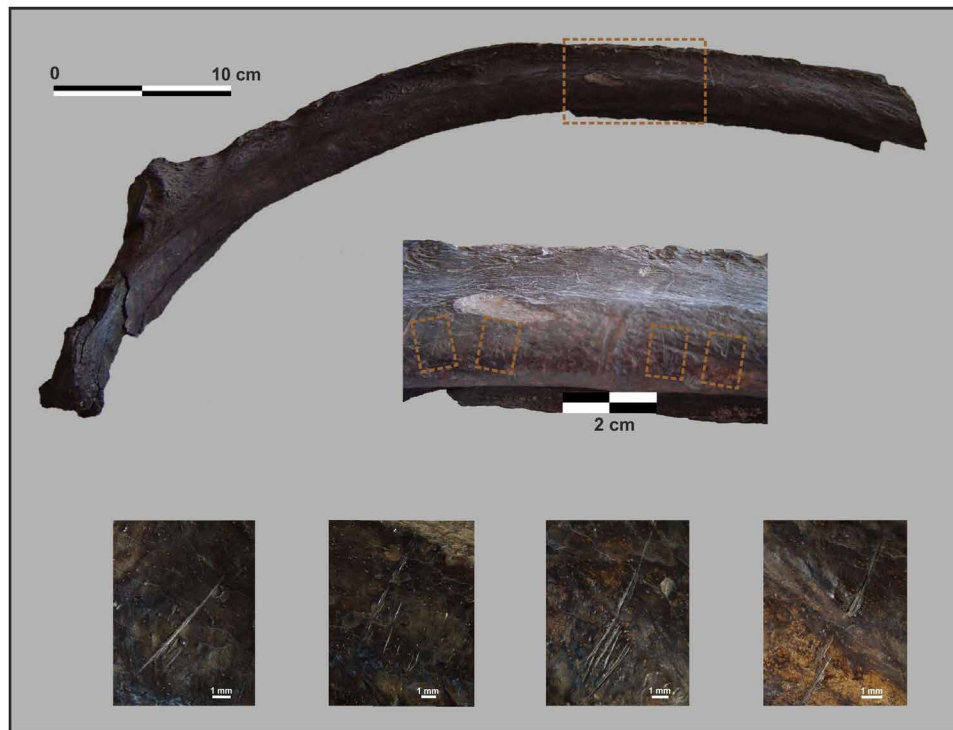
**Fig. 3. Lithic tools found at Campo Laborde site.** (A) Lanceolate bifacial projectile point stem (FCS.CLA.33). (B) Striations and small pits related with hafted micropolishing ( $\times 200$  magnification). (C) Distal half of a broken side scraper (FCS.CLA.183). (D) Proximal half of a broken side scraper (FCS.CLA.1990). (E) Biface (FCS.CLA.1989). [Photo credit: Pablo Messineo. INCUAPA-CONICET, Facultad de Ciencias Sociales (UNICEN)].

of the giant ground sloth and the Patagonian hare (see cultural modification below), all other modern and small vertebrates species are interpreted as the result of incremental accumulation of fossil bones (“bone rain”) that occur at the site due to natural deaths (eto-ecological processes) and owl activity (29) that postdate human-*Megatherium* interaction. In relation to the rest of the other megamammal species (*Neosclerocalyptus* sp. and *Doedicurus* sp.), it is difficult to interpret their incorporation into the deposit as a result of human activity since they are only represented by a single bone each (absence of any evidence of a human selective pattern) and they do not show any trace of human action on bones (cut marks, impact points, anthropic fractures, or burning).

In contrast, in addition to the close stratigraphic, vertical and horizontal association with lithics (Fig. 2 and fig. S2), other evidence supports the butchering and processing of *M. americanum* and *D. patagonum*. Evidence of butchering included stone tool modifi-

cations on an *M. americanum* rib (FCS.CLA.9) and a *D. patagonum* tibia (FCS.CLA.227), as well as flakes and helical fractured bones of megafauna taxa caused by human agency (fig. S3A). We made all diagnoses and identifications on the original specimens under a Leica Stereo Zoom S6D Trinocular stereoscopic microscope with magnifications ranging from 6.3 to 40 $\times$  under adjustable incident light and with a digital Leica DMC 4500 camera.

