ANTHROPOLOGY

Direct hydroxyproline radiocarbon dating of the Lapedo child (Abrigo do Lagar Velho, Leiria, Portugal)

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The 1998 discovery of a nearly intact Gravettian human burial in the Lapedo Valley (Leiria, Portugal) propelled the Lagar Velho rockshelter to worldwide fame. The ochre-stained skeleton of the Lapedo child, a juvenile aged around four or five, exhibited a mosaic of Neanderthal and anatomically modern human features argued to reflect admixture between the two human populations. Here, we present direct compound-specific radiocarbon dates for the child's skeleton [27,780 to 28,550 calibrated years before present (cal B.P.)] and five associated bones from the burial and underlying contexts. We reassess the chronology and archaeological interpretation of the burial in light of these new dates and demonstrate the suitability of hydroxyproline radiocarbon dating for poorly preserved Paleolithic samples that otherwise fail routine radiocarbon pretreatment methods.

INTRODUCTION

The Lagar Velho rockshelter (39°45′20.28″N, 8°44′5.72″W; WGS 1984 datum) features an Upper Paleolithic stratigraphic succession preserved at the base of a north-facing limestone cliff in the Lapedo Valley, Central Portugal (Fig. 1). The site was discovered by chance in November 1998 when, while surveying the locality to confirm the discovery of rock art by student P. Ferreira, J. Maurício and P. Souto investigated a nearby overhang that appeared to be an ideal locale for Paleolithic occupation. A few years before, the site had been terraced by the landowner to install a shed; a deposit of LGM (Last Glacial Maximum) age tucked in a joint running along the shelter's wall, the "Hanging Remnant," is all that remains of the more than 3 m of archaeological infilling destroyed by the terracing (Fig. 2).

In a niche with loose sediment at the interface between the terraced surface and the wall, J. Maurício and P. Souto found a few ochre-stained, juvenile human hand bones. A week later, the discovery was confirmed by C.D. and J.Z., who subsequently led a salvage excavation to investigate whether those bones could stand for a shallow burial that remained largely undisturbed. The salvage work, which lasted between 12 December 1998 and 8 January 1999, eventually revealed the nearly intact, ochre-stained skeleton of a 4- to 5-year-old child that was meticulously studied and monographically published (*1*–*3*) (Fig. 3). Radiocarbon dating of animal bone and charcoal recovered from the burial context suggested that the event had taken place between 27.7 and 29.7 ka (thousand years) ago, but despite four attempts, it was not possible to obtain a reliable radiocarbon date for the individual.

Gravettian mortuary ritual is well known across Europe, represented by 38 graves including 46 individuals (*4*), plus 7 individuals from the mass burial at Předmostí, Czech Republic, and at least 6 from the Grotte de Cussac, France (*5*, *6*). As such, these remains provide the oldest sample of early modern humans from Europe sufficiently large to enable reliable inferences on their biology and behavior and to discuss issues of status as related to age and sex. For instance, (i) the Lapedo child revealed a morphological "mosaic" of anatomically modern human and Neanderthal features, evidencing a shared ancestry (*1*, *2*); (ii) the contrast between age-at-death burial patterns in the Middle Paleolithic (reflecting mortality) and Gravettian (reflecting a strong bias against juveniles) has been taken to mean that the latter was underpinned by social rules based on key life history events, namely, weaning and puberty (*7*, *8*); and (iii) the variation in mortuary ritual and personal ornamentation permits inferences regarding interindividual or interregional diversity, e.g., in terms of language or ethnicity (*9*).

Reliable dating is a prerequisite for a meaningful discussion of these issues, as change through time over the 10 millennia duration of the technocomplex may play a substantial role in explaining the observed variation. Many of these burials lack reliable or precise radiometric dating (*4*); examples of the impact of improved direct dating in assessing these burials are the reassignment to the Gravettian of the Cro-Magnon remains, long thought to be of Aurignacian age (*10*, *11*), and the backdating by some five millennia of the Sunghir burials (*12*). Also revealed is the clustering around 28 ka cal B.P. (calibrated years before present) of southern European cases, consistent with the similarity observed in the personal ornamentation of the bodies, namely, with regard to the practice of head dressing using marine shell beads (*9*, *13*).

Direct dating of the Lapedo child

Four attempts [three by the Centre for Isotope Research at the University of Groningen, The Netherlands, and one by the Oxford Radiocarbon Accelerator Unit (ORAU), UK] were originally made to obtain a direct radiocarbon age for the Lapedo child (*14*). The ORAU carried out a routine acid-base-acid (ABA) collagen extraction protocol upon femoral fragments (sample A) via a continuous flow system (*15*). This ABA protocol comprised a period of demineralization with 0.5 ml of HCl, followed by a rinse in dilute base (0.1 M NaOH) to remove humic components. This was then followed by a final rinse in 0.5 M HCl to remove any dissolved CO_2 before gelatinization, filtration, and lyophilization. The direct date (OxA-8417; 21,420 ± 220 B.P.) and associated elemental data obtained for the Lapedo child femoral fragment at the ORAU are provided in Table 1. Because the C/N atomic ratio of the collagen (3.7) was outside of the

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Fig. 1. The site. Overview of the rockshelter from the northwest in December 1998, at the time of discovery. Image credit: J.Z.

expected range for good quality collagen (*16*, *17*) and the collagen yield (% weight) was well below 1%, the sample was considered to have failed following internationally and internally accepted quality control parameters. The high C/N atomic ratio is likely due to the incomplete removal of carbon contamination.

Rib fragments (sample E) were selected for dating at Groningen. The poor condition of the bones prompted the use of more dilute HCl (0.1 M) to prevent aggressive demineralization of the bone. The results are given in Table 1. GrA-10972 and GrA-12194 are duplicate results with an average age of 17,520 ± 113 B.P., while GrA-13360 (21,980 ± 100 B.P.) was obtained using the residue from collagen extraction. All three dates are inconsistent with the original minimum age for the burial provided by a rabbit vertebra touching the left tibia of the child (sample C, described below): 23,920 ± 220 B.P. [OxA-8422, 27,730 to 28,640 cal B.P., 95.4% probability, calibrated with OxCal v4.4.4 (*18*) and IntCal20 (*19*), as all calibrated radiocarbon ages herein reported]. Given that the ribcage was consolidated in UHU glue and removed en bloc during the original excavation,

