Refining techniques for radiocarbon dating small archaeological bone samples

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Chapter Four

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Direct radiocarbon dates of mid Upper Palaeolithic human remains from Dolní Věstonice II and Pavlov I, Czech Republic

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ABSTRACT

The ritual human burials and scattered fragments of human bones excavated from Dolní Věstonice II and Pavlov I (Czech Republic) in the 20th century provide a large body of evidence on morphology and funerary practices in the Gravettian as well as the population history of European *Homo sapiens* during the Upper Palaeolithic. A series of radiocarbon dates on charcoal and animal bone places the occupation of the sites predominantly between 31,000–29,000 cal BP (Early-Evolved Pavlovian) but direct radiocarbon dating of the human remains has not been previously undertaken. In 2013, human bones from Dolní Věstonice II and Pavlov I were sampled for aDNA analysis, including three skeletons from a triple burial (DV13, DV14, DV15), two skeletons from single burials (Pav1, DV16) and two unarticulated human bones (DV42, DV43). Small amounts of bone material were left over from the aDNA sampling, providing the first opportunity to directly date seven of the human individuals. Non-destructive pre-screening with near-infrared (NIR) spectroscopy indicated that sufficient collagen was preserved in the bone material for radiocarbon dating. We sampled very small amounts (32–202 mg) of bone material for collagen extraction, ultrafiltration and accelerator mass spectrometer (AMS) dating. Each collagen extract was dated multiple times using both graphite targets (ca. 800 µg C) and the gas ion source (< 100 µg C) of the AixMICADAS to obtain accurate and precise radiocarbon ages. The direct dates confirm the Pavlovian origin of the human remains and indicate that several of the radiocarbon dates carried out in the 1980s on associated charcoals were likely affected by low-level contamination of modern carbon. The results add seven individuals to the small collection of reliably dated Upper Palaeolithic humans in Europe.

1. Introduction

Human remains excavated from Gravettian contexts across Eurasia have been the focus of considerable palaeobiological research, including numerous elaborate ritual burials that offer fascinating insights into the biology and behaviour of mid Upper Palaeolithic people (see inventories in Henry-Gambier, 2008; Pettitt, 2011; Trinkaus et al., 2014; Vanhaeren and d'Errico, 2002). The large hunter-gatherer settlements at Dolní Věstonice and Pavlov, located on the northeastern slopes of the Pavlov Hills in Czech Republic (Fig. 1), have yielded extensive evidence of Pavlovian behaviour (a local variant of the Eastern Gravettian culture). The Dolní Věstonice-Pavlov region, which includes the large site clusters of Dolní Věstonice I (DVI), DVII, DVIII, Pavlov I and Pavlov II, is particularly notable for some of the earliest examples of carved mammoth ivory and fired clay objects of human and animal figurines, notably the fired clay Venus of Věstonice from DVI (Absolon, 1933; Vandiver et al., 1989). Over the course of the 20th century, several ritual human burials and numerous disarticulated human bones were excavated from these sites, including the famous triple burial from DVII (Klima, 1987; Sázelová et al., 2018; Sládek et al.,

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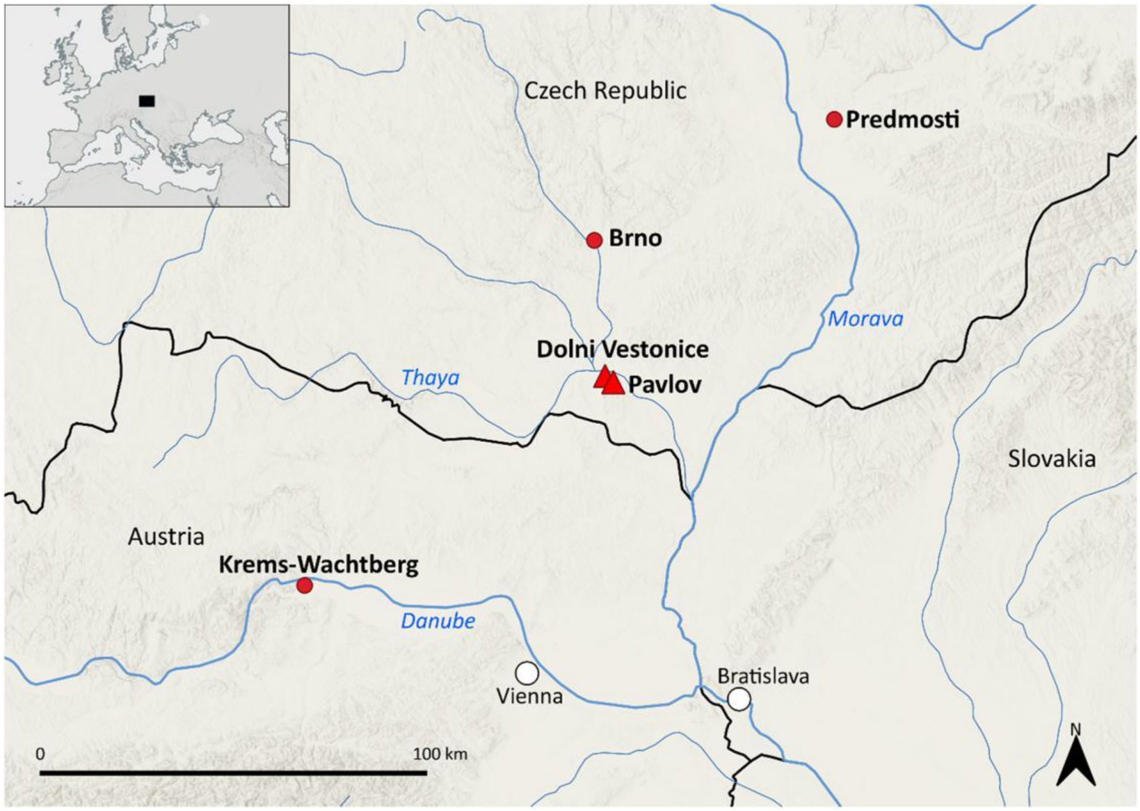

Fig. 1. Map of the Middle Danube region of central Europe showing the locations of Dolní Věstonice, Pavlov, and other nearby sites containing Gravettian burials mentioned in the text.

2000; Svoboda, 1987, 2016; Trinkaus and Svoboda, 2006; Trinkaus et al., 2010; Trinkaus et al., 2017; Vlcek, 1997). A series of radiocarbon dates on charcoal and bone measured at the Oxford and Groningen laboratories place the main occupations of DVII and Pavlov I between ~31,000–29,000 cal BP (Svoboda et al., 2016).