Cut marks on the right rib of *M. americanum* are located on the interior surface. They represent four areas of marks with a transverse orientation to the rib axis (Fig. 4). This element shows unambiguous stone tool cut marks that are perpendicular to the cortical surface, straight V-shaped in cross section, and with internal microstriations, two of them showing multiple parallel marks (Fig. 4). We infer that these marks are associated with defleshing the animal. Cut marks on a *D. patagonum* tibia are located on the posterior surface of this



**Fig. 4. Cut marks on *M. americanum* rib (FCS.CLA.9).** [Photo credit: Pablo Messineo. INCUAPA-CONICET, Facultad de Ciencias Sociales (UNICEN)].

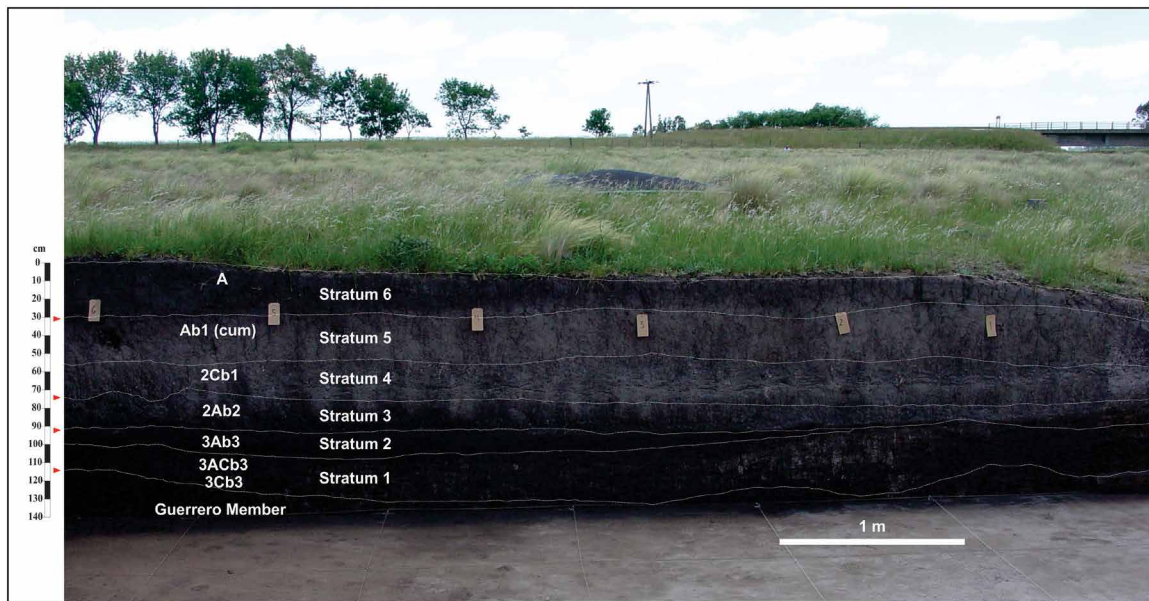
element and have a length of ~1 cm, with an oblique/transverse orientation, generally parallel to the axial axis of the bone (fig. S4). These cut marks were interpreted as a result of activities related to the skinning the animal (29).

In addition, two bone tools were made from megamammal ribs. One of them corresponds to the right distal end of *M. americanum* rib (FCS.CLA.47), which is a fracture-based utilitarian bone tool and has a rounded and polished fracture edge (fig. S3B). We solely placed these traces on the end section of the fracture edge (fig. S3C), whereas adjoining segments of the fracture edge and the rest of the rib do not present any type of bone modifications (i.e., abraded, smoothed, and polished). The second bone tool is a fragment of a rib (FCS.CLA.184) from an unidentified megafaunal species (same size of *Megatherium*) that has, at least, five negative flaking scars along the external compact bone produced during the manufacture of the tool (fig. S3D). One bone flake (FCS.CLA.26) was refitted onto one of these negative scars (fig. S3E). The bone tool and flake were separated by ~0.75 m horizontally (Fig. 2). The distal end edge of this bone is rounded and polished with parallel striations and microflaking on the external cortical surface (fig. S3F). This is probably related to its use. The rest of the bone does not show these types of modifications. In the use-wear analysis, the wear polish was interpreted as the result of contact between the bone tool and a hard material. It is important to remark that no other bones in the collection show evidence of abrasion or polishing, not even in a lower degree (as happens occasionally in swamp environments). These observations support the human manufacture of the two bone tools (28).

