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A 14C chronology for the Middle to Upper Palaeolithic transition at Bacho Kiro Cave, Bulgaria

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Published Version:

A 14C chronology for the Middle to Upper Palaeolithic transition at Bacho Kiro Cave, Bulgaria / Fewlass, H., S. Talamo, L. Wacker, B. Kromer, T. Tuna, Y. Fagault, E. Bard, S. P. McPherron, V. Aldeias, R. Maria, N. L. Martisius, L. Paskulin, Z. Rezek, V. Sinet-Mathiot, S. Sirakova, G. M. Smith, R. Spasov, F. Welker, N. Sirakov, T. Tsanova and J.-J. Hublin. - In: NATURE ECOLOGY & EVOLUTION. - ISSN 2397-334X. -ELETTRONICO. - 4:6(2020), pp. 794-801. [10.1038/s41559-020-1136-3]

This version is available at: https://hdl.handle.net/11585/770560 since: 2022-07-07

Published:

DOI: http://doi.org/10.1038/s41559-020-1136-3

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The final published version is available online at: 10.1038/s41559-020-1136-3

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A ¹⁴C chronology for the Middle–to–Upper Palaeolithic transition at Bacho Kiro Cave, Bulgaria

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21 Abstract

22 The stratigraphy at Bacho Kiro Cave, Bulgaria, spans the Middle to Upper Palaeolithic transition, 23 including an Initial Upper Palaeolithic (IUP) assemblage argued to represent the earliest arrival of Upper Palaeolithic Homo sapiens in Europe. We applied the latest techniques in ¹⁴C dating to an extensive 24 25 dataset of newly excavated animal and human bones to produce a robust, high precision radiocarbon 26 chronology for the site. At the base of the stratigraphy, the Middle Palaeolithic (MP) occupation dates to 27 >51,000 BP. A chronological gap of over 3000 years separates the MP occupation from the occupation of the cave by Homo sapiens, which extends to 34,000 cal BP. The extensive IUP assemblage, now 28 29 associated with directly dated Homo sapiens fossils at this site, securely dates to 45,820 - 43,650 cal BP (95.4% probability), likely beginning from 46,940 cal BP (95.4% probability). The results provide 30 31 chronological context for the early occupation of Europe by Upper Palaeolithic Homo sapiens.

32

33 Introduction

Bacho Kiro Cave in Bulgaria (Fig. 1a) has an archaeological sequence spanning the late Middle 34 Palaeolithic (MP) through the early Upper Palaeolithic. First excavated in 1938¹ and again in 1971-1975², 35 36 the cave is particularly notable for its distinctive lithic assemblages from Layers 11 and 11a² consisting of 37 elongated Levallois-like blades, retouched points, end-scrapers and splintered pieces, along with pendants made of animal bone and teeth²⁻⁴. A radiocarbon date of >43,000 ¹⁴C BP (GrN-7545) (Table 1) 38 on charcoal from Layer 11 made it perhaps one of the earliest Upper Palaeolithic (UP) assemblages in 39 Europe². Originally named 'Bachokirian'², the assemblage is now recognised as part of the Initial Upper 40 Palaeolithic (IUP)^{3,5} and is argued to represent one of the earliest occurrences of Upper Palaeolithic 41 *Homo sapiens* in $Europe^{4,6}$. 42

43 In 2015, the National Archaeological Institute with Museum (NAIM-BAS, Sofia) and the Department of 44 Human Evolution at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA, Leipzig) re-45 opened the cave with the primary goals of re-sampling the lithic assemblages and re-dating the IUP Layers 11 and 11a⁷. Two excavation sectors adjacent to the area of the 1970s excavations were 46 47 established (Fig. 1b) and excavated to bedrock. The Main Sector (Fig. 1c) encompasses the sequence as previously described². The Niche 1 is a small and low ceilinged lateral chamber located to the east, 48 49 preserving only the lower portion of the sequence, including the IUP and underlying Middle Palaeolithic 50 (Fig. 1d). Layer designations were kept separate between these two areas, with the layers from Niche 1 51 having an "N1-" prefix. The two areas are approximately 4 m apart (Fig. 1b; Extended Data Fig. 1). 52 Previous excavations removed the deposits connecting the two sectors, but field observations of 53 sedimentary characteristics, morphology and archaeological content allow some layers to be correlated (Supplementary Text 1). Here we use the new stratigraphic nomenclature (Fig. 1) wherein Layer 11² 54 55 corresponds to Layer I in the Main Sector and Layer N1-I in the Niche 1 and Layer 11a² to Layers J and 56 N1-J.

57 The recent excavations confirm the previously reported stratigraphy and archaeological sequence² 58 (Extended Data Fig. 2). In the Main Sector (Fig. 1c), the stratigraphy begins with the upper part of Layer 59 J, which overlies bedrock and continues through to Layer A0 at the current surface of the cave deposit. 60 The Niche 1 stratigraphy (Fig. 1d) starts with Layer N1-K deposited directly on the bedrock, continuing 61 through Layer N1-J and the distinctive Layer N1-I, into deposits ending with Layer N1-3a. Based on 62 archaeological and geological observations, Layers N1-J, N1-I and N1-H/G in the Niche 1 clearly 63 correspond, respectively, to Layers J, I, and G in the Main sector. The labelling of layers in the upper part 64 of the Niche 1 stratigraphy with numbers (N1-3a-e) reflects the lack of correlation of these layers to 65 lettered layers (A-J) in the Main Sector, although the erosive lower contact of Layers C and N1-3b can be 66 used as a stratigraphic marker in these two areas.