The human skeletal collection from Dolní Věstonice-Pavlov is the largest sample of human remains existing for this time period. Extensive analysis of this collection has greatly contributed to our understanding of the morphological variability, pathology and burial practices of Pavlovian people (Trinkaus and Svoboda, 2006). In 2013, 13 human bones from DVII and Pavlov I were sampled for aDNA analysis (Mittnik and Krause, 2016) and the results constitute a relatively large proportion of the genetic data used to investigate the population history of Upper Palaeolithic Europe (Fu et al., 2016). Given their importance within the assemblage of Upper Palaeolithic human skeletal material, it became increasingly important to directly date the human remains. Here we show the results of a new radiocarbon dating program of seven individuals using small amounts of bone material (< 200 mg) which were left over following aDNA analysis. We used rigorous methods of sample pretreatment established for small and precious Palaeolithic bones and obtained replicate radiocarbon measurements from each bone using two AMS dating methods (Fewlass et al., 2019). The directly dated individuals include the three skeletons from the triple burial (DV13, DV14, DV15), two skeletons from single burials (Pav1, DV16) and two unarticulated human bones (fibula fragment DV42 and femoral fragment DV43). aDNA analysis was also carried out for isolated human bones DV40, DV41, DV45, DV56 and DV57 from DVII but no material remained for dating (Mittnik and Krause, 2016).

2. Archaeological context of the human remains

2.1. Dolní Věstonice II

DVII has been excavated in a series of salvage excavations since the 1980s. The site complex is located 220–240 m above sea level (asl) on a loess elevation above the Dyje River. It is made up of distinct settlement units with central hearths, formed through repeated, short-term occupations. Although the area is large (almost 500 m^2), the occupation of DVII was less intensive than nearby DVI or Pavlov I and lacks the iconic artistic and decorative objects (Svoboda, 2006a). In addition to the ritual burials (DV13, DV14, DV15, DV16) a high number of unarticulated human bone fragments were found scattered throughout the cultural layers (Fig. 2; Sládek et al., 2000; Svoboda, 2006a; Trinkaus et al., 2000). The site is one of the most extensively dated Pavlovian settle-ments (Svoboda et al., 2016). A cluster of dates around 27,000 ^{14}C BP (end of the Early Pavlovian phase) was associated with some un-articulated human remains but the majority of the human burials were associated with dates from the Evolved Pavlovian phase between 27,000–25,000 ^{14}C BP (Table 1; Svoboda et al., 2016; Trinkaus and Svoboda, 2006).

In 1986, a triple human burial (Fig. 3) was discovered during excavation of the upper southern periphery of the site-top area (settle-ment units K7–9; Klima, 1987). The central figure (DV15) was lain on his back and showed evidence of congenital abnormalities (Trinkaus et al., 2016). The left figure (DV13) was also on his back, slightly twisted towards the central figure. The figure on the right (DV14) was lying on his front. The arms of the DV13 and DV14 overlapped the central figure, testifying that DV15 was laid out first. All three have been identified as adolescent-young adults (Trinkaus et al., 2016). Genetic analyses confirmed that the three individuals were male and found that DV14 and DV15 were closely maternally related (Mittnik and Krause, 2016). The burial included a small number of perforated

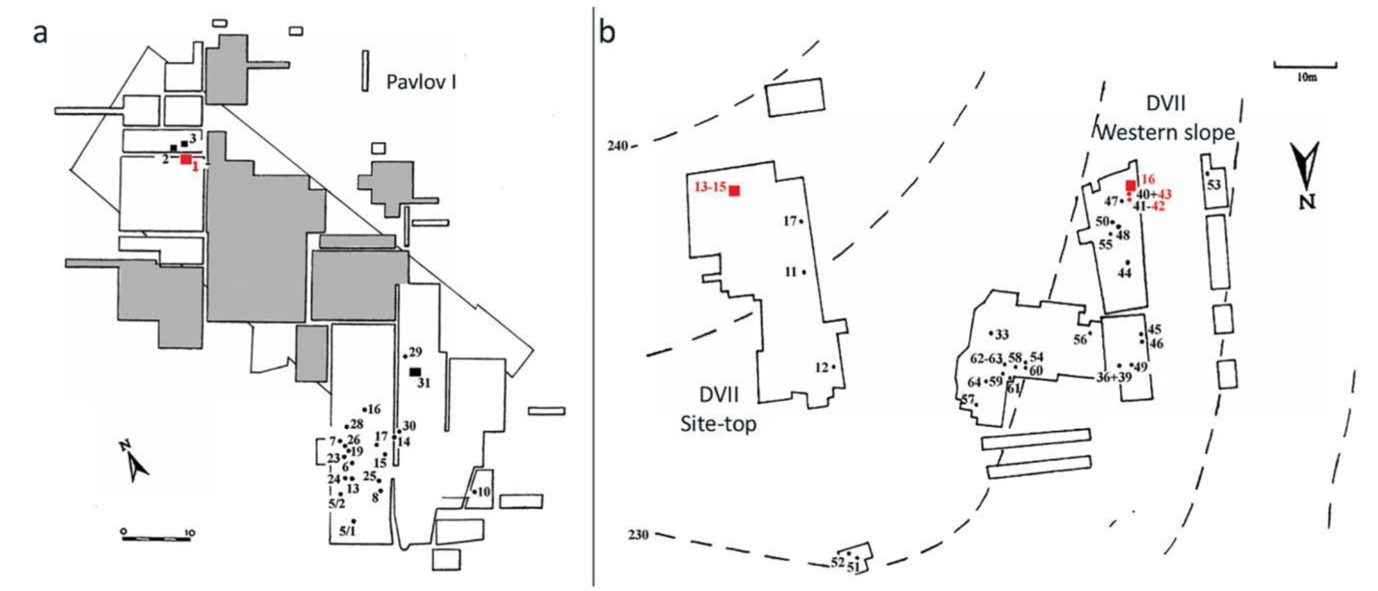

Fig. 2. Plan of (a) Pavlov I and (b) Dolní Věstonice II showing the locations of the human burials (squares) and unarticulated human bones (circles) excavated from the site. The human remains dated in the study are shown in red and those in black are documented elsewhere (Sládek et al., 2000; Trinkaus et al., 2010). In (b) the 230 m and 240 m asl contour lines are shown, with the site sloping down towards the north-northwest. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

canine teeth and ivory pendants and large concentrations of ochre, notably present as thick crusts on the skulls of the three individuals and pelvic area of the central figure. A large amount of charcoal was present in the burial, including the charcoal sample that produced the asso-ciated date (GrN-14831; Table 1).