Geologic studies identified stratigraphic units typical of the Late Pleistocene-Holocene Pampas (Fig. 5 and fig. S5). In the lower section of the profile (~1.30 to 1.35 m BGL), lacustrine sediments corresponding to the Luján Formation (30) are present. Most of the

archeological deposit (i.e., bones and lithics) was located from approximately 0.95 to 1.30 m BGL in stratum 1. This paleoswamp unit rested unconformably on Guerrero Member sediments and consisted of alternating layers of silty clays and sandy muds whose deposition was punctuated by pedogenesis (Fig. 5 and fig. S5). We excavated small numbers of microflakes ( $n = 25$ ) and small bone fragments in an overlying paleosol, 3Ab3, between 0.85 to 0.95 m BGL (fig. S6). These two units are located in a transition between the Río Salado and Guerrero Members (20, 30). Above the archeological deposits are two additional buried soils; the stratigraphic sequence is capped by the modern soil (A). We recovered no archeological evidence from sediments above 0.85 m BGL (Fig. 5 and fig. S5). The lithics are directly associated with the sloth's stratigraphic position and occur from 0.85 to 1.35 m BGL, mainly in stratum 1 (figs. S5 and S6). The scarce materials found in the Guerrero Member and in the paleosol 3Ab3 are due to the vertical migration of microflakes and small nonidentifiable bones from the stratum 1 (3ACb3 and 3AC3; see refitting among lithic materials).

The chronology of Campo Laborde has been difficult to establish due to extreme degradation and loss of bone organic matter and the bones' severe humate contamination. Initially, only 7 of 12 bones had detectable collagen (table S3). *M. americanum* fossils containing collagen yielded ages ranging from  $6740 \pm 480$   $^{14}\text{C}$  years BP (AA-71667) to  $9730 \pm 290$   $^{14}\text{C}$  years BP (AA-71665). We obtained two soil organic matter samples from the northern profile of the site. One of the samples came from paleosol 3Ab3 and gave a date of  $7960 \pm 100$   $^{14}\text{C}$  years BP (LP-1983). The second sample was taken from stratum 1, the paleoswamp where there was the highest proportion of bones and stone materials and provided an age of  $8090 \pm 190$   $^{14}\text{C}$  years BP (LP-2003). These results should be taken with caution because dates obtained from soil organic matter must be considered



**Fig. 5. Stratigraphy of the Campo Laborde site.** [Photo credit: Pablo Messineo. INCUAPA-CONICET, Facultad de Ciencias Sociales (UNICEN)].

minimum soil ages and do not date the time of human occupation. Since this group of ages roughly dated this component between ~9700 and 6750  $^{14}\text{C}$  years BP, Campo Laborde was interpreted as an Early Holocene giant ground sloth procurement site along the border of an ancient swamp, where megafauna were killed and butchered (20, 21). Although the possibility that the giant ground sloth was scavenged by humans cannot be ruled out (31, 32), we believe that the sloth would have been hunted on the basis of the location of the event (driving prey into a swamp is a frequent hunting strategy) and the finding of a the broken bifacial projectile point that could be interpreted as one of the hunting tools (27).