67 Overall, the sequence is characterised by an exceptionally high artefact density in Layers I and N1-I (15 finds >2 cm per litre of sediment) and low densities in other layers. During the new excavations, we 68 69 recovered ~14,000 bones and ~2,000 lithics (>2 cm), with >70% of these coming from Layers I and N1-I. 70 These quantities allow the lithic and bone industry to be correlated with previously excavated material and securely characterised as IUP^{2,3} (Supplementary Table 1). In both the old and new lithic collections, 71 72 the material from Layers J and N1-J is technologically consistent with the Layer I/N1-I assemblage, but 73 the find density is much lower (0.6 finds per litre of sediment). The contact between N1-J and the 74 underlying Layer N1-K is gradual, making the separation between the two sometimes difficult to 75 recognise in the field, and there are some artefacts at the base of N1-J that are consistent with the 76 Middle Palaeolithic assemblage of the underlying Layer N1-K (Levallois flakes from coarse-grained 77 syenite porphyry). In addition to changes in typology and technology from flakes to blades, this 78 transition is marked by a shift in raw material use, from coarser syenite porphyry to fine-grained flint². 79 Layers H—D and N1-H—N1-3a contain no lithic artefacts and a very low density of animal bones. Layers 80 C, B, A2 and A1 in the upper part of the stratigraphy contain characteristic Upper Palaeolithic artefacts, 81 including retouched blades, backed bladelets, carinated end-scrapers, burins, bone tools and pendants. 82 However, their lithic assemblages are poor in diagnostic technological attributes to any particular Upper 83 Palaeolithic industry (Supplementary Table 1).

In the 1980s and 1990s, radiocarbon dating was attempted on material from the 1970s excavation^{2,8}.
Although several of the samples produced dates of great antiquity, the sequence of dates was
inconsistent with the stratigraphy (Table 1). In particular, a wide range (>43,000 - 34,800 ± 1,150 ¹⁴C BP)
was obtained from Layer 11 and a much younger date (33,750 ± 850 ¹⁴C BP) from the underlying Layer
11a. This suggested that either the site was affected by post-depositional mixing between layers, or
storage of the material was problematic, and/or that modern carbon contamination had been
insufficiently removed from some of the samples prior to ¹⁴C dating, leading to an under-estimation of

their true ages⁸. Since then the establishment of more stringent methods of sample pretreatment, 91 including acid-base-wet oxidation (ABOx) pretreatment for charcoal^{9,10}, and ultrafiltration of bone 92 collagen¹¹⁻¹³, have greatly improved the reliability of radiocarbon dates on Palaeolithic samples¹⁴. 93 During ZooMS (Zooarchaeology by Mass Spectrometry)¹⁶ screening of a subset of undiagnostic bone 94 fragments from Bacho Kiro Cave, several elements from Layer N1-I (n=4), Layer B (n=1) and from the 95 1970s collection (Layer B/C; n=1) were identified as hominin⁴. In 2017, a morphologically diagnostic 96 97 hominin molar was found in Layer J in the Main Sector. DNA analysis confirmed the attribution of these 98 seven elements to Homo sapiens. The five newly identified Homo sapiens from Layers N1-I and J, described in detail in Hublin et al.⁴, are securely associated with the IUP assemblage and represent an 99 100 early dispersal of Homo sapiens into Europe in the Upper Palaeolithic. Given the implications of this 101 assemblage, we sought to establish the range of the IUP at Bacho Kiro Cave and resolve the previous age 102 anomalies by conducting a large-scale dating program. In this paper, we present an extensive dataset of 103 high-precision accelerator mass spectrometer (AMS) radiocarbon dates, which includes direct dating of the newly discovered *Homo sapiens* bones⁴. 104 105

106 Results

We successfully extracted collagen from 139 of the 147 pretreated bones (Supplementary Table 2). The average collagen yield across all layers was 8.3% with several bones in the lowest layers yielding up to 15% (Extended Data Fig. 3), which is much greater than the minimum level of 1% generally required for radiocarbon dating¹⁷ and exceptional for a site of this age range. Isotopic and elemental analysis showed that all collagen extracts are within the range of well-preserved collagen, suitable for ¹⁴C dating¹⁷. Although the C:N values of all extracts (range: 3.0 - 3.4) were within the range of well-preserved collagen, four extracts had C% and N% values slightly above the normal range (marked in red in

Supplementary Table 2), potentially indicating the presence of exogenous material. Only one of these was selected for dating and the age was identical to other bones in close proximity with acceptable C% and N% values. The FTIR spectra of all extracts were characteristic of pure collagen, with no evidence of exogenous material.