The burial of DV16, an adult male, was excavated in 1987 (Fig. 3) from settlement unit S1 on the western slope of DVII (Svoboda, 1987, 2006b, 2016). The skeleton was laid on his right side in a flexed position facing the central hearth which was only 20 cm from his knees. The head and pelvic area were covered in a thick layer of ochre, and four perforated carnivore canines were found in association with the skeleton. The dated charcoal sample (GrN-15276) was associated with the burial (Table 1). Extensive root etching on the skeletons of DV13–16, which probably occurred during the Interpleniglacial (MIS3) shortly after deposition, indicates the burials were shallow or the bodies were laid directly on the ancient surface and may have been covered by superstructures for protection (Svoboda, 2006b, 2016; Trinkaus et al., 2010).

DV42 and DV43 were both excavated in 1987 from the lower part of the western slope but were only identified as human during laboratory processing of the archaeozoological material in 1998 (Holliday et al., 2006; Trinkaus et al., 2000). DV42 is a fragment of human fibula, which was located approximately 1 m from the central hearth in settlement unit S2, where the associated charcoal date (GrN-15279) comes from (Table 1; Svoboda et al., 2016). DV43, a fragment of human femur, was excavated from a depression with faunal bones and traces of fire and red ochre located approximately 1 m from the central hearth and 1 m from the DV16 burial in unit S1 (Svoboda et al., 2016). The associated date (GrN-15277) is from charcoal taken from the S1 hearth (Table 1).

2.2. Pavlov I

Pavlov I was systematically excavated between 1952 and 1972 (north-west and south-east sections) by B. Klima and again between 2013 and 2015 (south-east and south-west sections) by J. Svoboda (Svoboda et al., 2016). Based on dates from the Klima excavations it was thought that Pavlov I formed through a high intensity of repeated occupations during the Evolved Pavlovian period (27,000–25,000 ^{14}C BP; Svoboda, 2006a), but the more recent excavations show that the large site complex has a longer deposition of cultural deposits from the Early Pavlovian (Svoboda et al., 2016). The burial of an adult male (Pavlov 1) was excavated from the north-western part of the site in 1957 (Vlcek, 1997). Post-depositional slope movements caused some displacement of the skeleton. No ochre or artefacts were related to the burial but the skeleton was partially covered by mammoth scapulae. A human maxilla and two mandibles (Pavlov 2–4) were excavated from the same area and isolated human teeth and bone fragments were found throughout the cultural layers (Sázelová et al., 2018; Svoboda et al., 2016; Trinkaus et al., 2017). The charcoal date (GrN-20391; Table 1) was the only date from the north-western portion of the site but was not directly associated with the burial (Svoboda, 2006b).

Table 1
Previously published conventional radiocarbon dates and calibrated ranges (cal BP) of charcoals associated with the human remains from DVII and Pavlov I. DVII dates are reported in Svoboda et al. (2016). Pavlov I date is reported in van der Plicht, (1997). Radiocarbon ages were calibrated using the IntCal13 calibration curve (Reimer et al., 2013) in OxCal 4.2 (Bronk Ramsey, 2009). Dates have been rounded to the nearest 10 years.

Sample ID	Context	Material dated	Lab code	^{14}C age (BP)	Error (1σ)	2σ calibrated range (cal BP)
Pav1	Single burial	Charcoal (unrelated to burial)	GrN-20391	26,170	450	31,110–29,420
DV13	Triple burial	Charcoal in burial	GrN-14831	26,640	110	31,070–30,670
DV14						
DV15						
DV16	Single burial	Charcoal in burial	GrN-15276	25,570	280	30,540–29,040
DV42	Unarticulated	Charcoal in S2 hearth	GrN-15279	26,920	250	31,320–30,700
DV43	Unarticulated	Charcoal in S1 hearth	GrN-15277	25,740	210	30,580–29,400

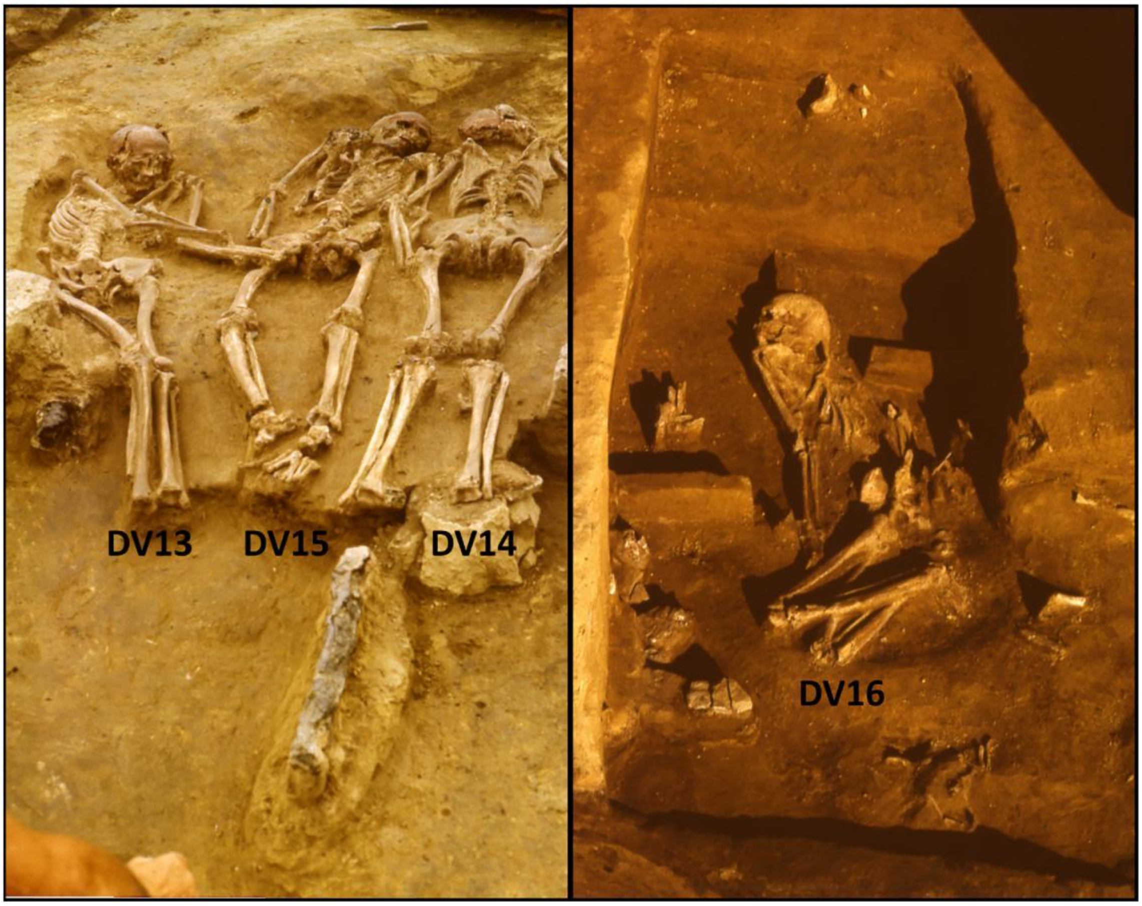

Fig. 3. Photos taken during the excavation of the DVII human burials in the 1980s, showing the DV13, DV14 and DV15 individuals in the triple burial (left) and the single burial of DV16 (right). Photos by J. Svoboda.