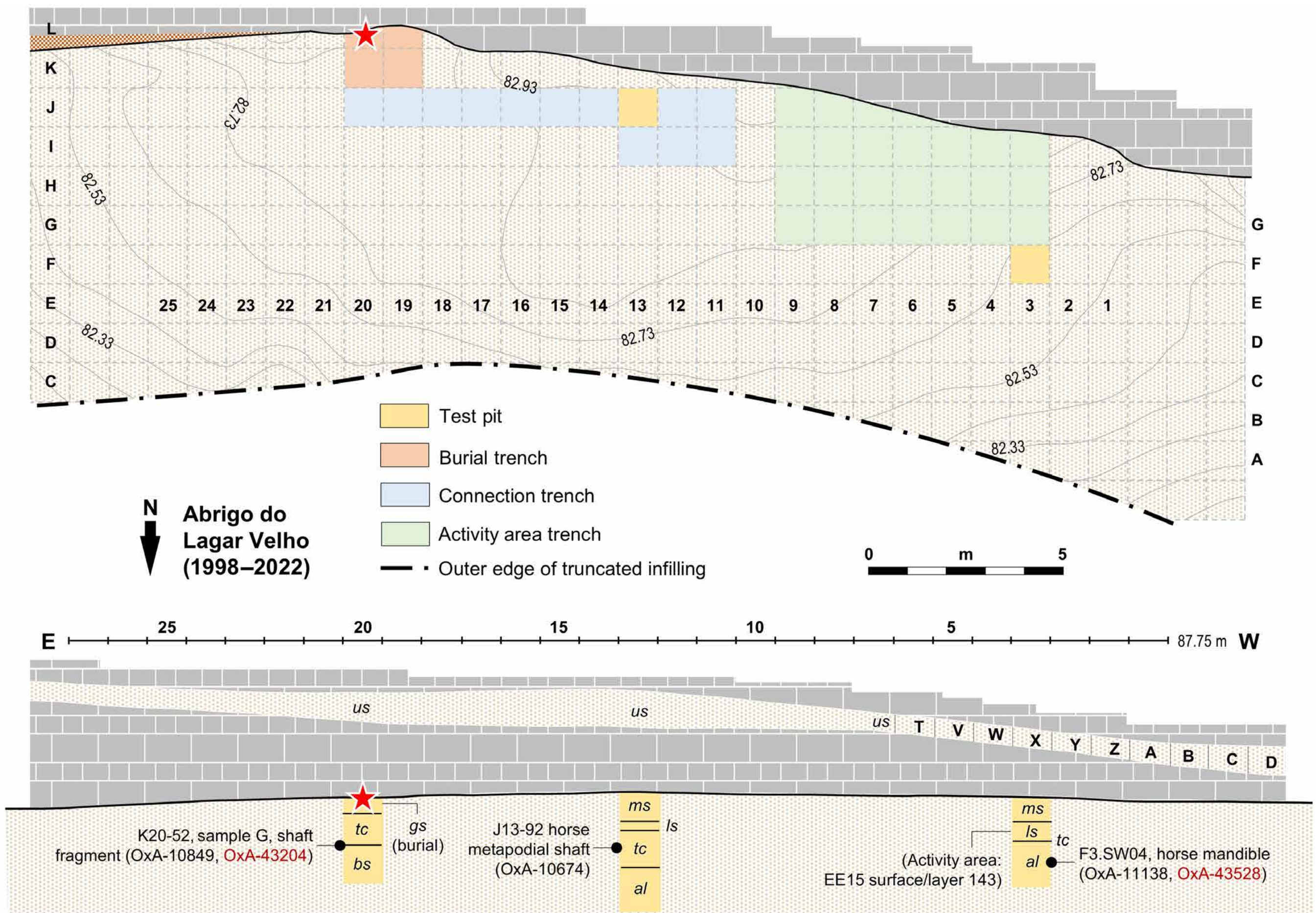

Fig. 2. Plan and schematic profile. Area of the 1998 salvage excavation of the grave (grid units K and L/19 and 20), excavated areas of the LGM deposit preserved in the Hanging Remnant (T to Z and A to D), and location of the test pits and trenches open during subsequent field seasons. The star marks the position of the grave. Lab numbers and sample descriptions refer to the samples from the basal infilling dated 1999 to 2002 (the lab numbers in red are for their HYP redating). Elevations are in meters above sea level. The sedimentary complexes are designated after (*52*): *bs*, lowest slope; *al*, alluvial; *tc*, transitional; *gs*, fine gravel and sand; *ls*, lower slope; *ms*, intermediate slope; *us*, upper slope.

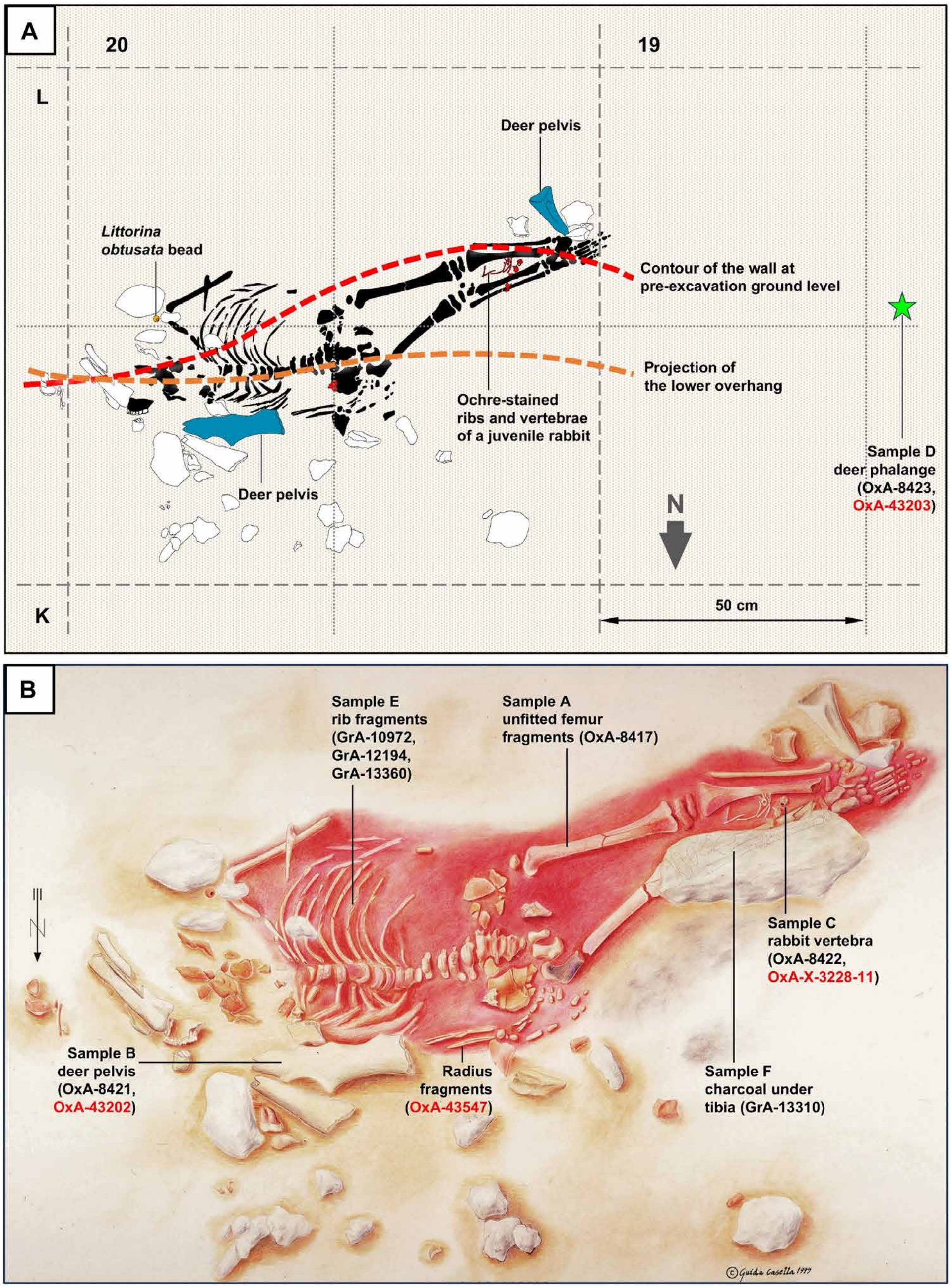

Fig. 3. The grave. Plan (**A**) and drawing (**B**) after (*21*). The position of the dated samples is indicated (the lab numbers in red are for their HYP redating). Artist credit: (A) J.Z. and (B) G. Casella.

and that a number of rib fragments were glued together with a nitrocellulose-based cement, it is likely therefore that the younger-than-expected dates are a result of incomplete removal of modern carbon contamination derived from the glue.

