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

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1 **A <sup>14</sup>C chronology for the Middle–to–Upper Palaeolithic transition at Bacho Kiro Cave, Bulgaria**

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18

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20

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  
24 Palaeolithic *Homo sapiens* in Europe. We applied the latest techniques in <sup>14</sup>C dating to an extensive  
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  
28 the cave by *Homo sapiens*, which extends to 34,000 cal BP. The extensive IUP assemblage, now  
29 associated with directly dated *Homo sapiens* fossils at this site, securely dates to 45,820 – 43,650 cal BP  
30 (95.4% probability), likely beginning from 46,940 cal BP (95.4% probability). The results provide  
31 chronological context for the early occupation of Europe by Upper Palaeolithic *Homo sapiens*.

32

33 **Introduction**

34 Bacho Kiro Cave in Bulgaria (Fig. 1a) has an archaeological sequence spanning the late Middle  
35 Palaeolithic (MP) through the early Upper Palaeolithic. First excavated in 1938<sup>1</sup> and again in 1971-1975<sup>2</sup>,  
36 the cave is particularly notable for its distinctive lithic assemblages from Layers 11 and 11a<sup>2</sup> consisting of  
37 elongated Levallois-like blades, retouched points, end-scrapers and splintered pieces, along with  
38 pendants made of animal bone and teeth<sup>2-4</sup>. A radiocarbon date of >43,000 <sup>14</sup>C BP (GrN-7545) (Table 1)  
39 on charcoal from Layer 11 made it perhaps one of the earliest Upper Palaeolithic (UP) assemblages in  
40 Europe<sup>2</sup>. Originally named 'Bachokirian'<sup>2</sup>, the assemblage is now recognised as part of the Initial Upper  
41 Palaeolithic (IUP)<sup>3,5</sup> and is argued to represent one of the earliest occurrences of Upper Palaeolithic  
42 *Homo sapiens* in Europe<sup>4,6</sup>.

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  
46 Layers 11 and 11a<sup>7</sup>. Two excavation sectors adjacent to the area of the 1970s excavations were  
47 established (Fig. 1b) and excavated to bedrock. The Main Sector (Fig. 1c) encompasses the sequence as  
48 previously described<sup>2</sup>. The Niche 1 is a small and low ceilinged lateral chamber located to the east,  
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  
54 (Supplementary Text 1). Here we use the new stratigraphic nomenclature (Fig. 1) wherein Layer 11<sup>2</sup>  
55 corresponds to Layer I in the Main Sector and Layer N1-I in the Niche 1 and Layer 11a<sup>2</sup> to Layers J and  
56 N1-J.

57 The recent excavations confirm the previously reported stratigraphy and archaeological sequence<sup>2</sup>  
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  
68 finds >2 cm per litre of sediment) and low densities in other layers. During the new excavations, we  
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  
71 and securely characterised as IUP<sup>2,3</sup> (Supplementary Table 1). In both the old and new lithic collections,  
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 flint<sup>2</sup>.  
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).

84 In the 1980s and 1990s, radiocarbon dating was attempted on material from the 1970s excavation<sup>2,8</sup>.  
85 Although several of the samples produced dates of great antiquity, the sequence of dates was  
86 inconsistent with the stratigraphy (Table 1). In particular, a wide range (>43,000 – 34,800 ± 1,150 <sup>14</sup>C BP)  
87 was obtained from Layer 11 and a much younger date (33,750 ± 850 <sup>14</sup>C BP) from the underlying Layer  
88 11a. This suggested that either the site was affected by post-depositional mixing between layers, or  
89 storage of the material was problematic, and/or that modern carbon contamination had been  
90 insufficiently removed from some of the samples prior to <sup>14</sup>C dating, leading to an under-estimation of

91 their true ages<sup>8</sup>. Since then the establishment of more stringent methods of sample pretreatment,  
92 including acid-base-wet oxidation (ABOx) pretreatment for charcoal<sup>9,10</sup>, and ultrafiltration of bone  
93 collagen<sup>11-13</sup>, have greatly improved the reliability of radiocarbon dates on Palaeolithic samples<sup>14</sup>.