118 It has previously been suggested that the level of deamidation measured in ZooMS analysis could be an 119 efficient pre-screening tool to identify bones with well-preserved collagen for ¹⁴C dating¹⁸. The large 120 dataset in this study allowed us to robustly compare deamidation rates of two collagen peptides (P1106 121 and P1705) with collagen yields following extraction for ¹⁴C dating. No correlation was observed 122 between deamidation rates of peptides P1106 and P1705 and collagen yields, indicating that 123 deamidation rates would be an unsuitable method for pre-screening for ¹⁴C sampling, at least for Bacho

124 Kiro Cave (Supplementary Text 4; Supplementary Fig. 5).

125 In total, we AMS dated 95 bones, including 6 Homo sapiens. 63% of the dates obtained from animal 126 bones are from specimens that were anthropogenically modified (Fig. 2; Supplementary Text 5). The 127 AMS measurements of the collagen backgrounds which were used in the age correction of all samples were highly reproducible within and between each magazine (~500 mg bone extractions: 2016 mean 128 $F^{14}C=0.00168$, SD=0.00018; 2018 mean $F^{14}C=0.00220$, SD=0.00025; <100 mg bone extractions: 2018 129 mean $F^{14}C=0.00291$; SD = 0.00034; Supplementary Table 3). Due to the high reproducibility of the 130 131 background measurements, extended measurement time and high rate of transmission we were able to 132 reach exceptional levels of precision. The finite dates span 49,430 – 27,250 cal BP (95.4% probability; 133 Supplementary Tables 2, 4, 5). Nine of the bones dated beyond the radiocarbon range (>51,000 BP). All 134 of these come from the bottom of the stratigraphic sequence in the Niche 1, from Layer N1-K, the Layer 135 N1-J/K contact, and the lower part of Layer N1-J.

Eleven of the faunal collagen samples were dated in a second AMS lab (MAMS). The radiocarbon dates
from the two labs are in statistical agreement for eight of the 11 samples (from Layers C, E, F and N1-I).
However, combining dates from the two labs failed for three samples (R-EVA 1735, R-EVA 1737 and REVA 1739) from layer N1-I, all dating to >40,000 BP. The reasons for this are not understood so the dates
were excluded from further analysis.

Extended Data Figure 4 shows a comparison of the dates from graphite targets (*ca.* 2.5 mg collagen) and the gas ion source (<0.3 mg collagen) of the MICADAS system obtained for hominin bones F6-597 and BK-1653, carried out to cross-check the ages obtained. The level of precision achieved was excellent for both methods, despite a ten-fold reduction in sample size using the gas ion source, and the dates from the different methods measured in two labs are in statistical agreement (Supplementary Table 6), lending reliability to the results.

After Bayesian modelling in OxCal^{19,20}, the agreement index was 80.4 for model 1 (Main Sector), 78.8 for 147 model 2 (Niche 1), both above the generally accepted 60% threshold²¹ (Supplementary Table 5). The 148 high agreement index for the two separate areas indicates that the dates included are in keeping with 149 150 their stratigraphic positions. The dates from Layers N1-I/I and N1-J/J support the archaeological and 151 geological interpretations that these layers are correlated between the Main Sector and Niche 1. 152 However, the different spatial accumulation of Layer J between the two areas results in a low 153 agreement index (A_{model} = 28.9) for the model combining the dates (Supplementary Fig. 6). We therefore 154 consider the Bayesian models of the two areas separately (Fig. 3; OxCal code in accompanying file). 155

156

158 Discussion

Over the past two decades, the addition of an ultrafiltration step following collagen extraction has shown to be important for removing carbon contamination from Palaeolithic bones¹⁴. The new Bacho Kiro Cave results indicate that several of the 1980s and 1990s dates (Table 1) were affected by carbon contamination, making them appear younger than their actual ages. Our dates resolve the issues of the wide age range previously obtained for Layer I and the young age estimation obtained in Layer J (Table 1).

165 At the base of the sequence, resting on bedrock, Layer N1-K contains a small (n=82) Middle Palaeolithic 166 assemblage. All five dates from this layer are from Cervid/Saiga or Bos/Bison (including one with cut-167 marks) and are >51,000 BP. Overlying Layer N1-K, there are three age clusters represented in Layer N1-J, 168 which accumulated relatively slowly (Supplementary Fig. 4). First, an anthropogenically modified Ursidae 169 bone (ETH-86788) and a Cervid/Saiga bone (ETH-93195) from the very bottom of Layer N1-J are also >51,000 BP. Second, a minimum of 5,000 ¹⁴C years separates these two dates from the next occupation 170 represented by a cut-marked horse bone (ETH-86787), also excavated from the lower part of Layer N1-J, 171 172 indicating that hominins were present sometime between 49,430 – 46,940 cal BP (modelled age, 94.5% 173 probability; Fig. 3b). A small number of lithics were recovered from the lower part of Layer N1-J. Some 174 are consistent with the overlying IUP (n=6) and some are consistent with the underlying Middle 175 Palaeolithic (n=8), which is in agreement with the findings of the 1970s excavations². The gradual 176 contact between N1-J and the underlying N1-K makes it difficult to clearly distinguish between the two 177 layers during excavation of this area. From a radiocarbon perspective, it is impossible to know whether 178 the lower part of Layer N1-J relates more to the overlying IUP or more to the underlying Middle 179 Palaeolithic. However, the high resolution of the radiocarbon data suggests temporally distinct 180 occupations in this lower part, which makes an in-situ transition between the Middle Palaeolithic and

181 IUP less likely. The last occupation phase — in the upper part of Layer N1-J — spans from 46,940 – 182 45,130 cal BP (modelled range, 95.4% probability; Fig. 3b) and is associated with a low density of IUP 183 artefacts which share the techno-typological characteristics of those in the overlying Layer N1-I. The 184 dates from Layer J in the Main Sector (Fig. 3a) are at the younger end of the Layer N1-J range (95.4% 185 modelled range: 45,690 – 44,390 cal BP), which supports the geoarchaeological interpretation that only 186 the upper part of this layer is preserved in the Main Sector (where it overlies bedrock and abuts against 187 it towards the south; Supplementary Text 1).