Dating of the burial context

Five bone samples from the burial environment were selected for analysis during the original dating program, designed to obtain an estimate for the timing of the burial event. Sample D, a red deer third phalange, was collected from sediment outside the grave but at the same elevation and provides an age of 24,520 ± 240 B.P. (OxA-8423; 28,080 to 29,210 cal B.P., 95.4% probability) for the deposition of the sedimentary complex (*gs*) that the burial pit was excavated into (Fig. 2). Sample C, a rabbit vertebra in direct contact with the child's left tibia and one of five spread across the lower legs in a semiarticulated manner provides a minimum age for the burial of 23,920 ± 220 B.P. (OxA-8422; 27,730 to 28,630 cal B.P., 95.4% probability). On the basis of a number of arguments, it was suggested that the rabbit that this vertebra and associated skeletal elements belonged to was intentionally placed into the grave with the child, as opposed to being introduced to the burial context through burrowing (as discussed below). Two other samples provide a maximum age for the burial (Fig. 2):

Table 1. Direct dates obtained for the Lapedo child during the original dating program. Radiocarbon dates obtained on bulk collagen at the Oxford Radiocarbon Accelerator Unit (OxA dates) and at the Center for Isotope Research, University of Groningen (GrA dates). Conventional radiocarbon ages (CRA) are expressed in years B.P. (Stuiver and Polach, 1977). Collagen yield is the percent yield of extracted collagen as a function of the starting weight of the bone analyzed. %C is the carbon present in the combusted collagen sample. Stable carbon isotope ratios ($\delta^{13}C$) are expressed in per mil (‰) relative to vPDB. C/N denotes the atomic ratio of carbon to nitrogen and is acceptable if it ranges between 2.9 and 3.5 in the case of collagen. N/A, not available.

Lab code	Material and context	CRA	Cal B.P. (95.4% probability)	Sample weight (mg)	Collagen yield (mg)	Collagen yield (wt %)	$\delta^{13}C$	C/N
GrA-10972	L20.189 (sample E), human bone collagen (rib)	17,380 ± 160 B.P.	20,540–21,430	474	10.9	2.3	−21.75	N/A
GrA-12194	Duplicate of GrA-10972	17,660 ± 160 B.P.	20,930–21,910	(474)	(10.9)	(2.3)	−21.64	N/A
GrA-13360	Residue from collagen extraction for GrA-10972	21,980 ± 100 B.P.	25,960–26,420	(474)	26.5	5.6	−25.57	N/A
OxA-8417	L20-103 (sample A), human bone collagen (unfitted fragments of the child's left femur)	21,420 ± 220 B.P.	25,200–26,220	520	2.8	0.5	−20.8	3.7

Sample G, a shaft fragment from a large mammal bone, was recovered 1 m below the grave, at the interface between complex *tc* and complex *bs*, thereby providing also a minimum age of 27,100 ± 900 B.P. (OxA-10849; 29,800 to 33,900 cal B.P., 95.4% probability) for the latter's erosional truncation; and sample F3.SW04, a horse mandible from the *al* complex, the basal alluvium, which was dated to 29,800 ± 2500 B.P. (OxA-11318; 29,900 to 43,100 cal B.P., 95.4% probability).

One charcoal sample from a single *Pinus sylvestris* branch (sample F) recovered from beneath the right leg of the child and a red deer pelvis touching the right shoulder of the child (sample B) were dated to test hypotheses about ritual behavior (Fig. 3). The charcoal sample (sample F) yielded an age of 24,860 ± 200 B.P. (GrA-13310; 28,690 to 29,700 cal B.P., 95.4% probability) and was interpreted as evidence of ritual burning of the branch before the deposition of the child's body. The red deer pelvis (sample B) returned a date of 24,660 ± 260 B.P. (OxA-8421; 28,230 to 29,630 cal B.P., 95.4% probability). Its contemporaneity with the red deer third phalange (sample D) and the *P. sylvestris* branch (sample F), combined with its direct contact with the child and greater degree of preservation when compared to other red deer remains in nearby deposits, was interpreted as evidence that it had been intentionally incorporated in the grave at the time of burial (*14*, *20*–*22*).

Hydroxyproline dating

Currently at the ORAU, the vast majority of radiocarbon measurements on archaeological bones are performed on bulk collagen, which is purified following chemical pretreatment protocols specific to the condition of the sample. These protocols are characterized by a modified Longin (*23*) collagen extraction procedure, followed by gelatinization and lyophilization (AG) or gelatinization, ultrafiltration, and lyophilization [AF; see (*24*) for full details]. The purpose of these protocols is to remove as much carbon contamination as possible, which can be derived directly from the burial environment, from handling of the specimen during excavation, from laboratory activities, and from the application of consolidants for conservation purposes.

Routine pretreatment protocols are not always sufficient to entirely remove exogenous carbon. Although ultrafiltration has been shown to reduce the amount of contamination in many cases (*25*, *26*), it cannot remove contaminants that exceed the molecular weight cutoff (Vivaspin 15 30-kDa MWCO Sartorius ultrafilters are in routine use by the ORAU) or contaminants that are chemically cross-linked to collagen. Compound-specific radiocarbon analysis (CSRA) has therefore emerged as an alternative pretreatment method that involves isolating and dating a single amino acid present in bone collagen, thereby providing a highly pure source of autochthonous carbon for dating (*27*). Hydroxyproline is the targeted amino acid because it constitutes around 12% of the total amino acids in mammalian collagen and is almost unique in such high abundances elsewhere in nature (*28*). The method has enabled a growing number of accurate dates to be obtained for samples that previously yielded younger-than-expected ages due to insufficient removal of contamination (*12*, *29*–*40*). Because initial attempts to directly date the Lapedo child were hampered by poor collagen preservation and incomplete removal of exogenous carbon from the burial environment (ORAU) or from conservation materials (Groningen), this individual is an ideal candidate for hydroxyproline dating (HYP).

RESULTS

A sample of the Lapedo child's right radius, crushed in situ, was selected for HYP dating (see Materials and Methods and Fig. 4). Because the collagen yields for the original dating attempts were particularly low, approximately 4 g of bone was sampled. The three bone samples from the burial environment previously radiocarbon dated in the late 1990s and early 2000s (the red deer pelvis, the rabbit vertebra, and the red deer third phalange; samples B, C, and D respectively) were also redated on newly extracted HYP, as were the two bone samples originally dated at the same time that provide maximum ages for the burial event (the shaft fragment from a large mammal bone and the horse mandible; samples G and F3.SW04, respectively). In total, we present six compound-specific radiocarbon dates obtained on HYP. The results are given in Table 2. The HYP date for the right radius of the Lapedo child is 23,950 ± 150 B.P. (27,780 to 28,550 cal B.P., 95.4% probability). The new HYP dates for samples B, C, and D are 24,600 ± 230 B.P. (28,200 to 29,260 cal B.P., 95.4% probability), 23,590 ± 430 B.P. (27,150 to 28,790 cal B.P.,

Fig. 4. The child's radius. Fragments of the child's right radius, shattered in situ, used for the OxA-43547 hydroxyproline date. Image credit: C.D.