94 During ZooMS (Zooarchaeology by Mass Spectrometry)<sup>16</sup> screening of a subset of undiagnostic bone  
95 fragments from Bacho Kiro Cave, several elements from Layer N1-I (n=4), Layer B (n=1) and from the  
96 1970s collection (Layer B/C; n=1) were identified as hominin<sup>4</sup>. In 2017, a morphologically diagnostic  
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,  
99 described in detail in Hublin *et al.*<sup>4</sup>, are securely associated with the IUP assemblage and represent an  
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  
104 the newly discovered *Homo sapiens* bones<sup>4</sup>.

105

## 106 **Results**

107 We successfully extracted collagen from 139 of the 147 pretreated bones (Supplementary Table 2). The  
108 average collagen yield across all layers was 8.3% with several bones in the lowest layers yielding up to  
109 15% (Extended Data Fig. 3), which is much greater than the minimum level of 1% generally required for  
110 radiocarbon dating<sup>17</sup> and exceptional for a site of this age range. Isotopic and elemental analysis showed  
111 that all collagen extracts are within the range of well-preserved collagen, suitable for <sup>14</sup>C dating<sup>17</sup>.

112 Although the C:N values of all extracts (range: 3.0 – 3.4) were within the range of well-preserved  
113 collagen, four extracts had C% and N% values slightly above the normal range (marked in red in

114 Supplementary Table 2), potentially indicating the presence of exogenous material. Only one of these  
115 was selected for dating and the age was identical to other bones in close proximity with acceptable C%  
116 and N% values. The FTIR spectra of all extracts were characteristic of pure collagen, with no evidence of  
117 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  $^{14}\text{C}$  dating<sup>18</sup>. 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  $^{14}\text{C}$  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  $^{14}\text{C}$  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  
128 were highly reproducible within and between each magazine (~500 mg bone extractions: 2016 mean  
129  $F^{14}\text{C}=0.00168$ ,  $\text{SD}=0.00018$ ; 2018 mean  $F^{14}\text{C}=0.00220$ ,  $\text{SD}=0.00025$ ; <100 mg bone extractions: 2018  
130 mean  $F^{14}\text{C}=0.00291$ ;  $\text{SD} = 0.00034$ ; Supplementary Table 3). Due to the high reproducibility of the  
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.

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

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

147 After Bayesian modelling in OxCal<sup>19,20</sup>, the agreement index was 80.4 for model 1 (Main Sector), 78.8 for  
148 model 2 (Niche 1), both above the generally accepted 60% threshold<sup>21</sup> (Supplementary Table 5). The  
149 high agreement index for the two separate areas indicates that the dates included are in keeping with  
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 ( $A_{\text{model}} = 28.9$ ) 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

157

158 **Discussion**

159 Over the past two decades, the addition of an ultrafiltration step following collagen extraction has  
160 shown to be important for removing carbon contamination from Palaeolithic bones<sup>14</sup>. The new Bacho  
161 Kiro Cave results indicate that several of the 1980s and 1990s dates (Table 1) were affected by carbon  
162 contamination, making them appear younger than their actual ages. Our dates resolve the issues of the  
163 wide age range previously obtained for Layer I and the young age estimation obtained in Layer J (Table  
164 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  
170 >51,000 BP. Second, a minimum of 5,000 <sup>14</sup>C years separates these two dates from the next occupation  
171 represented by a cut-marked horse bone (ETH-86787), also excavated from the lower part of Layer N1-J,  
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 excavations<sup>2</sup>. 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  
190 Interstadial 12 (GI12) at  $46,950 \pm 1000$  BP in the GICC05 NGRIP ice core<sup>22</sup> and beginning at  $\sim 47,600$  BP in  
191 the Hulu Cave speleothem  $\delta^{18}\text{O}$  records in China<sup>23</sup>. In closer proximity to Bacho Kiro Cave, mild climatic  
192 conditions are indicated in a speleothem  $\delta^{13}\text{C}$  record from Ascunsa Cave (AC) in the South Carpathians<sup>24</sup>  
193 ( $\sim 400$  km NW of Bacho Kiro Cave) and palaeoclimatic records from the Black Sea<sup>25,26</sup> and northern  
194 Greece<sup>27</sup>. However, correlations between calibrated  $^{14}\text{C}$  dates and palaeoclimate archives linked to  
195 calendar ages are limited by the accuracy and precision in the calibration curve<sup>28</sup>, especially approaching  
196 the limit of the method, and in the various palaeoclimate records themselves<sup>29</sup>. As such these  
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.