Excavations in 2016 and 2017 (Fig. 2 and fig. S7A) yielded a taxonomically indeterminate rib fragment, 2 *M. americanum* metapodials (fig. S7B), 1 *Lama guanicoe* cervical vertebra (tables S1 and S2), 2 orthoquartzite tools (Fig. 3, D and E, and fig. S7, C and D), and 58 microflakes and debitage made from orthoquartzite, chert, silicified dolomite, and quartz. All the lithic materials and bone remains were found in stratum 1, the paleoswap, between 0.95 and 1.30 m BGL. One of the tools is a knife manufactured on a biface (FCS.CLA.1989; 81.6 cm by 55.8 cm by 15.3 cm); one of the convex edges has been retouched and has an angle of ~40° to 50°, which makes it suitable for cutting activities (Fig. 3E). The other tool is the proximal half of a broken side scraper (FCS.CLA.1990) that refits with the other distal half (FCS.CLA.183) found ~3.5 m away in 2003 (Figs. 2 and 3, C and D). The edge of the distal-half side scraper was resharpened after breaking (Fig. 3C), indicating that the tool was broken during use and that one-half was repaired and used again. In 2003, four silicified dolomite microflakes that came from different grids and levels also refitted (Fig. 2). One of the refits was between proximal (FCS.CLA.238) and distal (FCS.CLA.251) flakes that were separated by ~2 m horizontally (Fig. 2). The other refit connects proximal (FCS.CLA.239) and medial (FCS.CLA.242) flakes separated by ~3 m horizontally (Fig. 2). In this last refitting, one fragment come from the level 1.30 to 1.35 m BGL (in the limit between the Guerrero Member and the paleoswap) and the other piece come from the level 1.20

to 1.25 m BGL (stratum 1), supporting the conclusion that the lithic material in Campo Laborde is associated with the paleoswap and the small quantity of microflakes in the Guerrero Member and in the stratum 2 (paleosoil 3Ab3) correspond to vertically dislocated materials. This vertical migration of microflakes may be the result of the depositional environment (lentic environment of alluvial plain margin) and pedogenic processes that subsequently affect the deposit. Likewise, the presence of few burrows and a small grouping of bones (*Ctenomys* sp.) in a limited sector of the site supports the action of fossorial animals and could explain the migration of microflakes downward and upward into the Guerrero Member and the stratum 2.

### 2016–2017 AMS $^{14}\text{C}$ DATING PROGRAM

Accurate and precise radiocarbon chronologies are the foundation for studying Late Pleistocene megamammal extinction times and rates and for assessing what degree of temporal overlap exists between extinct taxa and humans. Once initial stratigraphic association is shown between megamammal fossils and human-manufactured lithic or bone artifacts, and these associations are determined taphonomically valid, the geologic age of these sites is determined by directly dating bones of the extinct mammals and bone tools. Indirect dating of physically associated macroflora, microfauna, molluscs, nonextinct mammal taxa, or organic matter from enclosing sediments or soils is not acceptable.

Excepting bones from tar seeps, fossil bones from Campo Laborde and similar localities are the most difficult to radiocarbon date accurately. Campo Laborde fossils have zero to low quantities of collagen, the collagen is poorly preserved chemically and is difficult to extract, and the protein (collagen) is heavily contaminated with sedimentary and soil organic matter, usually occurring as humates (fulvic acids, humic acids, and humins). The single most intransigent chemical problem to overcome is the covalent bonding of humates to collagen, which occurs through the Maillard reaction (33). Separating

humates from collagen and isolating indigenous collagen and its amino acids require chemical pretreatments not commonly used by radiocarbon laboratories.

Humate contamination cannot be overcome by using standard bone collagen preparation techniques (e.g., decalcification, alkali leaching, gelatinization, or ultrafiltration). During burial, the Maillard reaction covalently binds humic and fulvic acids to proteins (17, 33), resulting in very dark brown to light yellowish brown collagen (34). Although alkali treatment extracts some humates, and gelatinization removes other, nonbound humates, covalently bound humic and fulvic acids can only be separated from collagen by breaking the collagen-humate bounds by hydrolyzing the collagen in 6 M HCl for 24 hours at 110°C (35). The resulting hydrolyzate contains free, cationic amino acid hydrochlorides and weakly charged to uncharged fulvic acids. The collagen's amino acids are isolated for  $^{14}\text{C}$  dating by passing the hydrolyzate through hydrophobic XAD-2 resin, which binds to the fulvic acids and allows the amino acids to pass through XAD-2 resin.