188 The appearance of the IUP in Layer N1-J coincides with a period of climatic warming indicated in various 189 palaeoclimate records across the Northern Hemisphere, including the beginning of Greenland Interstadial 12 (GI12) at 46,950 ± 1000 BP in the GICC05 NGRIP ice core²² and beginning at ~47,600 BP in 190 the Hulu Cave speleothem δ^{18} O records in China²³. In closer proximity to Bacho Kiro Cave, mild climatic 191 conditions are indicated in a speleothem δ^{13} C record from Ascunsa Cave (AC) in the South Carpathians²⁴ 192 (~400 km NW of Bacho Kiro Cave) and palaeoclimatic records from the Black Sea^{25,26} and northern 193 Greece²⁷. However, correlations between calibrated ¹⁴C dates and palaeoclimate archives linked to 194 calendar ages are limited by the accuracy and precision in the calibration curve²⁸, especially approaching 195 the limit of the method, and in the various palaeoclimate records themselves²⁹. As such these 196 197 correlations are tentative. Further work on local climatic conditions at Bacho Kiro Cave is on-going and 198 will be discussed further in future.

The evidence for the age range of Layer I/N1-I is extremely robust. Twenty-five dates on human remains and anthropogenically modified bones set the modelled age range for the IUP from these correlated layers from 45,820 – 43,650 cal BP (95.4% probability). The radiocarbon dates from the four dated *Homo sapiens* bones span the full range of dates coming from anthropogenically modified bones. We chose to focus more on the dating of Layer N1-I in the Niche 1 where this layer is more extensively exposed and

204 more clearly delineated in the stratigraphic sequence. Nevertheless, the four dates from Layer I in the 205 Main Sector fall entirely within the range of Layer N1-I, supporting the archaeological and geological link 206 made between these two areas. The dates from the contact zone I/J in the Main Sector fall within the 207 range of Layer I and the upper part of Layer J. The age ranges from the upper part of Layer N1-J and 208 from Layer N1-I, together with high artefact densities, imply relatively continuous human use of the cave 209 during this interval. The radiocarbon evidence supports the archaeological and geological interpretation 210 that Layer I represents an intensification of the IUP occupation that began during the formation of Layer 211 J.

212 Site formation processes and the low number of artefacts in the layers above Layer I make it difficult to 213 determine when the IUP ended at Bacho Kiro Cave. Layers N1-H, N1-G and G are thick water laid 214 deposits with a low density of artefacts at the base (Layers N1-H and G). These were likely re-deposited 215 from Layer N1-I/I by a stream originating from the cave's inner karst system. These layers essentially 216 seal the underlying Layers N1-K, N1-J/J and N1-I/I⁴. Layers F and E are thick with very low densities of 217 bones and no lithic artefacts. The tight age range from these layers overlap with the youngest age range 218 of Layer I and suggest a rapid rate of sedimentation for Layers G through E. Although no lithics were 219 excavated from Layers F-D during the new excavations, the low density of artefacts recovered during the 1970s excavation indicate that the IUP characteristics continue from Layer J to Layer D⁴ (Supplementary 220 221 Table 1). In the new collection, a relative increase in artefact density occurs in Layer C (42,110 – 36,340 222 cal BP), Layer B (39,000 – 34,970 cal BP) and Layer A2 (35,440 – 34,350 cal BP). The lithic artefacts within 223 these layers are not characteristic of the IUP but rather of various phases of the subsequent Upper 224 Palaeolithic (specifically bladelet production, platforms consistent with the appearance of soft hammer 225 percussion in Layer C, and backed bladelets similar to Gravettian types in Layer A1; Supplementary Table 226 1). In the Main Sector, the Upper Palaeolithic occupation extends to 34,350 cal BP (modelled range) in 227 Layer A2. In Layer A1, the date of 27,610 – 27,250 cal BP (95.4% probability) on a cut-marked Bos/Bison

bone (ETH-86796) is consistent with the Gravettian backed bladelets and can be considered the
youngest preserved layer. The Dansgaard-Oeschger climatic cycles in the Northern Hemisphere between
the end of GI12 and the end of GI8 (~44,000 – 36,500 BP²²) may have been a driver of the demographic
turnover seen in archaeological and genetic studies during this interval in Europe^{30,24}, which is indicated
at Bacho Kiro Cave by the change in technology seen between the IUP in Layers J and I and the UP forms
in Layers C and above.

The Niche 1 has a shorter Upper Palaeolithic sequence than the Main Sector and the same differences in artefact densities. Nevertheless, we attempted to date the layers above Layer N1-I in part to help correlate its stratigraphy to that of the Main Sector. Unlike the rest of the deposits in this area, collagen preservation was very poor in these layers, and only four of the 11 bones had sufficient collagen yields for dating. At least one of the resulting dates (ETH-86776 or ETH-86775) is inconsistent with its stratigraphic position (Supplementary Table 2), and it was not possible to make any connections between the upper layers of the Niche 1 and the Main Sector based on the radiocarbon evidence.