199 The evidence for the age range of Layer I/N1-I is extremely robust. Twenty-five dates on human remains  
200 and anthropogenically modified bones set the modelled age range for the IUP from these correlated  
201 layers from 45,820 – 43,650 cal BP (95.4% probability). The radiocarbon dates from the four dated *Homo*  
202 *sapiens* bones span the full range of dates coming from anthropogenically modified bones. We chose to  
203 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/I<sup>4</sup>. 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  
220 1970s excavation indicate that the IUP characteristics continue from Layer J to Layer D<sup>4</sup> (Supplementary  
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*

228 bone (ETH-86796) is consistent with the Gravettian backed bladelets and can be considered the  
229 youngest preserved layer. The Dansgaard-Oeschger climatic cycles in the Northern Hemisphere between  
230 the end of GI12 and the end of GI8 (~44,000 – 36,500 BP<sup>22</sup>) may have been a driver of the demographic  
231 turnover seen in archaeological and genetic studies during this interval in Europe<sup>30,24</sup>, which is indicated  
232 at Bacho Kiro Cave by the change in technology seen between the IUP in Layers J and I and the UP forms  
233 in Layers C and above.

234 The Niche 1 has a shorter Upper Palaeolithic sequence than the Main Sector and the same differences in  
235 artefact densities. Nevertheless, we attempted to date the layers above Layer N1-I in part to help  
236 correlate its stratigraphy to that of the Main Sector. Unlike the rest of the deposits in this area, collagen  
237 preservation was very poor in these layers, and only four of the 11 bones had sufficient collagen yields  
238 for dating. At least one of the resulting dates (ETH-86776 or ETH-86775) is inconsistent with its  
239 stratigraphic position (Supplementary Table 2), and it was not possible to make any connections  
240 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<sup>31,32</sup>.

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  
254 one of the largest <sup>14</sup>C datasets from a single Palaeolithic site processed by one team. This large effort  
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.

262 Even as the precision of AMS measurements increases, one of the constraints of dating samples so close  
263 to the limit of the <sup>14</sup>C method is the imprecision of the calibration curve in this time range. The output of  
264 the Bayesian modelling presented here may change as the resolution of the calibration curve  
265 improves<sup>23,33,34</sup>. On-going work in this area is crucial for enhancing our understanding of the timing of  
266 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  
271 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  
273 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  
282 “I/J” to indicate that they come from the contact zone between these two layers.

### 283 *Bone pretreatment*

284 The bones were pretreated in the Department of Human Evolution at the MPI-EVA, following the  
285 collagen extraction plus ultrafiltration protocol described in Fewlass et al<sup>31</sup> (see Supplementary Text 2  
286 for further details). To preserve as much material as possible for aDNA and palaeoproteomic analysis,  
287 small aliquots of the hominin bones were sampled (80-110 mg) for direct <sup>14</sup>C dating. The quality of all  
288 the collagen extracts was assessed based on collagen yield, elemental (C%, N%, C:N) and stable isotopic  
289 values ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ )<sup>17</sup>. All collagen extracts were analysed with Fourier transformed infra-red (FTIR)  
290 spectroscopy prior to dating to look for evidence of incomplete demineralisation, degraded collagen or  
291 the presence of any exogenous material in the extracts<sup>35-37</sup>.