Low collagen content per se is not a hindrance to  $^{14}\text{C}$  dating fossil bones or teeth. One concern is that larger masses of bone are needed to compensate for low weight percentages of collagen (e.g., needing 5 g of bone instead of 0.1 g). A greater problem with low protein content is the remaining collagen has undergone increasingly more diagenesis, which decreases the protein's stability during HCl decalcification and KOH treatment and decreases the collagen's solubility during gelatinization. The result is that less protein is recoverable, and although the bone initially contained marginal amounts of collagen, no collagen may ultimately be recoverable.

Previous  $^{14}\text{C}$  dating of extinct fauna from Campo Laborde (table S3) yielded Holocene ages ranging from  $6740 \pm 480$  (AA-71667) to  $9730 \pm 290$   $^{14}\text{C}$  years BP (AA-71665). We obtained these ages using three different chemical fractions: (i) acid-base-acid purified collagen (alkali-extracted decalcified collagen), (ii) gelatin, and (iii) ultrafiltered (> 30 kDa) gelatin (table S3 and Materials and Methods).

In 2016–2017, only 1 of 10 bones processed from Campo Laborde contained collagen. The one successful specimen was an *M. americanum* Metacarpal V (FCS.CLA.154; Fig. 2) that was first dated in 2007 as  $9730 \pm 290$   $^{14}\text{C}$  years BP (AA-71665; table S3) and was redated. AMS  $^{14}\text{C}$  measurements for this paper used XAD-2 resin purification to date total amino acids from hydrolyzed collagen and fulvic acids separated from the collagen hydrolyzate (Table 1, table S4, and Materials and Methods). XAD purification followed Stafford *et al.* (35) and Stafford (34) and comprised decalcification in HCl, extraction with KOH, gelatinization and filtering through 0.45- $\mu\text{m}$  membranes, 6 M HCl hydrolysis, and passing the hydrolyzate through XAD-2 resin. Fulvic acids bound to the XAD resin were eluted by washing the resin with 0.05 M HCl to remove excess acid, eluting the fulvic acids with 0.05 M NaOH, drying the liquid under vacuum, and finally, acidifying the fulvic acids solution with 1 M HCl. AMS  $^{14}\text{C}$  measurements on XAD hydrolyzates produced three radiocarbon ages (RC years  $\pm$  1 SD) for the Metacarpal V:  $10,570 \pm 170$   $^{14}\text{C}$  years BP (CAMS-171851),  $10,655 \pm 35$   $^{14}\text{C}$  years BP (CAMS-171852), and  $10,690 \pm 380$   $^{14}\text{C}$  years BP (CAMS-171861; Table 1). AMS  $^{14}\text{C}$  measurements (fraction modern or Fm) on fulvic acids solution represent an integration of all ages of fulvic acids that accumulated in the bone since its deposition. While the average Fm value for the three XAD dates was  $0.2660 \pm 0.0078$ , the fulvic acids had a higher (more modern) averaged Fm =  $0.3238 \pm 0.0099$ . Calculated “ages” for the fulvic acids are given to demonstrate that fulvic acids contain more

$^{14}\text{C}$  than the collagen and cause more recent (Holocene) ages when non-XAD chemistry is used to date the bones (Table 1 and table S4).

We conclude that humates remaining in collagen caused the previous Campo Laborde bones to date younger than their actual age and that only XAD purification chemistry is acceptable. While well-preserved collagen with no-to-negligible humates can sometimes be dated accurately using either ultrafiltered or 0.45- $\mu\text{m}$  filtered gelatin (34, 36), both filtration methods and lesser purity fractions as decalcified collagen and alkali-extracted collagen will produce erroneous  $^{14}\text{C}$  dates.