241 The date of the human bone F6-597 (35,960 – 35,210 cal BP, 95.4% modelled range) from Layer B agrees 242 with the range of dates from the fauna in this layer. The hominin bone BK-1653 identified using ZooMS 243 from the 1970s collection was labelled Layer "6a/7", which corresponds to the contact of Layers B and C 244 in the new excavation. This bone was excluded from the modelling because of uncertainties over its 245 exact stratigraphic context, but its age (34,810 – 34,210 cal BP, 95.4% probability) fits with the dates on 246 fauna from Layer A2. Both human bones from the Upper Palaeolithic levels of the site (BK-1653 and F6-247 597) were dated using both the gas ion source and graphite methods to cross check the obtained ages 248 (Extended Data Fig. 4; Supplementary Table 6). The high level of agreement between the two methods 249 measured in two different AMS laboratories serves as further evidence of the suitability of the gas ion 250 source of the MICADAS for dating precious and/or small archaeological bone samples^{31,32}.

251 Conclusion

252 The chronology presented here for Bacho Kiro Cave constitutes an extensive set of high-quality collagen 253 samples radiocarbon dated at exceptional precision. To the best of our knowledge, this study represents one of the largest ¹⁴C datasets from a single Palaeolithic site processed by one team. This large effort 254 255 was made to resolve the questions left open by the previous dates from this eponymous site. The 256 integrity of the stratigraphic sequence is clearly indicated by the dates. The extensive dataset allows us 257 to securely place the IUP from correlated Layers I and N1-I in the interval from 45,820 – 43,650 cal BP 258 (95.4% probability). The start date for the IUP at Bacho Kiro Cave falls during the accumulation of Layer 259 N1-J, likely from 46,940 cal BP (95.4% probability). Direct radiocarbon dating of the four Homo sapiens 260 bones from Layer N1-I confirms their association with the IUP assemblage, and represents the earliest 261 direct evidence of our species in Europe in the Upper Palaeolithic.

Even as the precision of AMS measurements increases, one of the constraints of dating samples so close to the limit of the ¹⁴C method is the imprecision of the calibration curve in this time range. The output of the Bayesian modelling presented here may change as the resolution of the calibration curve improves^{23,33,34}. On-going work in this area is crucial for enhancing our understanding of the timing of major events in hominin adaptations and demographic processes during this time period.

267

268 Materials and Methods

269 Sample selection for radiocarbon dating

270 Bones were selected for radiocarbon dating from finds excavated during the 2015-2017 field seasons

- spanning the stratigraphy in both the Main Sector (Supplementary Figs. 1-2) and the Niche 1
- 272 (Supplementary Figs. 3-4). In total, 141 animal and 6 hominin bones were selected for collagen
- extraction (Supplementary Table 2). Where possible, animal bones that had signs of anthropogenic

274 modification (cut-marks, impact fractures) on their surfaces were selected (53% of the sample set) (Fig. 275 2). A particular focus was given to sampling the IUP in Layer N1-I, where the layer was extensively 276 exposed. Due to the exceptionally high density of bone in this layer (Supplementary Fig. 4), we were 277 able to select a high number of bones (77%) with anthropogenic modifications. A small number of 278 samples were also taken from Layer I in the Main Sector to confirm the stratigraphic link between the 279 two areas through radiocarbon dating. During excavation of the contact zone between Layers I and J in 280 the Main Sector, precise attribution of the finds to either Layer I or J was sometimes impossible to make, 281 due to the sediment moistness and the presence of large limestone rubble. These finds are labelled as "I/J" to indicate that they come from the contact zone between these two layers. 282

283 Bone pretreatment

The bones were pretreated in the Department of Human Evolution at the MPI-EVA, following the 284 collagen extraction plus ultrafiltration protocol described in Fewlass et al³¹ (see Supplementary Text 2 285 286 for further details). To preserve as much material as possible for aDNA and palaeoproteomic analysis, small aliquots of the hominin bones were sampled (80-110 mg) for direct ¹⁴C dating. The quality of all 287 288 the collagen extracts was assessed based on collagen yield, elemental (C%, N%, C:N) and stable isotopic values $(\delta^{13}C, \delta^{15}N)^{17}$. All collagen extracts were analysed with Fourier transformed infra-red (FTIR) 289 290 spectroscopy prior to dating to look for evidence of incomplete demineralisation, degraded collagen or the presence of any exogenous material in the extracts³⁵⁻³⁷. 291

292 AMS measurement

Collagen extracts from 6 human bones and 89 animal bones and teeth were selected for radiocarbon
 dating based on stratigraphic position, signs of anthropogenic surface modification and the level of
 collagen preservation (Supplementary Table 2). The collagen extracts were graphitised using the AGE
 III³⁸ and dated using the MICADAS³⁹ in the Laboratory of Ion Beam Physics at ETH Zürich, Switzerland

(lab code: ETH). The latest model of the MICADAS, equipped with Helium stripping gas⁴⁰ and permanent 297 magnets⁴¹, has been in operation at ETH Zürich since the beginning of 2016 and produced high ion 298 currents, a high rate of transmission, and a low and stable instrument background (~53,000 ¹⁴C BP). 299 300 Oxalic Acid II standards and collagen backgrounds extracted alongside the samples were measured in 301 the same magazine and used in the age calculation (AMS determinations of collagen and instrument 302 backgrounds are included in Supplementary Table 3). Data reduction was performed using BATS software⁴². An additional 1‰ was added to the error calculation of the samples, as per standard 303 304 practice.