95.4% probability), and 24,610 ± 150 B.P. (28,610 to 29,170 cal B.P., 95.4% probability), respectively; they are statistically indistinguishable from the AG dates obtained during the original dating program, suggesting that the AG pretreatment procedure was sufficient to remove any carbon contamination from the samples or that no contamination was present in the bones.

The new HYP date for the horse mandible from the *al* complex, sample F3.SW04 (25,400 ± 160 B.P.; 29,250 to 30,000 cal B.P., 95.4% probability), is statistically younger than the original ultrafiltration date obtained during the first dating program, and the HYP date for the shaft fragment from a large mammal bone (sample G; 25,540 ± 150 B.P.; 29,300 to 30,090 cal B.P., 95.4% probability), while statistically indistinguishable from the corresponding AG date, only narrowly passes the χ^2 test ($t = 3.5$). Given the excellent high-performance liquid chromatography (HPLC) background (>50,000 B.P.) and the acceptable C/N values of the HYP samples, the younger HYP dates are unlikely to be the result of the addition of modern carbon to the HYP fractions. The parsimonious explanation is the introduction of dead carbon to the original ultrafiltered samples.

The original dating of the horse mandible, sample F3.SW04 (OxA-11318), took place in March 2002, 2 years after the first introduction of ultrafiltration at ORAU. The collagen yield was particularly poor (0.1%), below the threshold that would normally categorize a sample as a failure (*41*). In late 2002, an intercomparison between radiocarbon laboratories revealed that known-age bone samples dated at ORAU were returning older dates than expected, which was later determined to be the result of incomplete removal of a fossil-derived humectant present on the ultrafilter membranes (*42*, *43*). Up to 50 μg of C from this humectant was shown to be mixed in with sample collagen during the ultrafiltration process if the filters were washed three times with ultrapure water, and between 0.4 and 1 mg of C if they were only washed twice (*43*). The exceptionally low collagen yield of sample F3.SW04 resulted in 350 μg of carbon, and if we assume that 50 μg of this (>14%) is fossil carbon derived from the ultrafiltration process, we would expect the measured age to be approximately 1150 years older than the true age. Fossil-derived carbon contamination is also likely to have affected the original ultrafiltration date for sample G (OxA-12730), which was dated in 2001 and also had a particularly low collagen yield (0.4%). Given the inaccuracy and imprecision of the original ultrafiltered dates and the likelihood that they were affected by this fossil carbon contamination, the new HYP dates offer much more reliable age estimates for the mammal bone shaft fragment (sample G) and the horse mandible (sample F3.SW04).

In light of this evidence and to test the hypothesis, two other bone samples from Portuguese sites dated at ORAU at the same time as the two Lagar Velho samples were AF* redated in 2024: OxA-11129 (11,755 ± 80 B.P.) from Final Magdalenian level 3 of Galeria da Cisterna (Almonda karst system) (*44*) and OxA-11235 (23,410 ± 170 B.P.) from Late Gravettian level 2b of Lapa do Anecrial (*45*). The new results for these samples are in both cases some 800 years younger: 10,917 ± 43 B.P. (OxA-44991) and 22,620 ± 180 B.P. (OxA-44990), respectively. This experiment corroborates that contamination by fossil-derived carbon present in the ultrafilters in use at ORAU at the time of dating is the explanation for the anomalously old AF* results for sample G and sample F3.SW04.

DISCUSSION

The new HYP radiocarbon date for the Lapedo child (27.8 to 28.5 ka cal B.P.) is consistent with the original estimate of the timing of the burial event (~28 to 30 ka cal B.P.); however, it is inconsistent with the ages of the two red deer bones spatially associated with the burial (*22*), which fall in the 28.2 to 29.2 ka cal B.P. interval and are statistically older.

One of these red deer bones, sample B (the red deer pelvis touching the right shoulder of the child), was originally considered to be part of the burial ritual—either as an offering of food, or as a tool for the construction of the burial pit (*20*–*22*). The absence of animal gnawing marks and surface weathering led to the suggestion that the individual could have died shortly before the burial took place, as a result of either hunting by humans or death by natural causes in situ. The fact that the pelvis fragment was in excellent surface condition compared to much of the rest of the contemporaneous faunal assemblage at the site gave further weight to the hypothesis that it represents the ritual offering of deer meat. However, given that the pelvis is statistically older than the child on the basis of the HYP dates, this hypothesis can now be rejected.

The fact that the red deer pelvis (sample B) and the red deer phalanx recovered from sediment adjacent to the burial pit (sample D) are statistically identical in age further rejects the hypothesis of intentional placement of meat at the time of burial. However, these data do not reject the other hypothesis originally laid out that the two red deer bones in the grave—the dated pelvis by the shoulder and the undated pelvis by the feet—were used for "construction" (e.g., as wedges to help with the positioning of the body in the burial pit). Nevertheless, red deer remains are common in the sedimentary infilling of the rockshelter, and although many such remains exhibit signs of carnivore activity and weathering consistent with exposure to water, other red deer bones with good surface condition akin to the red deer pelves by the child's right shoulder (sample B) and feet were recovered from adjacent, undisturbed deposits (*22*, *46*). Therefore, it is also possible that the two pelves simply stand for preexisting material that was disturbed during the inhumation.

Once the skeleton was removed from the burial pit, it could be observed that the bones were extensively ochre stained on all surfaces, indicating that the coloration was likely derived from decomposition

Table 2. Original radiocarbon dates and new hydroxyproline dates for the Lapedo child burial and adjacent contexts. OxA, published date ID for the Oxford Radiocarbon Accelerator Unit; GrA, published date ID for the Center for Isotope Research, University of Groningen; P-No, internal chemistry ID for the Oxford Radiocarbon Accelerator Unit; P-Code, internal chemistry protocol code for the Oxford Radiocarbon Accelerator Unit (AG, no ultrafiltration; AF, ultrafiltration; HYP, hydroxyproline); LV sample, field-assigned ID. $F^{14}C$ corrected for chemistry (including HPLC), graphitization, and AMS measurement. $\delta^{15}N$, C/N, and $F^{14}C$ not available for the GrA sample. Boldface used to highlight the new HYP dates.