A geologic profile 20 m upstream from Campo Laborde was studied (37), with paleosoil 3Ab3 being reinterpreted as a black mat (highly organic marsh deposit). The study produced a date of  $5680 \pm 40$   $^{14}\text{C}$  years BP (Beta-254925) on organic material from the black mat and a date of  $8550 \pm 50$   $^{14}\text{C}$  years BP (Beta-254924) from a biogenic cavity fill below the paleoswamp (where we recovered most of the archeological evidence). This cavity fill was interpreted as an “intrusion of younger organic matter from overlying early Holocene marshes” (37). In addition to the  $^{14}\text{C}$  dates, an Optically Stimulated Luminescence age of  $12,120 \pm 2120$  years BP (X-3565) was measured for the paleoswamp. Last, *Succinea* sp. shells were dated as  $10,620 \pm 60$   $^{14}\text{C}$  years BP (Beta-254926) and  $10,420 \pm 60$   $^{14}\text{C}$  years BP [Beta-254928 (35)]. On the basis of these results, Toledo and Scheweninger (37) concluded that the bone samples from several Pampean archeological sites (e.g., La Moderna and Paso Otero 5) had been contaminated by younger organic matter and that Campo Laborde “...appear(s) to have a terminal Pleistocene age (12–14 ka CAL BP? and not a basal Holocene age as previously proposed, ...)”.

## DISCUSSION

The interpretation of Campo Laborde as a kill (or procurement) and butchering site makes it difficult to discuss the broader subsistence pattern during Late Pleistocene times in the Pampas (23, 38) because this type of site can bias the interpretations about the subsistence strategies as a whole. However, on the basis of the evidence from other sites, the role of megafauna appears to have greater importance in the Pampas during this period in comparison with other regions of South America (38). The presence of the orthoquartzite stem allows a comparison with the other projectile points from the Pampas. The main projectile point type in the region is the Fishtail point, which has been dated between  $\sim 11,800$  and  $10,000$   $^{14}\text{C}$  years BP (39–42), although the only site in the region with a clear association between this point type and extinct megamammals is Paso Otero 5 (43), which dates between  $10,440 \pm 100$   $^{14}\text{C}$  years BP and  $9560 \pm 50$   $^{14}\text{C}$  years BP. While Fishtail points are the most abundant in the Pampas, other Late Pleistocene projectile types have been recorded in the region (44) and in the Southern Cone (45). Among them, Tigre points in the Pampas of Uruguay show a wide stem with a convex base and have been dated between  $\sim 10,420$  and  $9730$   $^{14}\text{C}$  years BP (44). However, no Tigre points have been found in the Pampas of Argentina. In any case, the stem found in Campo Laborde (with a similar concave base but different in dimensions and technological features) suggests that, around  $10,650$   $^{14}\text{C}$  years BP, at least two projectile point models were used to hunt Pleistocene megamammals in the Pampas of Argentina. Moreover, the bifacial knife of Campo Laborde also confirms the use of this technology in the Pampas at the end of the Pleistocene and shows similarities with other comparable artifacts (42, 44).

**Table 1. AMS radiocarbon measurements for Campo Laborde *M. americanum* Metacarpal V (FCS.CLA.154) and humates separated during chemical pretreatment.** Accepted age is  $10,655 \pm 35$   $^{14}\text{C}$  years BP (CAMS-171852) due to having the highest mass bone (3.058 g) and lowest SD. NA, not available; C.I., confidence interval.

AMS Lab no.	Chemical fraction dated	Bone processed (g)	Carbon dated ( $\mu\text{g}$ )	Fm $\pm$ SD	$^{14}\text{C}$ date $\pm$ 1 SD RC years BP	CAL BP (2 $\sigma$ ) 95.4% C.I. (4 $\sigma$ )
<b>Original University of Arizona analysis</b>						
AA-71665	Gelatin	—	22	0.298 $\pm$ 0.011	9730 $\pm$ 290	10,250–11,982
<b>CAMS bone dates</b>						
CAMS-171861	XAD KOH-extracted decalcified collagen	1.025	40	0.2642 $\pm$ 0.0122	10,690 $\pm$ 380	11,304–13,207
CAMS-171873	Fulvic acids eluted from CAMS-171861 XAD resin	—	165	0.3397 $\pm$ 0.0030	8670 $\pm$ 80	NA
CAMS-171851	XAD decalcified collagen	1.137	90	0.2684 $\pm$ 0.0056	10,570 $\pm$ 170	11,924–12,732
CAMS-171875	Fulvic acids eluted from CAMS-171851 XAD resin	—	30	0.2983 $\pm$ 0.0167	9720 $\pm$ 450	NA
CAMS-171852	XAD KOH-extracted decalcified collagen	3.058	410	0.2654 $\pm$ 0.0010	10,655 $\pm$ 35	12,547–12,677
CAMS-171874	Fulvic acids eluted from CAMS-171852 XAD resin	—	165	0.3335 $\pm$ 0.0021	8670 $\pm$ 80	NA