### 292 *AMS measurement*

293 Collagen extracts from 6 human bones and 89 animal bones and teeth were selected for radiocarbon  
294 dating based on stratigraphic position, signs of anthropogenic surface modification and the level of  
295 collagen preservation (Supplementary Table 2). The collagen extracts were graphitised using the AGE  
296 III<sup>38</sup> and dated using the MICADAS<sup>39</sup> in the Laboratory of Ion Beam Physics at ETH Zürich, Switzerland

297 (lab code: ETH). The latest model of the MICADAS, equipped with Helium stripping gas<sup>40</sup> and permanent  
298 magnets<sup>41</sup>, has been in operation at ETH Zürich since the beginning of 2016 and produced high ion  
299 currents, a high rate of transmission, and a low and stable instrument background (~53,000 <sup>14</sup>C BP).  
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  
303 software<sup>42</sup>. An additional 1‰ was added to the error calculation of the samples, as per standard  
304 practice.

305 Several collagen samples were split and dated in a second AMS lab to cross check the measurements.  
306 Eleven fauna collagen samples plus collagen extraction backgrounds were weighed into cleaned tin cups  
307 and sent to the Klaus-Tschira-AMS facility in Mannheim, Germany (lab code: MAMS), where they were  
308 catalytically converted to graphite and dated with the MICADAS-AMS<sup>43</sup>. Here, data reduction was also  
309 carried out using BATS software<sup>42</sup>, and errors were calculated from the blanks and standards measured  
310 in the same magazine. An additional 1‰ was included in the final error calculation, as per the standard  
311 practice at MAMS.

312 In addition to graphitisation and AMS measurement at ETH Zürich, small aliquots of collagen (<100 µg C)  
313 from *Homo sapiens* bones F6-597 and BK-1653 were measured using the gas ion source of the  
314 AixMICADAS<sup>40,31-32</sup> following the protocol described in Fewlass et al<sup>32</sup>.

### 315 *Calibration and Bayesian modelling*

316 Calibration and Bayesian chronological analysis was performed against the IntCal13 dataset<sup>20</sup> using  
317 OxCal 4.3<sup>19</sup>. Where multiple measurements were made from the same collagen extract, the R\_Combine  
318 function in OxCal 4.3<sup>19</sup> was used to combine them. As part of this function, a chi-squared ( $\chi^2$ ) test is  
319 performed to see if the dates are in statistical agreement<sup>44</sup>.

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  
322 failed the  $\chi^2$  test; one (ETH-71326) which was identified post-excavation as originating from next to the  
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  
325 that outliers could be manually eliminated<sup>21</sup>. Each layer was assigned a phase, and we used a general  
326 outlier model with prior outlier probabilities set to 0.05<sup>21</sup>. As OxCal compresses posterior date  
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 model<sup>21</sup>) in the  
334 three models. Based on this information, 14 of the 67 dates (shown in red in Supplementary Table 4 and  
335 discussed in Supplementary Text 6) were identified as outliers. When the dates from the two areas were  
336 combined (model 3), a higher number of dates were identified as outliers but only those identified as  
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  
339 agreement indices<sup>21</sup> (Supplementary Table 5).

#### 340 *ZooMS collagen fingerprinting*

341 All specimens (n=147) in the radiocarbon study were also analysed using MALDI-TOF-MS collagen  
342 peptide mass fingerprinting<sup>16,45</sup> in order to provide accurate species identifications for each specimen  
343 (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

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461  
462

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#### 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  
473 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.  
475 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**

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

486

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 <sup>14</sup>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 <sup>14</sup>C BP); **c)** *Bos/Bison* rib (R-EVA 2352/F5-182) from the contact zone