Lab code	P-No	P-Code	LV sample	Material and context	Species	Collagen yield (wt %)	$\delta^{13}C$	$\delta^{15}N$	C/N	$F^{14}C$	±	^{14}C age (B.P.)	±	Cal B.P. (95.4% probability)
OxA-8421	9932.0	AG	L20.33 (sample B)	Left pelvis of red deer touching the right shoulder of child	*Cervus elaphus*	3.2	−19.6	3.4	3.2	0.0465	0.0015	24,660	260	28,230–29,630
OxA-43202	**9932.1**	**HYP**	**L20.33 (sample B)**			**3.9**	**−26.7**	**2.6**	**5.1**	**0.04679**	**0.001314**	**24,600**	**230**	**28,200–29,260**
OxA-8422	9933.0	AG	L20.99 (sample C)	Rabbit vertebra touching the left tibia of child	*Oryctolagus cuniculus*	8.4	−21.2	3.8	3.3	0.0509	0.0014	23,920	220	27,730–28,640
OxA-X-3228-11	**9933.1**	**HYP**	**L20.99 (sample C)**			-	**−31.7**	**2.7**	**5.1**	**0.053046**	**0.002871**	**23,590**	**430**	**27,150–28,790**
OxA-8417	9931.0	AG	L20.103 (sample A)	Child's left femoral fragment	Human	0.5	−20.8	8.8	3.7	0.0695	0.0018	21,420	220	25,200–26,220
OxA-43547	**53,791**	**HYP**	**L20.168**	Child's right radius		**1.6**	**−26.9**	**9.3**	**5.2**	**0.0507**	**0.0009**	**23,950**	**150**	**27,780–28,550**
GrA-13310	N/A	N/A	L20.190 (sample F)	Charcoal lens under child's legs	*P. sylvestris*	N/A	−28.7	N/A	N/A	N/A	N/A	24,860	200	28,690–29,700
OxA-8423	9934.0	AG	L19.2 (sample D)	Third phalanx of red deer in sediment adjacent to burial pit	*C. elaphus*	2.4	−20.1	3.0	3.3	0.0473	0.0014	24,520	240	28,080–29,210
OxA-43203	**9934.1**	**HYP**	**L19.2 (sample D)**			**3.8**	**−23.3**	**4.5**	**5.1**	**0.046701**	**0.000884**	**24,610**	**150**	**28,610–29,170**
OxA-10849	12,730	AF	K20.52 (sample G)	Shaft fragment of large mammal at the truncated surface of complex *bs*	Large mammal (*Cervus* or *Equus* size)	0.4	−20.5	3.0	3.0	0.0342	0.004	27,100	900	29,800–33,900
OxA-43204	**12,730.1**	**HYP**	**K20.52 (sample G)**			**1.2**	**−25.3**	**6.1**	**5.0**	**0.041613**	**0.000791**	**25,540**	**150**	**29,300–30,090**
OxA-10674	11,207.1	AF	J13-92	Metapodial diaphysis from complex *tc*	*Equus* sp.	1.3	−20.3	3.4	3.3	0.04480	0.00130	24,950	230	28,760–29,860
OxA-11318	12,968.0	AF	F3.SW04	Horse mandible from complex *al* (basal alluvium)	*Equus* sp.	0.1	−21.3	2.8	3.4	0.0244	0.0075	29,800	2500	29,800–43,100
OxA-43528	**12,968.1**	**HYP**	**F3.SW04**			**6.6**	**−26.7**	**4.2**	**5.2**	**0.00086**	**0.001**	**25,400**	**160**	**29,250–30,000**

of a shroud that the child had been buried in—one that fully enveloped the body at the time of deposition (*47*). In direct contact with the red-stained underside of the child's leg bones, the sediment presented extensive black staining. The particles of Scots pine (*P. sylvestris*) charcoal retrieved from this lens, which yielded the GrA-13310 result (28,690 to 29,700 cal B.P., 95.4% probability), were found during anthracological analysis to represent a single branch (*48*). However, in the ensuing excavation of both the adjacent sediment and the underlying deposit, no charcoal was found. On the basis of these observations, the interpretation was put forth that the charcoal staining of the base of the burial pit was part of the event, representing, e.g., the lighting of a ritual fire, or the fire cleansing of the grave, before the deposition of the child's shrouded body. The fact that scattered charcoal had also been found slightly higher-up, among the sediment that contained the bones, could be explained as a by-product of localized micro-disturbance due to the action of microfauna in the soil or to the development of a network of rootlets (from the vegetation that grew on the shelter's ground surface in the few years between the terracing of the site and the discovery of the burial context) (Fig. 5).

However, even considering the totality of the dates' 95.4% probability intervals, the radiocarbon age of 24,860 ± 200 B.P. (GrA-13310; 28,690 to 29,700 cal B.P., 95.4% probability) obtained for the charcoal is at least 150 years older than the new HYP result for the child. For the ritual explanation of the charcoal to be plausible, the greater age must either be (i) the result of the "inbuilt age" of the wood or (ii) the result of an erroneous measurement. Scots pines have been observed to live up to 1000 years in rare cases, but because the anthracological analysis revealed that the charcoal was derived from a branch (that is shed during growth) rather than the trunk, such a large inbuilt age is an unlikely explanation. Alternatively, an erroneous radiocarbon measurement would require carbon with zero activity to be added to the sample. This might be possible due to contamination by a sufficient amount of geological material, especially given the poor preservation of the charcoal (*48*). The sampling comments made by the Groningen lab describe the charcoal as containing "pebbles that were removed by sieving and handpicking," which were likely calcareous particles derived from the surrounding karst environment, but given that the first stage of charcoal pretreatment for radiocarbon dating is a hydrochloric acid wash, it seems unlikely that enough calcium carbonate would survive. Therefore, the third and parsimonious explanation is that the charcoal was already present at the site quite some time before the burial, and as such, the ritual burning hypothesis must be rejected.

This conclusion is further supported by the new radiocarbon results obtained for the activity area (carcass processing and expedient stone tool knapping) extending 10 to 20 m to the west of the burial, at approximately the same elevation but in the *ls* complex: the "EE15/Layer 143" unit (Fig. 6). Its excavation, initially carried out in 2000 to 2004, was completed in 2018 to 2022 (*22*, *49*–*51*). This activity area is extensively black stained due to the presence of large amounts of charcoal and burnt bone, and we now know, on the basis of ultrafiltered bulk collagen results, that it dates to a limited time interval: The date for the top is 24,390 ± 220 B.P. (OxA-X-187-12; 27,990 to 29,110 cal B.P., 95.4% probability), and the dates for the

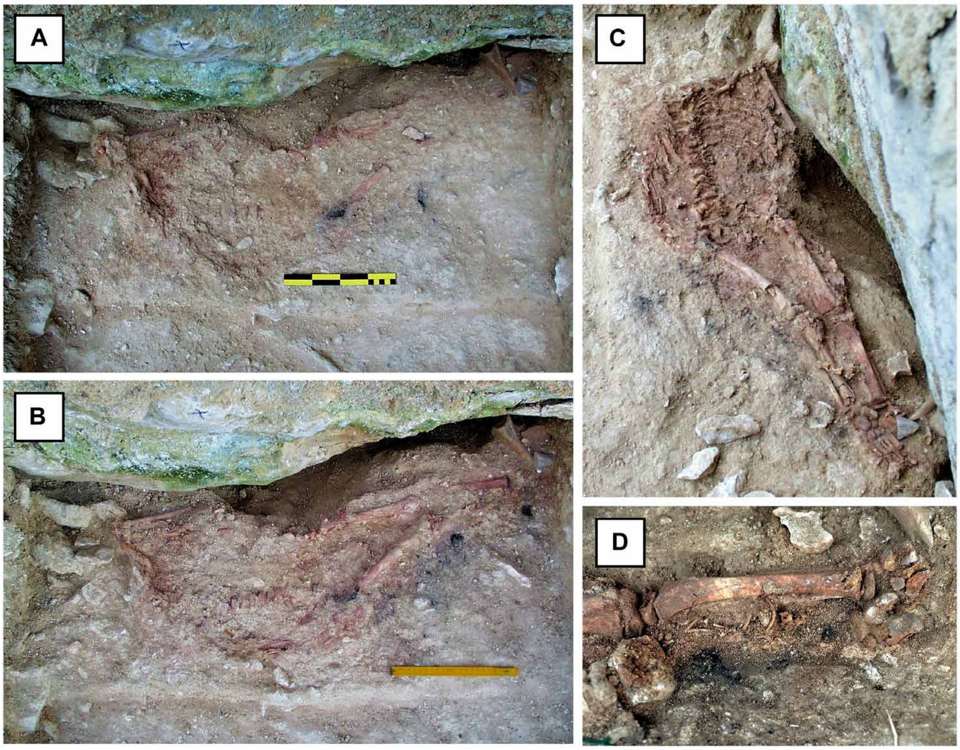

Fig. 5. The charcoal in the grave. (**A**) Scattered in the sediment that covered the bones (fourth day of excavation, 20 December 1998). (**B**) Emerging along the external edge of the burial pit (fifth day of excavation, 21 December 1998). (**C**) Scattered at the base of the burial pit, along the right child's right leg, once the skeleton had been fully exposed (11th day of excavation, 27 December 1998). (**D**) Under the child's right tibia, where the radiocarbon-dated charcoal sample was collected. After (*21*, *59*). Image credits: J.Z. and C.D.