The Pampas region has consistently produced younger last appearance dates for extinct megafauna than other regions of South America (9), leading some researchers to propose that it served as a refugium during the Late Pleistocene (7, 15). Pampas region sites with published Early Holocene radiocarbon dates on extinct megafauna include Arroyo Seco 2 (7, 10), La Moderna (7, 19), Paso Otero 4 (22), Arroyo Tapalqué (10), Centinela del Mar (10), and Río Cuarto (25). Many of these Holocene dates have been rejected by authors due to diagenetic concerns or failure to replicate dates or are otherwise suspect because the material dated is considered less reliable than bone collagen (e.g., tooth enamel bioapatite carbonate). Notably, none of these dates used amino acid–based methods, except some of the Arroyo Seco 2 dates (18), and therefore, this study raises the possibility that the Holocene  $^{14}\text{C}$  ages may be due to contamination by fulvic acids and other exogenous compounds that cannot be completely removed by traditional radiocarbon preparation methods. This contamination may be a particular problem in the Pampas, as compared with other parts of South America, because the open-air, fluvial, and lacustrine deposits typical of the region both produce low collagen yields through leaching and offer ample opportunity for plant- and animal-based humates to penetrate bones and bind with collagen.

## CONCLUSIONS

The recent excavations and new  $^{14}\text{C}$  dates support Campo Laborde being a kill and butchering site bordering a Late Pleistocene swamp. The lithic artifacts found around and within the giant ground sloth bone concentration suggest that hunters knapped directly around the car-

case. The refitting of side scraper fragments and microflakes supports the stratigraphic integrity of the deposit and that butchering activities and resharpening of artifacts occurred at the site. Microwear analysis on the broken projectile point stem indicates that it was hafted.

The new  $^{14}\text{C}$  measurements solidly date the killing and exploitation of the giant ground sloth to the Late Pleistocene. These new dates do not support extinct megamammals surviving into the Holocene at Campo Laborde and call into question Holocene survival of megafauna at most, if not all, Pampas localities. Certainly, more radiocarbon analyses using amino acid–based methods are necessary in sites such as La Moderna to test the supposedly Holocene age of the extinct megamammals in these sites.

Campo Laborde is the only confirmed giant ground sloth kill site in the Americas. New dating also reduces the time span between the arrival of humans and the extinction of the megamammals in the Pampas of Argentina in ~2000 years. Both of these results support previous proposals (3, 7) on the contribution of human impact in the extinction process of the South American megafauna. The Pampas data are evidence that some extinct species (e.g., *Megatherium*, *Equus*, and *Doedicurus*) were exploited by people, probably with a low level of predation, over at least two millennia, before their extinction.

## MATERIALS AND METHODS

### Radiocarbon sample preparation

The following summaries describe what chemical pretreatments were performed by each laboratory. The University of Arizona AMS Lab processed dates labeled as AA–. Dates shown as CAMS– were



chemically prepared and combusted by E.L.L. and were graphitized and AMS  $^{14}\text{C}$ -dated at the LLNL Center for Accelerator Mass Spectrometry, California.

#### **AA-55117, AA-55118, and AA-55119**

Bone samples were demineralized in 0.25 M HCl, and the acid-insoluble fraction was extracted with deionized (DI) water (pH 2) at 60°C. The hot water soluble fraction was dried and combusted to yield  $\text{CO}_2$  for graphitization.

#### **AA-71665 and AA-71667**

Bone samples were demineralized in dilute HCl, leached with NaOH, and acidified with dilute HCl using a continuous flow Acid-Base-Acid (ABA) extraction system to yield the ABA-treated bone fraction used for combustion and dating.