3. Materials and methods

The human remains were consolidated immediately following ex-cavation (acrylic resin to the skeletons from the triple burial and PVA to DV16 and the isolated fragments). Various protocols are applied to archaeological bone to remove conservatives prior to collagen extraction as their presence can significantly affect ^{14}C ages through the introduction of non-endogenous carbon (Brock et al., 2017). However, additional pretreatment steps (such as repeated organic solvent washes) increase the likelihood of damage to collagen and the introduction of lab-based contamination. Due to the limited material available from the DVII and Pavlov human remains we decided to apply our standard pretreatment method to very small aliquots of the available bone to reserve some material for further pretreatment if we found evidence of contamination in the first extracts.

During sampling for aDNA analysis, the outer bone surface was removed (Rohland and Hofreiter, 2007). All of the samples were taken from femora, except DV43 which was taken from a fibula. For six samples, a mixture of bone powder and small fragments of whole bone remained after sampling for aDNA. As it has been demonstrated that higher collagen yields are obtained when whole pieces of bone, rather than powdered bone, are pretreated (Fewlass et al., 2019), small whole bone fragments (32.3–70.4 mg material) were selected for pretreat-ment. For DV15, only bone powder was available so a larger sample (203 mg) was taken for collagen extraction.

Recently it has been shown that near-infrared (NIR) spectroscopy is a promising method for effective, fast and non-destructive pre-screening of archaeological bone for the presence of intact collagen (Sponheimer et al., 2019). Using the methods described in Sponheimer et al., (2019), NIR spectroscopy was used to analyse the bone powder of the DVII and Pavlov I burial remains prior to pretreatment to determine if sufficient collagen was preserved for radiocarbon dating. Powdered bone samples were scanned using a fiber-optic reflectance probe attached to a LabSpec 4 NIR spectrometer with a spectral range of 350 nm to 2500 nm. Subsequent data transformations and analyses were undertaken using Unscrambler X software (Camo Analytics, Oslo). A Savitzky-Golay transformation (derivative order = 2; polynomial order = 3; smoothing points = 31) was performed to correct for additive and multiplicative effects in the spectral data. A 1-factor model was used that restricts the spectra to the peaks at 2050 nm and 2180 nm, which have been shown to differ in relation to collagen content (Sponheimer et al., 2019).

Pretreatment was carried out in the Human Evolution department at the Max Planck Institute for Evolutionary Anthropology, Leipzig, following the modified Longin (1971) protocol described in Fewlass et al. (2019). Bone samples were fully demineralized in HCl 0.5 M, treated with a base wash (NaOH 0.1 M) to remove humic acid contamination and reacidified in HCl 0.5 M. The samples were then gelatinized in weakly acidic water (HCl pH 3) at 70 °C for several hours. The resulting gelatin was filtered to remove large particles (>80 µm; Ezee filters, Elkay labs, UK) and ultrafiltered (Sartorius VivaSpin Turbo 15) to concentrate the high molecular weight fraction (>30 kDa; Brown et al., 1988), which was then freeze-dried for 48 h. Ultrafilters were cleaned before use (Brock et al., 2007; Bronk Ramsey et al., 2004). A background bone (dating to >50,000 BP) was pretreated and measured alongside the samples to monitor and account for any contamination introduced in the laboratory.

To assess the quality of the extracts, collagen (ca. 0.5 mg) was packed into tin capsules and measured with a ThermoFinnigan Flash elemental analyser (EA) coupled to a Thermo Delta plus XP isotope ratio mass spectrometer (IRMS) to determine the elemental (C%, N%, C:N) and stable isotopic values ($\delta^{13}C$, $\delta^{15}N$). Stable carbon isotope ra-tios were expressed relative to Vienna PeeDee Belemnite (VPDB) and stable nitrogen isotope ratios were measured relative to atmospheric N_2 (AIR), using the delta notation (δ) in parts per thousand (‰). Repeated analysis of internal and international standards indicates an analytical error of ± 0.2‰ (1σ). In addition, a small amount (ca. 0.3 mg) of each collagen extract was homogenized in a mortar and pestle, mixed with ~40 mg IR grade KBr powder and pressed into a pellet using a manual hydraulic press (Wasserman). The pellets were analysed with an Agilent Technologies Cary fourier transform infra-red (FTIR) spectrometer with a deuterated triglycine sulfate (DTGS) detector. Sample spectra were recorded in transmission mode at 4 cm^{-1} resolution and averaged for 34 scans between 4000 and 4 cm^{-1} using Resolution Pro software (Agilent Technologies, Santa Clara). The spectra were evaluated and compared to library spectra of well-preserved collagen and bone to look for evidence of incomplete demineralisation, degraded collagen or the presence of any exogenous material in the extracts (D'Elia et al., 2007; DeNiro and Weiner, 1988; Yizhaq et al., 2005).

We made use of the hybrid nature of the ion source of the AixMICADAS (Bard et al., 2015; Wacker et al., 2010c) installed at CEREGE (Centre de Recherche et d'Enseignement de Geosciences de l'Environnement, Aix-en-Provence, France) to date each collagen extract multiple times with both graphite targets (2–3 mg bone collagen) and the gas ion source (Fewlass et al., 2017, 2019). Collagen was weighed into tin cups (ca. 2 mg), graphitized using the AGE 3 (Auto-mated Graphitisation Equipment; Wacker et al., 2010b) and dated using the AixMICADAS. Oxalic acid II standards and background collagen samples were measured in the same session and used in the age cal-culation of the archaeological samples. An external error of 1‰ was propagated in the error calculation as per standard practice.