504 between Layers I and J in the Main Sector with parallel cut-marks (ETH-86813:  $40,160 \pm 270$   $^{14}\text{C}$  BP); **d)**  
505 *Bos/Bison* long bone (R-EVA 2333/F5-107) excavated from Layer I used as a retoucher (ETH-86808:  
506  $41,350 \pm 310$   $^{14}\text{C}$  BP); **e)** *Bos/Bison* long bone (R-EVA 2311/CC7-2750) from layer N1-K with cut-marks  
507 (ETH-86793:  $>51,000$   $^{14}\text{C}$  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  
511 shown on the left side. The dates were calibrated against the IntCal13 dataset<sup>20</sup>, and the modelling was  
512 performed in OxCal 4.3<sup>19</sup>. Where more than one measurement was made from the same collagen  
513 extract, dates were combined (R\_Combine) in OxCal 4.3. **The distributions of dates from the Niche 1 are**  
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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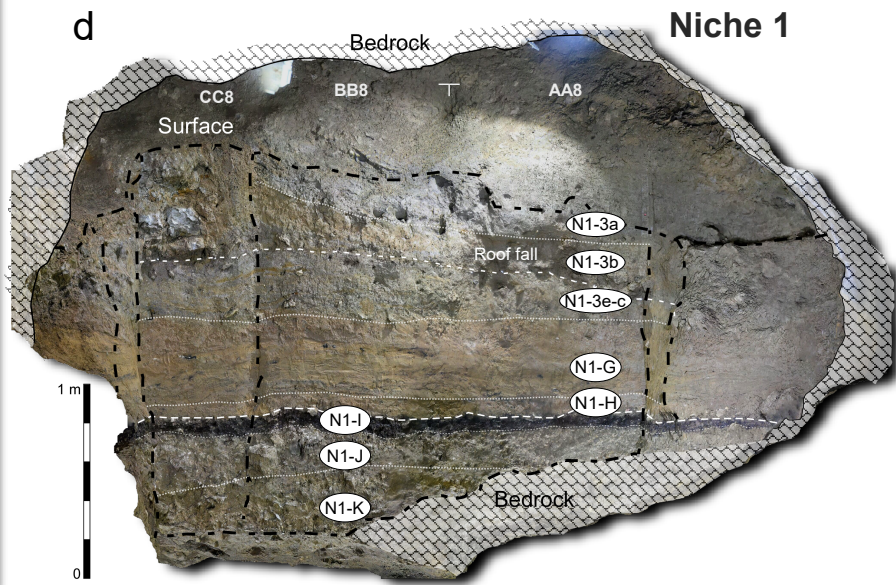
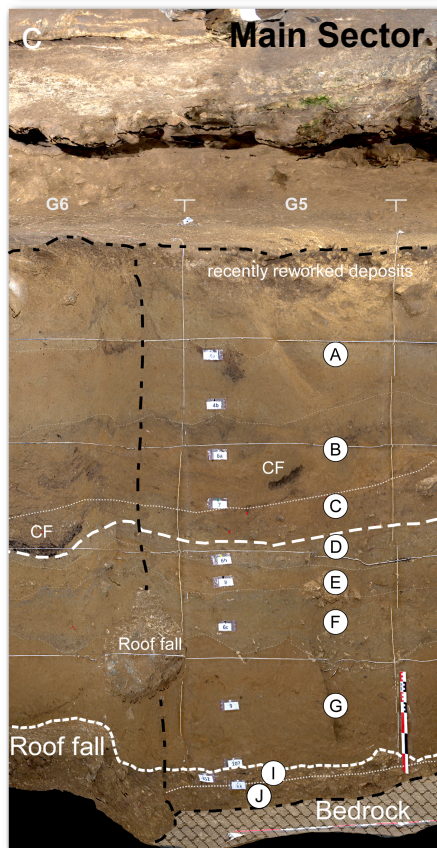
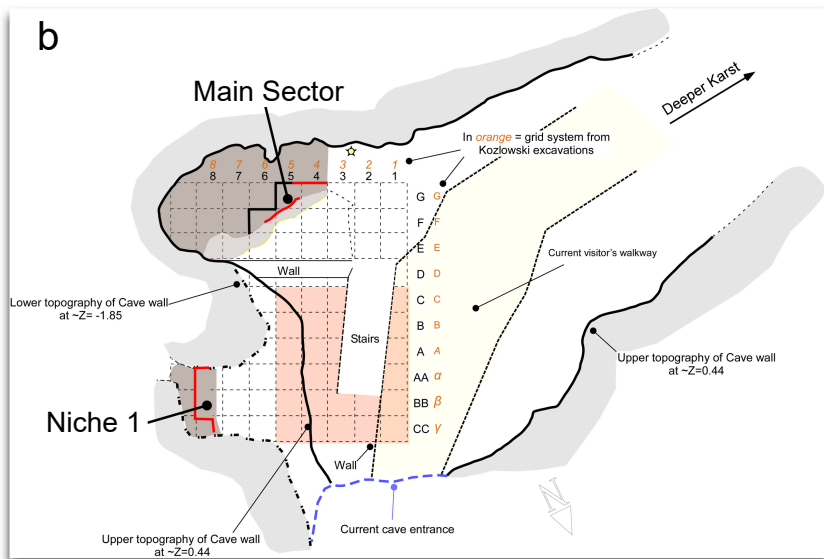
527 **Tables**