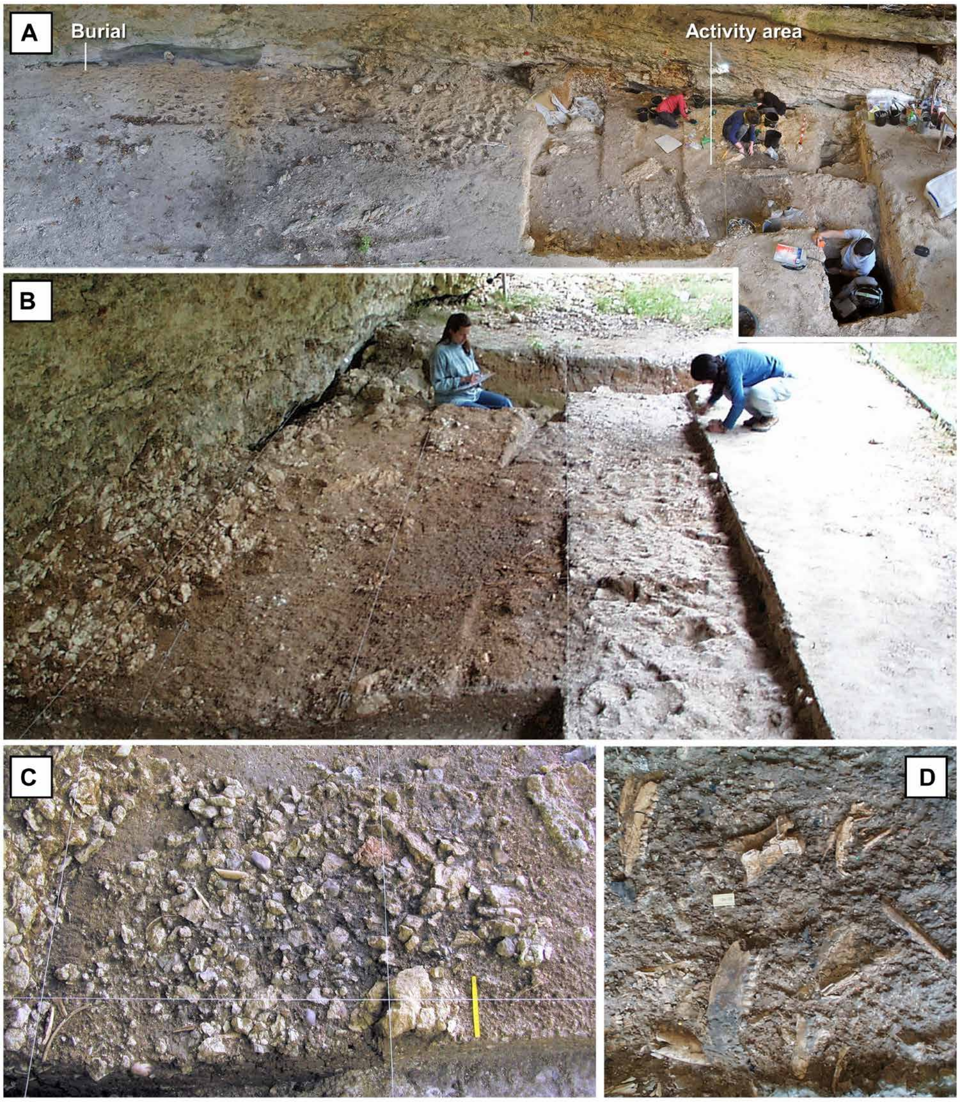

Fig. 6. The activity area west of the burial (unit EE15/layer 143). (**A**) Overview during the 2018 to 2022 phase of work; at this time, all trenches excavated to the east had been backfilled [after (*53*); photo courtesy of J. Daura, M. Sanz, A. C. Araújo, and A. Costa]. (**B** to **D**) At the beginning of the excavation, in 2001 [overview and details of a fire feature and of accumulations of faunal remains; after (*22*), image credit: J.Z.].

bottom are 24,650 ± 170 B.P. (OxA-42400; 28,600 to 29,200 cal B.P., 95.4% probability) and 24,660 ± 180 B.P. (OxA-42399; 28,580 to 29,220 cal B.P., 95.4% probability) (*49*). This is some two millennia earlier than indicated by the previously available ages: 22,493 ± 107 B.P. (Wk-9256, 26,440 to 27,120 cal B.P., 95.4% probability) on Scots pine charcoal and 23,042 ± 142 B.P. (Wk-9571, 27,150 to 27,670 cal B.P., 95.4% probability) on bone (*22*). Incomplete decontamination of the samples that yielded the results obtained more than 20 years ago is the parsimonious explanation for the discrepancy. Whichever the case may be, the fact remains that the three new dates for EE15/Layer 143 are indistinguishable from the GrA-13310 date for the charcoal under the child's legs, meaning that the hypothesis that all four sample the same event cannot be rejected.

Immediately west of the burial locus, the terracing work that allowed for the recognition of the site's archaeological significance caused extensive subsurface disturbance. As a result, the Connection Trench (Fig. 2) could not establish a direct stratigraphic link with the EE15/Layer 143 unit of the Activity Area Trench. However, burnt bone—e.g., a horse phalanx dated to 23,130 ± 130 B.P. (OxA-9571, 27,230 to 27,690 cal B.P., 95.4% probability), probably a minimum age only—was found in intact sediment at broadly the same elevation in a test pit open in square J13, halfway across (Fig. 2) (*22*, *52*). This evidence suggests the existence, predisturbance, of a low-density, discontinuous scatter of burnt material related to the EE15/Layer 143 activity area extending as far as the opposite end of the shelter where the grave was found. Given that the HYP date for the child is statistically significantly younger than the age obtained for the charcoal under the legs and that the latter is the same age as the ~30-cm-thick EE15/Layer 143 unit, it would seem that (i) the surface reached by the excavation of the burial pit correlates laterally with the deposit containing the activity recorded in that unit (explaining their identical radiocarbon ages) and (ii) the surface that the ~30-cm-deep burial pit was excavated from immediately postdates the end of the EE15/Layer 143 accumulation (as represented by the OxA-X-187-12 result, which appears to be slightly older than the child's, even though statistically indistinguishable).

The new HYP date for the juvenile rabbit vertebra (sample C) is statistically identical to both the child's and the original AG result. Abundant rabbit remains are present in the deposit surrounding the burial context. However, the pristine preservation of the deceased body's original positioning in the grave, the lack of any disturbances indicative of burrowing, the semiarticulated condition and ochre staining of the rabbit vertebrae found across the child's legs, and the fact that the other rabbit bones within the grave are both ochre stained and consistent with the same immature individual represented by the vertebrae suggest the intentional placement of a juvenile rabbit atop the burial of the child, before the backfilling of the burial pit, as an offering (*20–22*). This hypothesis is not falsified by the new HYP dates for the burial context.