Gas measurements were performed using the protocol described in Tuna et al. (2018) and Fewlass et al. (2019). Small aliquots of collagen (< 200 µg collagen) were measured into cleaned silver cups (800 °C, 2 h) and combusted in an Elementar Vario MICRO cube EA (Elementar Analysensysteme GmbH, Germany) which was directly coupled to the gas ion source of the AixMICADAS via the gas interface system (GIS; Ruff et al., 2010; Wacker et al., 2013). The sample CO_2 was mixed with helium (5% CO_2) and fed into the gas ion source at a flow rate of ca. 2 µg C/min. The EA-GIS system was flushed with helium to clean be-tween samples. Precleaned Ti gas targets were presputtered (2 min) in the ion source to remove remaining surface contamination. Oxalic acid NIST standards (from a gas canister) were measured to normalize and correct samples for fractionation. The long-term standard deviation of blanks ($F^{14}C = 0.001$) was used as the absolute blank error and an external error of 3.5‰ was added (Fewlass et al., 2017; Tuna et al., 2018). The background collagen (^{14}C free) was measured alongside the samples and used in the age calculation of archaeological samples. All data reduction was performed in BATS (Wacker et al., 2010a).

The radiocarbon ages were calibrated in OxCal 4.3 (Bronk Ramsey, 2009) against the IntCal13 dataset (Reimer et al., 2013). To combine the multiple dates we had from each collagen extract we used the R_Combine function in OxCal 4.3 using the $F^{14}C$ and error. As part of this function, a χ^2 test was performed to see if the dates agree statistically (Ward and Wilson, 1978). Following calibration, we used the Combine function to combine the weighted mean of the dates for each individual and the previous charcoal date from within the triple burial.

4. Results

Estimates of collagen preservation in the bone powder from NIR spectroscopy ranged from 7.2 to 9.5% (Table 2). The NIR estimate was identical to the extracted yield for DV14 and DV15 but slightly un-derestimated the collagen yields of Pav1, DV13 and DV16. This discrepancy may relate to the fact that powdered bone was scanned whereas whole bone pieces were extracted for all three individuals. The lower estimates for the powdered aliquots may result from the de-gradation of collagen during the process of transforming whole bone fragments into powder. In all cases, the NIR prescreening correctly determined that the bones had sufficient collagen preserved for ex-traction and analysis.

Following bone pretreatment, the quality of the extracted collagen was assessed based on the yield (as a percentage of the overall weight of the bone where modern bones typically yield ca. 22% weight collagen) and the elemental values determined by EA-IRMS. The collagen yields were excellent (8–14% collagen preserved) for Palaeolithic bone (the generally accepted lower limit for dating is 1%) and the elemental values (C%, N%, C:N) were within the accepted ranges for well-preserved collagen (C%: 30–45%; N%: 11–16%; C:N: 2.9–3.6), indicating that the collagen extracts were suitable for radiocarbon dating (Table 2; van Klinken, 1999). The $\delta^{13}C$ and $\delta^{15}N$ values of the seven individuals fall within the range of stable isotopic values seen for other mid Upper Palaeolithic humans in Eurasia (see Trinkaus et al., 2014). The new direct AMS dates from the seven individuals confirm they belong to the collection of mid Upper Palaeolithic human remains in central Europe. The weighted mean age for each human is shown in Table 2 and the calibrated ranges of the radiocarbon dates are shown in Fig. 4 (all AMS determinations are included in SOM Table S1). The radiocarbon dates determined through both the graphite and direct CO_2 dating methods agree within two standard deviations (2σ; 95.4%) for the six bones from DVII. Although the graphite date from the Pav1 bone collagen is within 2σ of the CO_2 dates from the same collagen extract, it is at the outer limit of this range and the dates just fail the χ^2 test of contemporaneity at the 95% confidence level (χ^2 test: T = 6.6 [5% 6.0], df = 2). The dates from the individuals buried in the triple grave are statistically indistinguishable which is in accordance with the genetic conclusion that DV14 and DV15 were closely maternally related (Mittnik and Krause, 2016) and the archaeological interpretation that the three individuals were interred at the same time (Klima, 1987). When the direct bone dates of the three humans and the previously dated charcoal from the triple burial are combined in OxCal (Fig. 4) the level of agreement is excellent (Acomb = 97.5%, An = 35.5%), giving a narrow calibrated age of 31,010–30,910 cal BP (1σ; 68.2% confidence level).

Although all C%, N% and C:N values of the collagen extracts are all within accepted ranges of well-preserved collagen, the C:N values of DV14, DV42 and Pav1 are at the higher end of this range (Table 2) which can indicate the presence of contaminating carbon (van Klinken, 1999). To assess if there was any evidence of contamination from the conservatives applied in the 1980s, the collagen extracts were analysed with FTIR (D'Elia et al., 2007). All extracts had spectra characteristic of well-preserved collagen with no evidence of exogenous material (Supplementary Online Material [SOM] Fig. S1). The agreement of ^{14}C ages between the collagen extracts from DV14, DV42 and Pav1 and the as-sociated charcoal samples and between DV14 and the other individuals in the triple grave imply that the ^{14}C ages are not significantly affected by carbon contamination from the conservatives applied after excava-tion. The removal of the outer bone surface followed by the acid-base-acid sequence, gelatinisation, ultrafiltration and multiple washes with H_2O during the pretreatment appear to have sufficiently removed any conservatives from the collagen extracts that may have caused erroneous ^{14}C results.

5. Discussion

For Pav1, DV13, DV14, DV15 and DV42, the new dates from the human bone collagen overlap with the associated/proximal charcoal dates at 1σ (68.2% probability) or 2σ (95.4% probability). For the single burial DV16, the dates from the human skeleton (27,220 ± 110 ^{14}C BP) are approximately 1850 ^{14}C years older than the date from charcoal within the grave (25,570 ± 280 ^{14}C BP; GrN-15276) and there is a very low level of agreement when they are combined in OxCal (Acomb = 0.9%, An = 50.0%). The DVII charcoal samples were

Table 2
Pretreatment data and accelerator mass spectrometer (AMS) radiocarbon determinations for the human bones pretreated from DVII and Pavlov I. Weighted mean ages and 1σ errors are given for replicate AMS measurements made from each collagen extract (shown in SOM Table S1). Calibrated ranges (cal BP) were determined in OxCal 4.3 (Bronk Ramsey, 2009), against the IntCal13 dataset (Reimer et al., 2013). All dates have been rounded to the nearest 10 years.