#### **CAMS-155863**

Approximately 200 mg of uncrushed, mechanically cleaned *M. americanum* rib bone (FCS.CLA.9) was decalcified in 0.25 M HCl at 4°C for 72 hours, replacing HCl daily, until the sample softened (the sample never attained the fully soft, spongy texture characteristic of demineralized collagen). The sample was then gelatinized in 0.1 M HCl at 58°C for 16 hours, after which the solution was filtered through a Whatman quartz fiber filter with vacuum suction and then ultrafiltered through 30-kDa Centriprep centrifugal filters that had been prerinsed via centrifugation four times in Milli-Q purified water. The ultrafiltered collagen was freeze-dried and combusted at 850°C for 12 hours in quartz tubes containing copper oxide (CuO) and silver (Ag). The resulting  $\text{CO}_2$  was purified and graphitized for AMS  $^{14}\text{C}$  measurement.

#### **CAMS-171851, CAMS-171852, and CAMS-171861**

Approximately 1 g of bone for samples CAMS-171851 and CAMS-171861 and 3 g for CAMS-171852 were removed from *M. americanum* Metacarpal V (FCS.CLA.154) and were mechanically cleaned with a Dremel tool. Bone fragments approximately 5 to 10 mm long were decalcified at 4°C in 0.2 to 0.5 M HCl for up to 35 days. Decalcification was considered complete when (i)  $\text{CO}_2$  effervescence had ceased, (ii) the resulting collagen had a translucent appearance and spongy texture, and (iii) no calcium phosphate density gradient was apparent after the sample had been stationary for several hours in fresh HCl. CAMS-171861 was subsequently alkali-extracted with 0.1 M KOH for 2 days at 38°C.

All decalcified collagen samples were rinsed in DI water and placed in sealed tubes with 6 M HCl on a heating block at 110°C for 22 hours to hydrolyze the collagen. Heating in strong HCl hydrolyzes the protein's peptide bonds to form free amino acids, releases humates bound to the collagen through the Maillard reaction, and causes other humate-related compounds to precipitate. The amino acids are cationic in strong acid, while the hydroxyl and carboxyl groups of the fulvic acids are fully protonated, making them neutral molecules that strongly bind to the nonpolar XAD-2 resin. The hydrolyzates were passed through XAD-2 columns containing approximately 2 ml of Restek Ultra-Clean XAD-2 resin retained between two 20- $\mu\text{m}$  Restek SPE frits; each column was fitted at the bottom with a 0.45- $\mu\text{m}$  Restek SPE filter cartridge. The XAD-filtered hydrolyzates were evaporated in a vortex evaporator, and between 1 and 6 mg of hydrolyzed collagen, were combusted in sealed quartz tubes containing copper oxide (CuO) and silver (Ag).

#### **CAMS-171873, CAMS-171874, and CAMS-171875**

Following the XAD-2 purification of the amino acid hydrolyzates for samples CAMS-171851, CAMS-171852, and CAMS-171861, the resin columns were washed with DI water (pH 8) to remove

excess HCl. Fulvic acids bound to the resin were eluted using a few tens of microliters of 0.1 M KOH; the elute was acidified with HCl, dried, and combusted in sealed quartz tubes containing copper oxide (CuO) and silver (Ag). All  $\text{CO}_2$  resulting from the combustions was graphitized, and targets were analyzed by accelerator mass spectrometry.

### **SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/5/3/eaau4546/DC1>

Fig. S1. View of *M. americanum* bones (ribs and vertebrae) and lithic tool (red arrow) recovered in 2003.

Fig. S2. Horizontal lithic distribution in Campo Laborde.

Fig. S3. Bone tools and flakes.

Fig. S4. Cut marks on *D. patagonum* tibia (FCS.CLA.227).

Fig. S5. Stratigraphic scheme of the Campo Laborde site.

Fig. S6. Vertical lithic distribution at Campo Laborde site.

Fig. S7. Bones and lithics exposed during new excavation.

Table S1. Stratigraphic and anatomical data for skeletal elements of giant ground sloth (*M. americanum*) and glyptodonts (*Neosclerocalyptus* sp. and *Doedicurus* sp.) recovered from Campo Laborde.

Table S2. Stratigraphic and anatomical data for skeletal elements (MNE) of modern fauna recovered from Campo Laborde.

Table S3. Previous AMS  $^{14}\text{C}$  dates from Campo Laborde.

Table S4. AMS radiocarbon measurements for known-age samples and backgrounds.

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