In conjunction with the new results for the EE15/Layer 143 deposit, the much-enhanced precision of the HYP results for the basal part of the succession shed new light on the chronology of the initial phases of the site's formation process. Using OxCal v. 4.4.4 (*18*), we integrated these results in a Bayesian model (Fig. 7). The model also includes the single radiocarbon age available for the *tc* complex, which was not redated in the current study: 24,950 ± 230 B.P. (OxA-10674; 28,760 to 29,860 cal B.P., 95% probability). This complex extends across the entire site, overlying the basal infilling and underlying the burial context and the EE15/Layer 143 activity area (Fig. 2). OxA-10674 is an AF result obtained in 2001 along with OxA-10849 and OxA-11318 (the two HYP-redated samples from the basal infilling discussed above; sample G and sample F3.SW04), but its collagen yield was much higher (1.3 wt %), and therefore, the potential impact of contamination by the ultrafilter humectant is unlikely to be substantial (<2% at the most). The model also assumes that the succession's basal units—the cryoclastic, slope-waste *bs* complex at the eastern end of the shelter, and the alluvial *al* complex extending westward of the shelter's central sector—are heteropic, as originally hypothesized and the new HYP results corroborate.

Under these premises, the modeling suggests that the accumulation of the basal infilling ended during the 30th millennium cal B.P. and was followed by valley incision, as indicated by the unconformity separating it from the overlying *tc* complex. Sea level lowering on the way to the LGM parsimoniously explains the onset of this process. Sedimentation resumed soon after the brief erosional hiatus denoted by *tc*'s scarred surface, producing the overlying *gs* (to the east) and *ls* (to the west) complexes. The former contains the child's grave, and the latter contains the EE15/Layer 143 activity area. Given the dating results, these units would seem to be heteropic. Bayesian modeling suggests that they formed rapidly, over a lapse of no more than three centuries within the first half of the 29th millennium cal B.P. The burial event took place immediately afterward, at the beginning of the second half of the millennium.

Correlation of the modeled ages with the Greenland isotope record suggests that (i) the basal infilling and the *tc* complex accumulated during Greenland Stadial (GS) 5.1, which began 30,600 years ago and ended 28,900 years ago; (ii) the episode of human use of the site represented by the EE15/Layer 143 activity area took place during the following, very short GI (Greenland Interstadial) 4, which lasted until 28,600 years ago; (iii) the burial event occurred at the very end of this milder climatic phase or at the beginning of the ensuing cold phase, GS 4, which ended 27,780 years ago; and (iv) the poorly developed soil horizon atop the *ls* complex represents a local manifestation of the GI 3 interstadial (27,780 to 27,540 years ago). Pending integration of the 1998 to 2004 geological, geomorphological, and sedimentological observations with the insights gained by the 2018 to 2022 work, these correlations are consistent with the paleoenvironmental inferences derived from the geoarchaeological study of the site, namely, (i) the *gs* and *ls* complexes formed under humid climatic conditions, (ii) the morphodynamic change undergone by the Lapedo valley at this time relates to the reequilibration of the river-slope system to accommodate the regional impact of the periods' glacio-eustatic and global climate changes, and (iii) said morphodynamic processes were of major magnitude, as documented by the ~2-m thickness of the *al*/*bs*-*gs*/*ls* succession, which accumulated over no more than about 1500 years.

This burial event would seem to have represented the last episode of a short interval of human use of the site. After a millennium of alluvial and colluvial riverside accumulation, valley incision made it possible for humans to settle on the elevated, sandy ground surface thus exposed on the left bank of the stream, at the foot of the shelter's wall. Over no more than three centuries within the first half of the 29th millennium cal B.P., the western end of the platform was used for the expedient processing of the carcasses of game hunted nearby, with the abundant bone remains left behind being exploited by the bearded vulture once humans went away [as the associated coprolites illustrate (*51*)]. Then, a child died. The burial was placed at the opposite end of the shelter away from the activity area, and the site was thereafter abandoned, much like other Gravettian rock shelters such as Abri Pataud (perhaps because of the significance it acquired from the child's death and burial, as speculated elsewhere) (*53*, *54*). More than two millennia would pass, and more than 1 m of sediment would accumulate before people would again take shelter at Lagar Velho, in LGM times.

While usually used for the dating of samples contaminated by museum consolidants and Paleolithic material close to the limit of radiocarbon dating, the direct date for the Lapedo child demonstrates that this compound-specific radiocarbon dating method can also be applied to poorly preserved samples that would otherwise fail routine pretreatment methods. Other morphologically and culturally important Paleolithic human remains such as those from the Mladeč Caves (Czech Republic), Abri Pataud, and Saint-Césaire (France) would be ideal candidates for this approach, particularly those that exhibit younger-than-expected direct radiocarbon ages such as the Mladeč 25c ulna (*55*), the AP/89-2-288 vertebra (*54*), and "Pierrette" the Neanderthal, respectively. Targeting HYP ensures that even if the collagen is poorly preserved and fragmented, autochthonous carbon is collected for dating. However, a substantial amount of poorly preserved bone must be available for destructive analysis to obtain enough HYP for a radiocarbon date. This may not always be possible, especially in the case of morphologically important Paleolithic specimens of a small size. Future advancements in gas-source AMS measurements, however, will likely reduce the carbon mass requirements for dating and permit the compound-specific dating of increasingly small and poorly preserved samples.

MATERIALS AND METHODS

The Lapedo child radius

The Lapedo child's right radius was selected for HYP dating. The terracing of the site damaged the skull and the right arm, which was only marginally protected by the niche overhang (Fig. 3). The right