Sample ID	Bone used (mg)	NIR prediction collagen (%)	Collagen yield (mg)	Collagen yield (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C%	N%	C:N	^{14}C age (BP)	Error (1σ)	1σ calibrated range (cal BP)	2σ calibrated range (cal BP)
Pav 1	50.4	7.2[a]	4.7	9.3	−19.5	13.6	41.2	14.3	3.4	25,490	90	29,720–29,410	29,910–29,260
DV13	42.3	9.3[a]	5.7	13.5	−19.3	12.9	38.5	14.0	3.2	27,040	100	31,170–30,980	31,250–30,880
DV14	37.9	9.4[a]	3.6	9.5	−20.2	13.3	39.0	13.1	3.5	26,760	100	31,040–30,830	31,120–30,730
DV15	201.5	8.0	16.2	8.0	−19.4	12.6	37.1	13.3	3.2	26,680	70	31,020–30,820	31,110–30,720
DV16	32.3	9.5[a]	4.5	13.9	−19.7	12.5	38.5	13.8	3.3	27,220	110	31,250–31,060	31,350–30,970
DV42	53.1		4.8	9.0	−19.8	12.7	39.2	13.5	3.4	26,880	110	31,110–30,900	31,180–30,790
DV43	70.4		7.2	10.2	−19.6	12.6	38.9	13.7	3.3	27,070	110	31,190–30,990	31,270–30,890

[a] Near-infrared spectroscopic estimates were made for powdered bone whereas whole pieces were pretreated for all the individuals except DV15, where only powder was available.

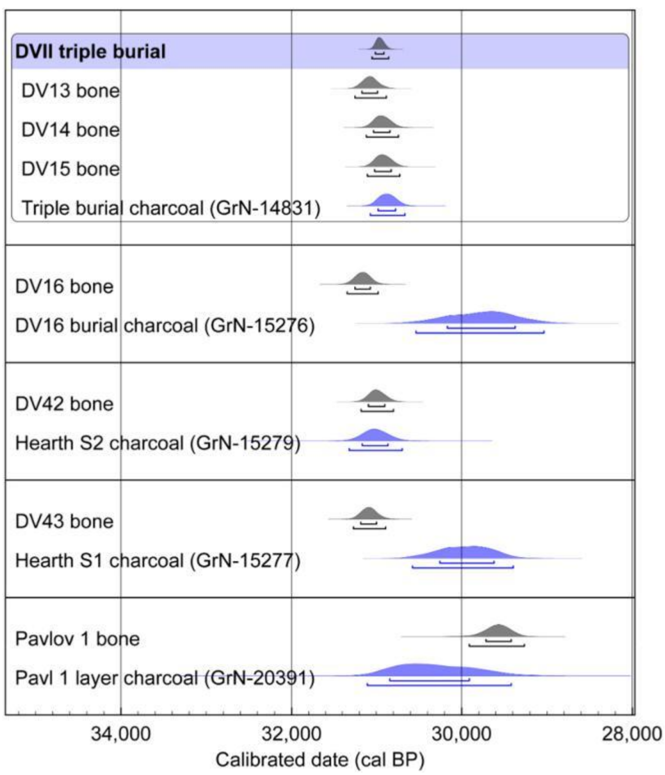

Fig. 4. Calibrated ranges of the new direct dates from the human remains (dark grey) and the associated charcoal dates produced in the 1980s (blue). The range shown for each bone date represents the weighted mean of replicate measurements from one collagen extract. The brackets beneath each distribution show the 1σ (68.2%) and 2σ (95.4%) probability ranges. The dates from the individuals in the triple burial and the associated charcoal date were combined (boxed). Calibrations were performed in OxCal 4.3 (Bronk Ramsey, 2009) using the IntCal13 dataset (Reimer et al., 2013). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pretreated before rigorous acid-base-wet oxidation (ABOX) pretreat-ment methods had been established to remove contamination from charcoal samples (Bird et al., 1999, 2014). Ultrafiltration of Palaeolithic bone collagen often produces older ages compared to non-ultrafiltered collagen from the same bone, which is attributed to the removal of small molecular weight contaminants from the final sample (Higham et al., 2006; Talamo and Richards, 2011). As any modern carbon con-tamination present in a sample at the time of dating will make an age younger, older dates are generally considered more accurate (Higham et al., 2006; Higham, 2011). It is therefore likely that the discrepancy in age between the DV16 bone and associated charcoal date is due to a small amount of modern carbon contamination not removed from the charcoal sample in the 1980s, which led to an underestimation in age. The new direct dates place the DV16 burial slightly older than the triple burial, falling within the late Early Pavlovian range of dates (around 27,000 ^{14}C BP; 31,000 cal BP) obtained from other charcoals at the site (Svoboda et al., 2016). The bone date for DV43 is also older than the date from the charcoal in the nearby S1 hearth. Either the hearth was made after the human had died, or the charcoal date is also an un-derestimation due to incomplete purification of the sample in the 1980s (more likely this scenario, considering DV16; Fig. 4). The new bone dates indicate that the Pavlov 1 burial dates slightly later than the burials at DVII.

Ritual and isolated human remains are present at a significant number of Upper Palaeolithic sites in addition to DV and Pavlov (see Pettitt, 2011). At several sites, human bones have been found scattered throughout the occupational sequences as well as in ritualistic burials. At Sunghir, Russia, the individuals Sunghir 1, 2 and 3 were interred in spectacularly rich burials (most recently dated between ~30,000–28,000 ^{14}C BP by compound specific radiocarbon dating; Marom et al., 2012; Nalawade-Chavan et al., 2014). Fragmented human bones (Sunghir 4 and 5) were found associated with the burials, potentially with cultural significance, and in the cultural layer (Sunghir 7) (Trinkaus and Buzhilova, 2018). This phenomenon has led to many questions about variable mortuary practices in the mid Upper Palaeolithic. The differential treatment of the dead at DV and Pavlov has been discussed previously in terms of both human behaviour and taphonomic factors (Sázelová et al., 2018; Svoboda, 2008; Trinkaus et al., 2000; Trinkaus et al., 2010; Trinkaus et al., 2019). The contemporaneous dates from the burials and the fragmentary unarticulated bones at DVII (Fig. 4) further demonstrate that postmortem treatment of different individuals varied concurrently, either naturally or though human intervention.