Several collagen samples were split and dated in a second AMS lab to cross check the measurements. Eleven fauna collagen samples plus collagen extraction backgrounds were weighed into cleaned tin cups and sent to the Klaus-Tschira-AMS facility in Mannheim, Germany (lab code: MAMS), where they were catalytically converted to graphite and dated with the MICADAS-AMS⁴³. Here, data reduction was also carried out using BATS software⁴², and errors were calculated from the blanks and standards measured in the same magazine. An additional 1‰ was included in the final error calculation, as per the standard practice at MAMS.

In addition to graphitisation and AMS measurement at ETH Zürich, small aliquots of collagen (<100 μg C)

from *Homo sapiens* bones F6-597 and BK-1653 were measured using the gas ion source of the

AixMICADAS^{40,31-32} following the protocol described in Fewlass et al³².

315 Calibration and Bayesian modelling

316 Calibration and Bayesian chronological analysis was performed against the IntCal13 dataset²⁰ using

317 OxCal 4.3¹⁹. Where multiple measurements were made from the same collagen extract, the R_Combine

function in OxCal 4.3¹⁹ was used to combine them. As part of this function, a chi-squared (χ^2) test is

319 performed to see if the dates are in statistical agreement⁴⁴.

320 Some of the dates were excluded from the Bayesian chronological analysis: nine dating beyond 51,000 321 BP; four from the upper N1-3 layers; ten from the Layer I/J contact zone; three from Layer N1-I which failed the χ^2 test; one (ETH-71326) which was identified post-excavation as originating from next to the 322 323 1970s backfill; and the human bone (BK-1751) recovered from the 1970s collection as the context is not 324 certain (Supplementary Table 2). Outlier analysis was performed for the rest of the dataset (n=67) so that outliers could be manually eliminated²¹. Each layer was assigned a phase, and we used a general 325 outlier model with prior outlier probabilities set to 0.05²¹. As OxCal compresses posterior date 326 327 distributions around the most precise date within a phase, the ordering of the data within each phase 328 does not affect the model outcome so the dates were ordered chronologically rather than by 329 stratigraphic depth. Dates from the Main Sector (model 1) and Niche 1 (model 2) were first considered 330 separately. As Layers I and J have been archaeologically and geologically correlated between the two 331 areas, the dates were then combined in a third model (model 3). The likelihood of individual dates being 332 outliers was considered based on their depositional histories, posterior outlier probabilities and 333 individual agreement indices (<60% indicates the date could be incompatible with the model²¹) in the 334 three models. Based on this information, 14 of the 67 dates (shown in red in Supplementary Table 4 and discussed in Supplementary Text 6) were identified as outliers. When the dates from the two areas were 335 combined (model 3), a higher number of dates were identified as outliers but only those identified as 336 337 outliers in the individual models were excluded from the final model. All models were then run again 338 without outlier analysis with the spurious dates removed and were assessed based on the model agreement indices²¹ (Supplementary Table 5). 339

340 ZooMS collagen fingerprinting

All specimens (n=147) in the radiocarbon study were also analysed using MALDI-TOF-MS collagen
 peptide mass fingerprinting^{16,45} in order to provide accurate species identifications for each specimen
 (Supplementary Text 3).

344 **Data Availability** 345 All data is available in the manuscript and supplementary materials. 346 **Code Availability** 347 OxCal script is included in the supplementary information. 348 349 References 350 1 Garrod, D., Howe, B. & Gaul, J. Excavations in the cave of Bacho Kiro, north-east Bulgaria. 351 Bulletin of the American School of Prehistoric research 15, 46-76 (1939). 352 2 Kozłowski, J. K. Excavation in the Bacho Kiro cave (Bulgaria): Final Report. (Państwowe 353 Wydawnictwo Naukowe, 1982). 354 3 Tsanova, T. Les Débuts du Paléolithique Supérieur dans l'Est des Balkans. Réflexion à Partir de 355 l'Étude Taphonomique et Techno-Économique des En-sembles Lithiques de Bacho Kiro (Couche 356 11), Temnata (Couches VI et 4) et Kozarnika (Niveau VII) Vol. 1752 (BAR International Series, 357 2008). 358 4 Hublin, J. J. et al. Initial Upper Palaeolithic Homo sapiens remains from Bacho Kiro Cave 359 (Bulgaria) Nature (Submitted). 360 5 Kuhn, S. L. & Zwyns, N. Rethinking the Initial Upper Paleolithic. Quaternary International 347, 361 29-38, doi:10.1016/j.quaint.2014.05.040 (2014). 362 6 Hublin, J. J. The modern human colonization of western Eurasia: when and where? Quaternary 363 Science Reviews 118, 194-210, doi:10.1016/j.quascirev.2014.08.011 (2015). 364 7 Sirakov, N. et al. Reopened Bacho Kiro - new data on Middle/Upper Palaeolithic transition and 365 Early-Middle stages of Upper Palaeolithic. (Bulgarian Academy of Sciences, Sofia, 2017). 366 8 Hedges, R. E. M., Housley, R. A., Bronk Ramsey, C. & Van Klinken, G. J. Radiocarbon dates from 367 the Oxford AMS system: Archaeometry datelist 18. Archaeometry 36, 337-374, 368 doi:10.1111/j.1475-4754.1994.tb00975.x (1994). 369 9 Bird, M. et al. Radiocarbon dating of "old" charcoal using a wet oxidation, stepped-combustion 370 procedure. Radiocarbon 41, 127-140 (1999). 371 10 Wood, R. E. et al. Testing the ABOx-SC method: dating known-age charcoals associated with the 372 Campanian Ignimbrite. Quaternary Geochronology 9, 16-26, doi:10.1016/j.quageo.2012.02.003 373 (2012). 374 11 Brown, T. A., Nelson, D. E., Vogel, J. S. & Southon, J. R. Improved collagen extraction by modified 375 Longin method. Radiocarbon **30**, 171-177 (1988). 376 12 Bronk Ramsey, C., Higham, T., Bowles, A. & Hedges, R. Improvements to the pretreatment of 377 bone at Oxford. Radiocarbon 46, 155-164 (2004). 378 13 Talamo, S. & Richards, M. A comparison of bone pretreatment methods for AMS dating of 379 samples >30,000 BP. Radiocarbon 53, 443-449 (2011). 380 14 Higham, T. European Middle and Upper Palaeolithic radiocarbon dates are often older than they 381 look: problems with previous dates and some remedies. Antiquity 85, 235-249 (2011). 382 15 Evin, J., Marien, G. & Pachiaudi, C. Lyon natural radiocarbon measurements VII. Radiocarbon 20, 383 19-57 (1978). Buckley, M., Collins, M., Thomas-Oates, J. & Wilson, J. C. Species identification by analysis of 384 16 385 bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass