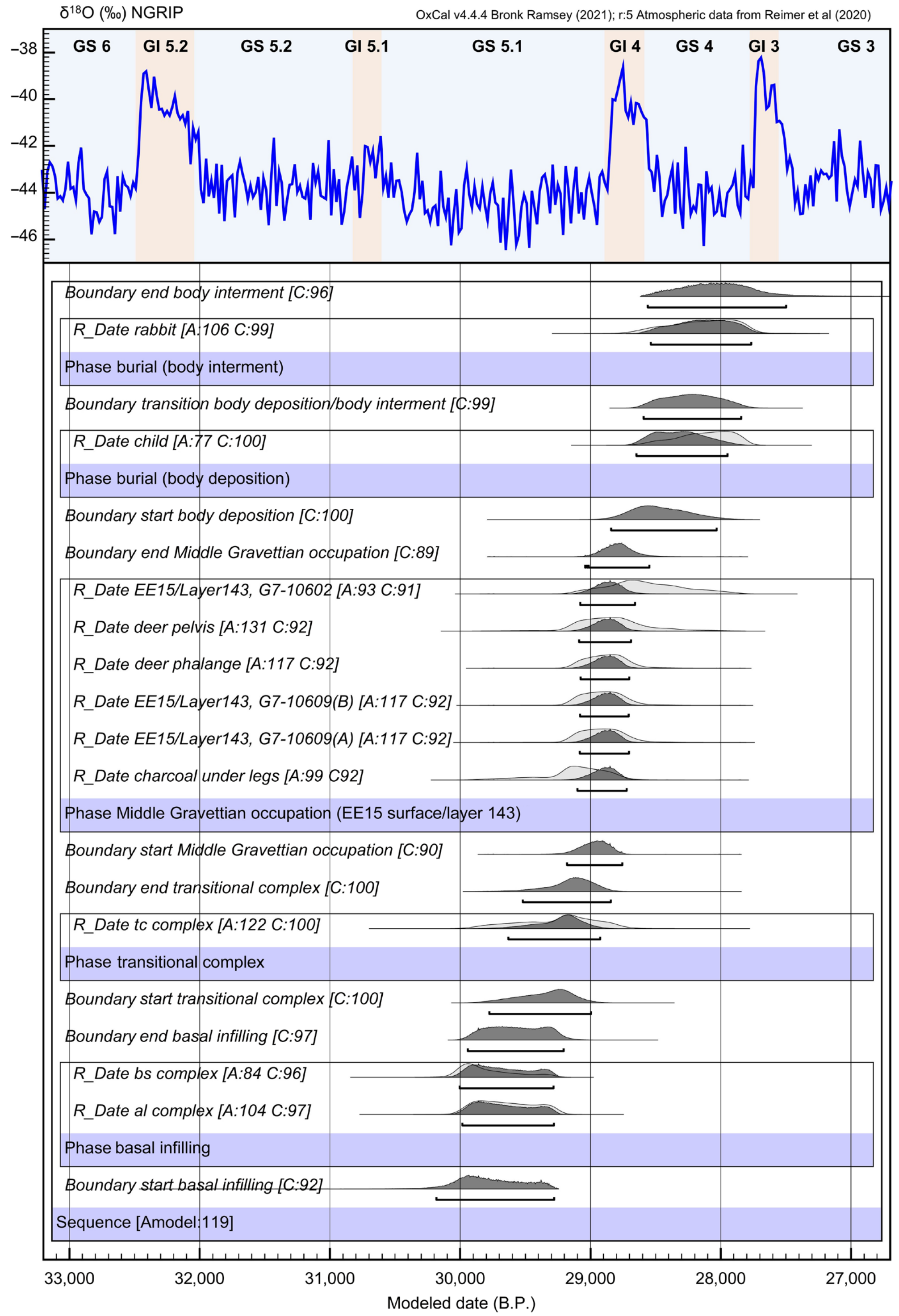

Fig. 7. Chronology of the burial context. Bayesian age model built in OxCal v.4.4.4 using the INTCAL20 calibration curve for the basal part of the Lagar Velho stratigraphic succession plotted against the Greenland ice core stadial/interstadial sequence (*60*) through the 27 to 33 ka ago interval. The burial event took place in the second half of the 29th millennium cal B.P.

humerus was lost, but almost all the teeth and the fragments that the cranium shattered into could be retrieved through excavation and sieving of the reworked sediment spread around the grave. The lower arm bones remained in situ, in their anatomical position, but were crushed in so many fragments that reconstruction was impossible, and so were targeted for HYP dating. Specifically, the submitted sample consisted of 6.6 g of bone shatter from the right radius (Fig. 4).

Collagen extraction

Collagen was extracted from the Lapedo child's right radius and samples B, D, G, and F3.SW04 following a modified AG protocol. Samples were initially cleaned by shot-blasting the surface of the bone with aluminum oxide powder (29 μm; OEA Labs, Exeter, UK). Bone samples of between 1 and 4 g (depending on availability and preservation) were then removed and crushed in a pestle and mortar. The collagen extraction procedure (AG) followed a modified Longin (*23*) method, involving an initial acid soak (0.5 M HCl) carried out over the course of 5 days, whereby the acid was replaced once per day until no further CO_2 evolved. This demineralization stage was carried out slowly at 5°C over 5 days (longer than the routine period of 2 days at the ORAU) to account for the poor preservation of the bones and larger sample sizes. The samples were then rinsed three times in ultrapure water to remove the acid, and subsequently treated with 0.1 M NaOH for 30 min to remove any humic contamination. The samples were rinsed again three times in ultrapure water and treated with 0.5 M HCl for 15 min to remove any CO_2 dissolved during the NaOH wash. Three final ultrapure water rinses were then performed. Gelatinization was carried out by adding 10 ml of pH 3 HCl to the samples at 75°C for 20 hours. Once gelatinized, the samples were filtered using 9-ml Ezee filters and frozen before lyophilization. The gelatinized collagen was then freeze dried for approximately 48 hours.

Collagen hydrolysis and amino acid separation by prep-HPLC

Between 14 and 50 mg of freeze-dried collagen from each sample (depending on availability) was hydrolyzed in 6 M HCl (1 ml per 10 mg of collagen) at 110°C for 24 hours and subsequently evaporated to dryness using a Genevac EZ-2 centrifugal evaporator (Warminster, PA, USA). Samples were then reconstituted in 700 μl of 0.2 M NaOH and 600 μl of ultrapure water to regulate the pH before HPLC injection.

Hydrolyzed collagen was then injected on a Varian Prostar HPLC system (Varian Analytical Instruments, Walnut Creek, CA, USA) equipped with two Prostar 210 Solvent Delivery Modules, a Prostar 320 UV/VIS detector set at 205 nm, a Prostar 510 column oven set at 30°C, a preparative scale mixed-mode Primesep A column (22 × 250 mm, particle size 5 μm; SIELC Technologies, Wheeling, IL, USA), a shorter Primesep A column (22 × 50 mm, particle size 5 μm, SIELC Technologies, Wheeling, IL, USA) installed upstream as a guard column, and a Prostar 701 X/Y fraction collector.

Following the protocol outlined by Devièse *et al.* (*27*), the hydrolyzed collagen was injected into an 18 ml min^{-1} flow of 100% ultrapure water as the initial mobile phase. The hydroxyproline fraction was collected and subsequently evaporated to dryness using the Genevac EZ-2 (Warminster, PA, USA). The mobile phase was then switched to 0.3% H_3PO_4 for the remainder of the program (275 min) to flush the column of the remaining amino acids. After evaporation to dryness of the mobile phase, the solid residue of hydroxyproline was reconstituted in 25 μl of Milli-Q water and transferred into a tin capsule (8 mm by 5 mm, OEA Laboratories Limited, Cornwall, UK) containing approximately 12 mg of diatomaceous silica (Chromosorb WAW 60/80 mesh; Supelco, PA, USA). The sample was then combusted following the ORAU graphite production protocols described in (*24*). Samples were then measured using the ORAU MICADAS 200-kV AMS system (Ionplus AG) and determinations were corrected for combustion and graphitization following (*56*).

Repeated hydroxyproline radiocarbon measurements of an Alaskan permafrost bison bone with an age beyond the limit of radiocarbon dating (>50,000 B.P.) and a known-age pig bone from the Mary Rose shipwreck [sank 1545 Anno Domini (AD)] were used to monitor the HPLC background and carry out quality control checks. The former provide data for the HPLC-specific background corrections outlined in Devièse *et al.* (*27*), which are integrated within the ORAU database.

Statistical analysis

We performed chi-square tests [error-weighted mean method (*57*) in OxCal v.4.4.4 using the *R_Combine* function, $\alpha = 0.05$] to test whether radiocarbon determinations made on the same bone (but using different pretreatment methods) were statistically different from one another. We used OxCal v. 4.4.4 (*18*) and the INTCAL20 calibration curve (*19*) to calibrate the radiocarbon determinations. We used Bayesian chronometric methods within OxCal v. 4.4.4 (*18*) to produce a refined chronological model for the burial and site, following established protocols described by Bronk Ramsey (*18*, *58*).

Supplementary Materials

This PDF file includes:
Supplementary Text

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Correction (9 May 2025): The original version of this paper erroneously used the wrong pretreatment code to refer to the redating of two bone samples. The erroneous codes "AG" have been revised to "AF*" in the Results section. The PDF and XML have been updated.