The DVII human burials are contemporary with other burials in the Middle Danube region of Central Europe (Fig. 5; Einwögerer et al., 2006; Svoboda, 2008). A large collection of human burial remains (>20 individuals) was excavated in the 19th and 20th centuries from Predmosti, located close to the Moravian Gate around 100 km north-east of DV/Pavlov (Fig. 1), but lamentably the majority of the collection was destroyed in a fire in 1945 (Svoboda, 2008). The few fragmentary remains have not been directly radiocarbon dated but the layer associated with the burials dates to the Evolved Pavlovian period (27,000–25,000 ^{14}C BP), which would make them roughly contemporaneous or slightly later than the DVII and Pavlov burials. A double burial of new-born infants (Burial 1), sealed with a large mammoth scapula and containing large amounts of ochre and ornaments, was excavated at Krems-Wachtberg, Austria (Fig. 1), along with another single infant burial (Burial 2; Einwögerer et al., 2006). The skeletons have not been directly dated but both burials were associated

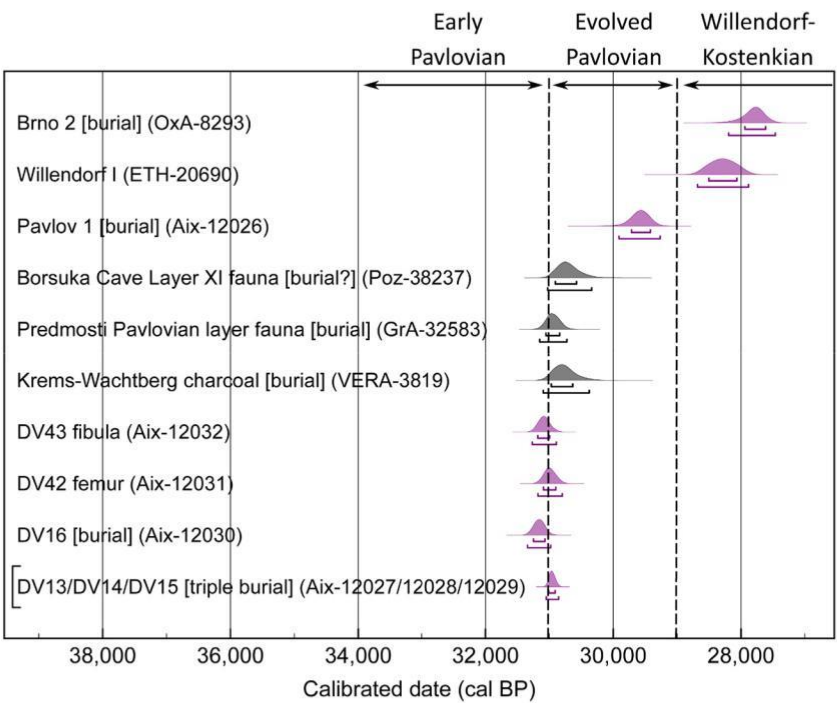

Fig. 5. Calibrated dates of the DVII and Pavlov 1 human remains in comparison to other central European mid Upper Palaeolithic human remains discussed in the text. Direct dates on human remains are shown in purple and associated dates are shown in black. DV35 is not shown as the date is thought to be anomalously young. Figure produced in OxCal 4.3 (Bronk Ramsey, 2009) using the IntCal13 (Reimer et al., 2013) dataset. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with a well-preserved living floor dated to ca. 27,000 ^{14}C BP and charcoal associated with Burial 1 gave a date of 26,520 + 210/−200 ^{14}C BP (VERA-3819; 68.2%: 30,970–30,630 cal BP), making it contemporaneous with the DVII triple burial (Einwögerer et al., 2009). A collection of six deciduous human teeth and 112 pendants made of large herbivore teeth from Borsuka Cave, Poland (~300 km NE of DV/ Pavlov), has also been interpreted as an infant burial, although the context is highly disturbed (Wilczyński et al., 2016). The human teeth have not been dated directly but two of the pendants were dated to 27,350 ± 450 ^{14}C BP (Poz-32394: 68.2%: 31,640–30,930 cal BP) and 25,150 ± 160 ^{14}C BP (Poz-38236: 68.2%: 29,400–28,980 cal BP) and a reindeer metatarsus from the associated Layer VI was dated to 26,430 ± 180 ^{14}C BP (Poz-38237) (Wilczyński et al., 2012; Wilczyński et al., 2016). Although not contemporaneous to each other, the dates suggest that the infant remains may originate from a similar time range as the remains from DV/Pavlov, and led to the association of the burial with the Pavlovian culture, despite a lack of associated diagnostic ar-tefacts (Wilczyński et al., 2016).

The only other directly dated human remains from the Middle Danube region fall within the later Willendorf-Kostenkian stage of the Gravettian (25,000–21,000 ^{14}C BP; Fig. 5). An isolated femur (Willendorf I) was excavated at Willendorf, a large open-air site complex located on the bank of the Danube River, Austria, in the 1880s. The bone yielded a direct radiocarbon age of 24,250 ± 180 ^{14}C BP (ETH-20690; 68.2%: 28,500–26,070 cal BP; Teschler-Nicola and Trinkaus, 2001), but information on its original context is lacking. The exceptionally rich Brno 2 burial (40 km north of DV and Pavlov; Fig. 1) was directly dated to 23,680 ± 200 ^{14}C BP (OxA-8293; 68.2%: 27,940–27,610 cal BP; Pettitt and Trinkaus et al., 2000). In closer proximity, an unarticulated human femur (DV35) from nearby DVI is the only previously directly dated human bone from the DV/Pavlov area and is dated to 22,840 ± 200 ^{14}C BP (OxA-8292; 68.2%: 27,420–26,980 cal BP; Trinkaus et al., 1999). However, DV35 was only identified as human in the 1990s so its exact context within DVI is uncertain. The much younger age compared to the other Early or Evolved Pavlovian dates from the site indicate that the sample was contaminated (Svoboda et al., 2016; Trinkaus et al., 1999). It is worth noting that Willendorf I, Brno 2 and DV35 were pretreated before the wide spread application of ultrafiltration and it has been suggested that the dates provide little more than confirmation that these human remains belong to the Gravettian (Trinkaus et al., 2014).

6. Conclusion

The results of this study confirm the Pavlovian origin of the seven human bones from DVII and Pavlov I, in two cases (DV16 and DV43) pushing back the age assigned to the human remains from associated charcoal dates. The collagen sample chemistry and the consistency of the ages from the triple burial and with the charcoal dates carried out in the 1980s lend confidence to the reliability of the results. This study further confirms the suitability of NIR spectroscopy as a collagen pre-screening method for radiocarbon dating archaeological bone (Sponheimer et al., 2019). The method is completely non-destructive which makes it ideal for prescreening precious archaeological bone prior to pretreatment to determine if collagen preservation is sufficient. Radiocarbon datasets such as reported here are crucial for refining our understanding of the chronology of Gravettian cultural evolution.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://

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Supplementary Online Material (SOM)

Direct radiocarbon dates of mid Upper Palaeolithic human remains from Dolní Věstonice II and Pavlov I, Czech Republic