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463 Acknowledgements

The re-excavation of Bacho Kiro Cave is a joint project between the National Institute of Archaeology and Museum, Bulgarian Academy of Sciences, Sofia and the Human Evolution department of the Max

Planck Institute for Evolutionary Anthropology, Leipzig. This work was funded by the Max Planck Society.

467 Graphitisation and AMS dating in Switzerland were funded by ETH Zürich. The AixMICADAS and its

- 468 operation are funded by the Collège de France and the EQUIPEX ASTER-CEREGE (PI E.B.). The authors
- acknowledge the vital contribution of all the excavators who have worked at Bacho Kiro Cave since
- 470 2015.

471 Author contributions

472 The study was devised by J.-J.H, S.T., S.M., Ts.Ts, N.S. and H.F. Archaeological excavation was undertaken

by Ts.Ts., N.S., Z.R., V.A. and S.M., who all contributed contextual information. The excavation lab and

474 collection was organised by V.S.-M. Lithic analysis was performed by Ts.Ts., N.S., S.S. and S.M.

200 Zooarchaeological analysis was performed by G.S. and R.S. N.M. classified the bone tools in the sample

476 set. Stratigraphic and micromorphological analysis was carried out by V.A. ZooMS was carried out by

- 477 F.W., L.P. and V.S.-M. Sample pretreatment and EA-IRMS analyses were carried out by H.F. FTIR analyses
- 478 were carried out by H.F. and R.M. Graphitisation and AMS dating at ETH Zürich was carried out by L.W.,

479	B.K. and H.F. Dating with the AixMICADAS was carried out by E.B., Y.F. and Th.T. Bayesian modelling was
480	carried out by H.F. and S.T. H.F. wrote the paper with input from all authors.

481 Competing Interests

482 The authors declare no competing interests.

483 Additional information

Supplementary information for this paper is available at XXX. Note that Supplementary Tables 2 - 5 are
 included as separate sheets in the accompanying supplementary Excel file.

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487

488 Figure Legends

489 Figure 1. Bacho Kiro Cave: a) location of the cave in Bulgaria, Balkan Peninsula, south-eastern Europe

490 (base map from naturalearthdata.com); b) site plan showing the location of excavations carried out in

491 1971-1975 (centre, light orange) and the new excavations, in the Main Sector (top) and in the Niche 1

492 (left). The locations of the profiles shown in c-d are marked by red lines; c) stratigraphic sequence in the

493 Main Sector, along squares G5 and G6 in 2015. Note the presence of Layer J overlying the bedrock at the

494 bottom of the sequence; **d)** Extract from a 3D model of Niche 1, showing the stratigraphic sequence.

495 Layer attributions from this sector have an 'N1-' prefix. Note the distinctive dark colour of Layer N1-I. In

496 c and d, numbers in white squares show the layer attributions from the 1970s excavations. Letters in

497 white circles show the layer attributions from the recent excavations.

498

499	Figure 2. A selection of bone specimens from Bacho Kiro Cave with human bone surface modification
500	that were radiocarbon dated in this study: a) Equidae bone (R-EVA 2298/CC7-2607) from the lower part
501	of Layer N1-J with cut-marks (ETH-86787: 44,890 ± 450 ¹⁴ C BP); b) Ursidae bone (R-EVA 2290/BB8-207)
502	from Layer N1-I with a large impact fracture scar, scrape marks and marks consistent with use as a
503	retoucher (ETH-86783: 40,340 ± 280 ¹⁴ C BP); c) <i>Bos/Bison</i> rib (R-EVA 2352/F5-182) from the contact zone

between Layers I and J in the Main Sector with parallel cut-marks (ETH-86813: 40,160 ± 270 ¹⁴C BP); d) *Bos/Bison* long bone (R-EVA 2333/F5-107) excavated from Layer I used as a retoucher (ETH-86808:
41,350 ± 310 ¹⁴C BP); e) *Bos/Bison* long bone (R-EVA 2311/CC7-2750) from layer N1-K with cut-marks
(ETH-86793: >51,000 ¹⁴C BP). Scale bar is 5 cm.