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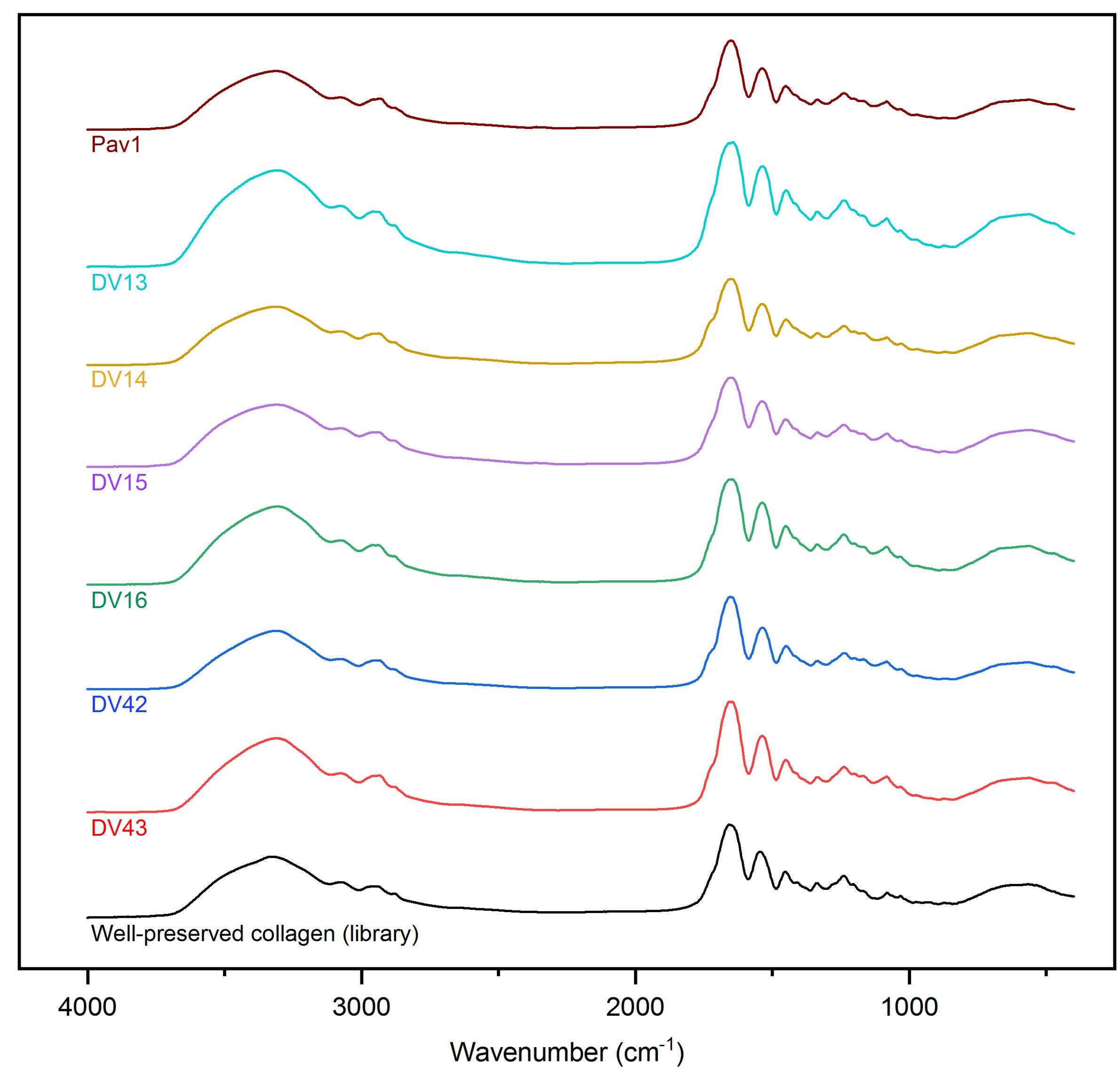

SOM Figure S1. Fourier transform infra-red (FTIR) spectra of the collagen extracts from the seven human bones from Pavlov I and Dolní Věstonice II. The bottom-most library spectra of well-preserved collagen is included for comparison.

SOM Table S1. New accelerator mass spectrometer (AMS) determinations from bone collagen from the Pavlov I and Dolní Věstonice II human remains showing the dating method used (CO_2 gas ion source or graphite target) and amount of carbon (µg C) measured by the elemental analyser (EA) following combustion of the sample. Based on series of measurements of various standards, the precision of AMS $\delta^{13}C$ is ca. 1 ‰ for graphite samples (Bard, et al., 2015) and ca. 2 ‰ for CO_2 gas samples (Tuna, et al., 2018). The $\delta^{13}C$ for gas samples is usually shifted by -2 ‰ with respect to the accurate analyses of graphite samples. Tuna et al. (2018) attributed this shift to isotopic fractionation in the elemental analyser + gas interface system for unknown samples, by contrast to the standard measured directly on a CO_2 bottle of oxalic acid. This small bias in the $\delta^{13}C$ calculation for unknown samples has no influence on their ^{14}C age determination. All dates have been rounded to nearest 10 years.

Sample ID	AMS lab code	Dating method	EA sample size (µg C)	^{14}C age (BP)	Error (1σ)	AMS $\delta^{13}C$ (‰)
Pavlov 1	Aix-12026.1.1	CO_2	85	26,220	370	-23.5
	Aix-12026.1.3	CO_2	97	25,790	250	-23.2
	Aix-12026.2.1	graphite	756	25,390	100	-18.8
DV13	Aix-12027.1.1	CO_2	80	26,950	390	-21.5
	Aix-12027.1.2	CO_2	98	26,990	390	-22.1
	Aix-12027.1.3	CO_2	94	27,510	290	-21.9
	Aix-12027.2.1	graphite	740	26,970	120	-18.4
DV14	Aix-12028.1.1	CO_2	92	26,670	370	-21.0
	Aix-12028.1.2	CO_2	91	26,490	360	-20.8
	Aix-12028.1.3	CO_2	102	27,190	410	-21.4
	Aix-12028.2.1	graphite	742	26,740	120	-18.6
DV15	Aix-12029.1.1	CO_2	93	27,310	400	-19.8
	Aix-12029.1.2	CO_2	84	26,890	390	-22.4
	Aix-12029.1.3	CO_2	93	26,990	380	-20.4
	Aix-12029.2.1	graphite	775	26,630	120	-16.2
DV16	Aix-12030.1.1	CO_2	85	27,960	430	-21.2
	Aix-12030.1.2	CO_2	97	27,380	420	-22.0
	Aix-12030.1.3	CO_2	99	26,950	380	-20.3
	Aix-12030.2.1	graphite	792	27,160	120	-18.1
DV42	Aix-12031.1.1	CO_2	87	27,040	380	-21.4
	Aix-12031.2.1	graphite	789	26,860	120	-21.8
DV43	Aix-12032.1.1	CO_2	88	26,570	360	-21.0
	Aix-12032.2.1	graphite	769	27,110	120	-18.7

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