508

509 Figure 3. Bayesian chronological models for Bacho Kiro Cave of material excavated during the 2015-2017 510 field seasons from the a) Main Sector (model 1) and b) Niche 1 (model 2). The AMS lab numbers are shown on the left side. The dates were calibrated against the IntCal13 dataset²⁰, and the modelling was 511 performed in OxCal 4.3¹⁹. Where more than one measurement was made from the same collagen 512 extract, dates were combined (R_Combine) in OxCal 4.3. The distributions of dates from the Niche 1 are 513 514 shown in green and from the Main Sector in black. The dates from the Homo sapiens bones from 515 Layer N1-I and Layer B are shown in purple. The radiocarbon likelihoods of calibrated dates (without 516 modelling) are shown in the lighter shade and the posterior distributions (after modelling) are darkly 517 shaded. Brackets show the 68.2% and 95.4% probability ranges of the calibrated dates. Dates marked 518 with an asterisk (*) are from bones bearing signs of anthropogenic modification. Note that 2 further 519 dates from the bottom of Layer N1-J date to >51,000 BP (beyond model range) as do 2 dates from the 520 N1-J/K contact and 5 dates from the underlying Layer N1-K. Further information is included in 521 Supplementary Tables 2, 4 and 5. OxCal code is included in Supplementary Text 7.

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527 Tables

529	Table 1. Previously published radiocarbon dates on material excavated from Bacho Kiro Cave from 1971-
530	1975.

Layer	Sample type	AMS lab number	¹⁴ C age	1σ error (years)	Reference	
7	Charcoal	OxA-3181	32,200	780	8	
6a/7	Bone	Ly1102	29,150	950	2,15	
6b	Charcoal	OxA-3182	33,300	820	8	
6b	Bone (no. 972)	GrN-7569	32,700	300	2	
11	Charcoal from hearth	GrN-7545	>43,000		2	
11	Bone	OxA-3213	38,500	1,700	8	
11	Charcoal	OxA-3183	37,650	1,450	8	
11	Tooth	OxA-3212	34,800	1,150	8	
11a	Bone	OxA-3184	33,750	850	8	
13	Bone (nos. 933 and 936)	GrN-7570	>47,000		2	









End Laver A2			4						
ETH-71296* IA	741		_4						
ETH-71295* IA.	1061			-					
ETH-71294 IA:	781			-					
l aver A2	-								
Transition Laver	B/Laver A2			-					
FTH-71298 IA-8	821								
ETH-86773/AIX	-12025 [A·104]			_					
ETH_71207 [A.	1021								
ETH_71300* [A	102]								
Laver B	102]								
Transition Laver	C/Laver B								
FTH-71302* IA	1001		<u> </u>						
ETH-71305* [A	1001	_							
ETH-71304* [A	981		-		h				
Laver C					U				
Transition Laver	D/I aver C				End Laver N1-L				=
ETH-86798* IA	1001	<u> </u>			ETH-86769 [A:20]		-		
Laver D		_			FTH-86783* [A·66]		_		_
Transition Laver	F/Laver D	<u>~</u>			ETH-86771 [A:96]				
ETH-71308 IA:8	R11				ETH-86780* [A·105]				_
ETH-71310 [A:	1151	_ <u>_</u>			ETH-71316* [A:105]		_		_
ETH-71309 [A.	1041				ETH-71318* [A·105]				_
ETH-71306/MA	MS-28675* [A:81]				ETH-71327* [A·104]				_
Laver F		_			ETH-71331* [A:104]				_
Transition Laver	F/Laver F	-			ETH-71322/MAMS-2948	3* [4.103]			
FTH-86799 IA-	7720y072				ETH-71315/MAMS-2867	7* [A·102]			
ETH-86801 [A:0					ETH-71320* [A·104]	/ [/(./02]			
ETH-71311 [A:1		<u> </u>			ETH-71325* [A:105]				
ETH-71312/MA	MS-28676* [A:98]	<u>~</u>			ETH-86784* [A:108]				
ETH-86800 IA	71	<u> </u>			ETH-71329* [A:107]				
Laver F					ETH-71314* [A:106]				
Start Laver F					ETH-71324/MAMS-2868	1* [A·107]			
End Laver I					ETH-86770 [A·110]				_
ETH-86807* [A	1061				ETH-71328* [A·107]				
ETH-868/09* [A	1111				ETH-71323/MAMS-2868	0* [<u>A</u> ·10 <u>4</u>]			
ETH-86810* [A	1241	<u> </u>			ETH-71330* [A·82]	0 [//04]			
ETH-86808* [A	941	<u> </u>			ETH-86772 [A·76]				
Laver I		-			Laver N1-				
Transition Laver	J/Laver I	_			Transition Laver N1-J/Lav	er N1-I		<u> </u>	
FTH-86819 IA	1131				FTH-93194 [A·105]				
ETH-86820* IA	1201				ETH-86789* [A:103]				
ETH-86821* IA	1141				ETH-86787* IA:1001				
ETH-86822* IA	1101				ETH-93196 [A:96]				
Laver J					Laver N1-J				
Start Laver J -					Start Laver N1-J				
Main Sector, Ba	cho Kiro Cave [Amode	el:80]			Niche 1, Bacho Kiro Cav	e [Amodel:79]			
					F 4 000 F 0 000				40.000
50,	000 45,00	u 40,0	35,0	100	54,000 52,000	50,000 48,	000 46,000	44,000	42,000
	Modeller	1 data (cal RD)				Modellad date	a (cal RD)		

Modelled date (cal BP)

Modelled date (cal BP)

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Niche 1

Main Sector

1970s excavation area