528

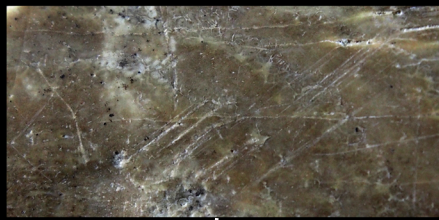
529 **Table 1.** Previously published radiocarbon dates on material excavated from Bacho Kiro Cave from 1971-  
530 1975.

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

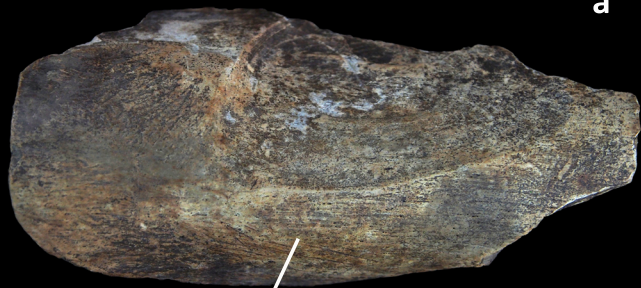
531







**b**



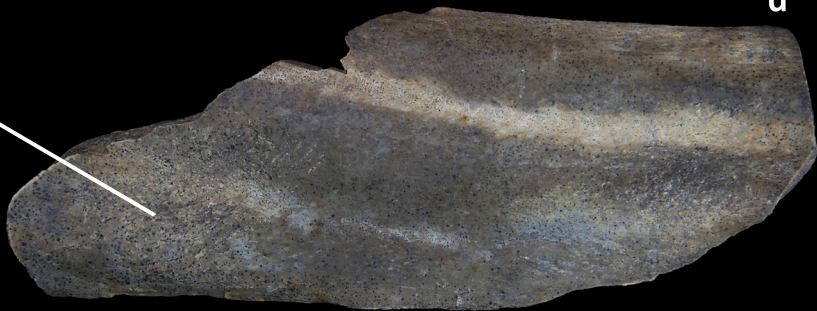
**a**



**c**

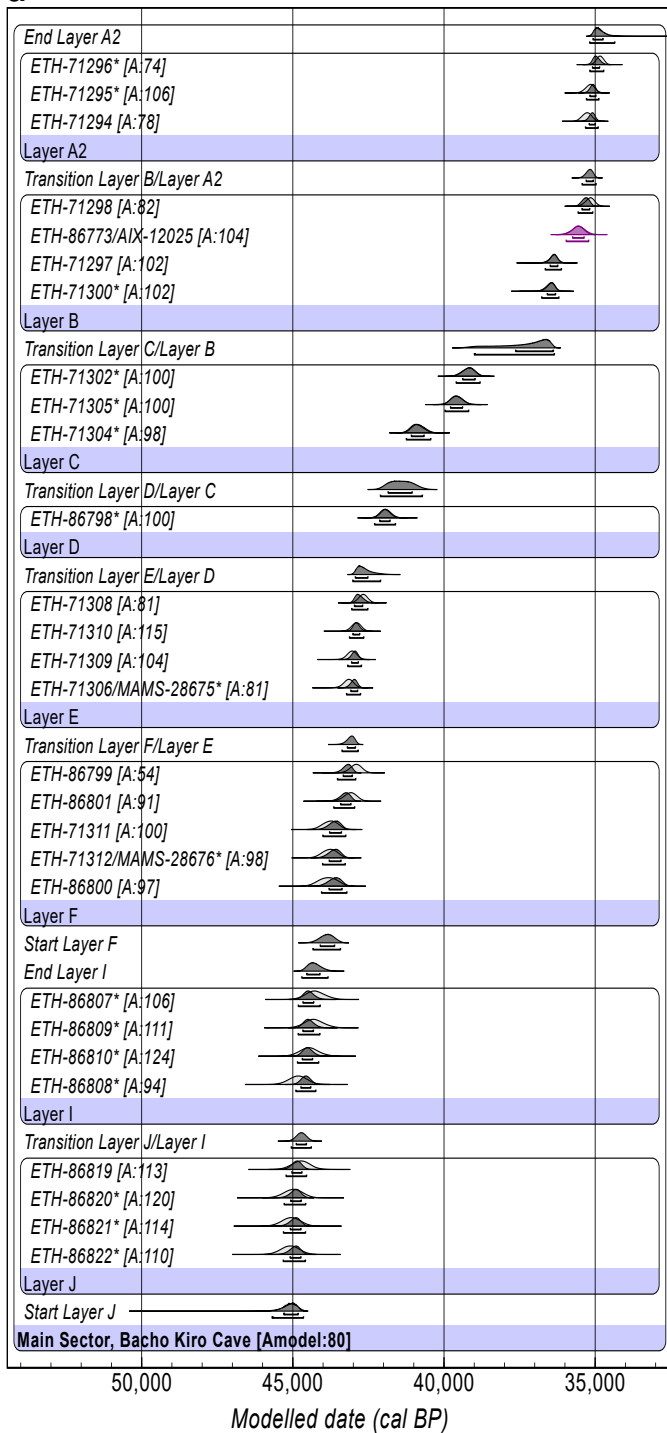
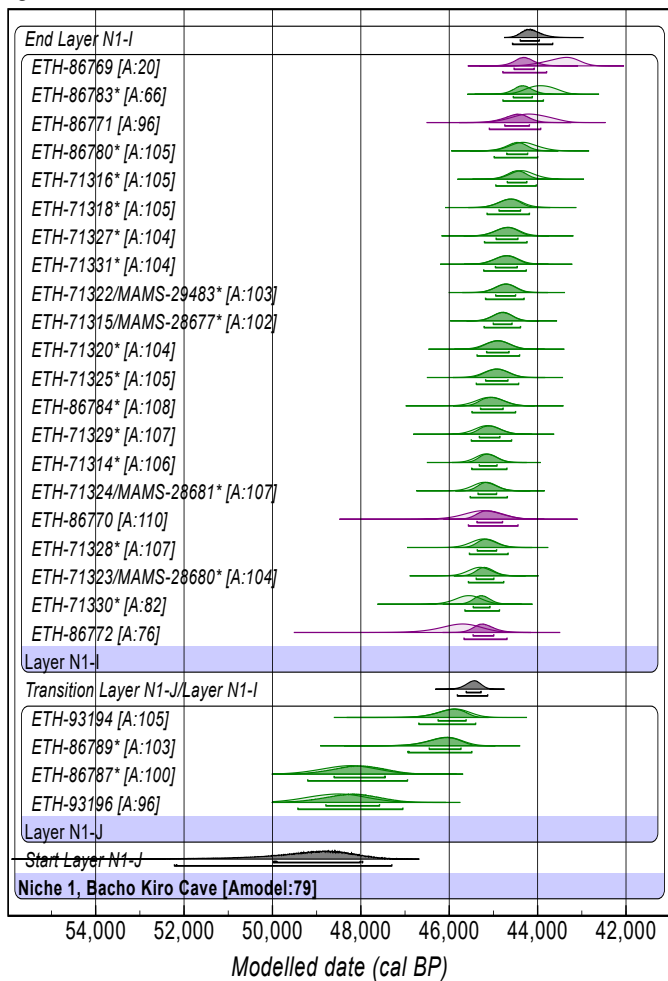


**d**



**e**



**a****b**



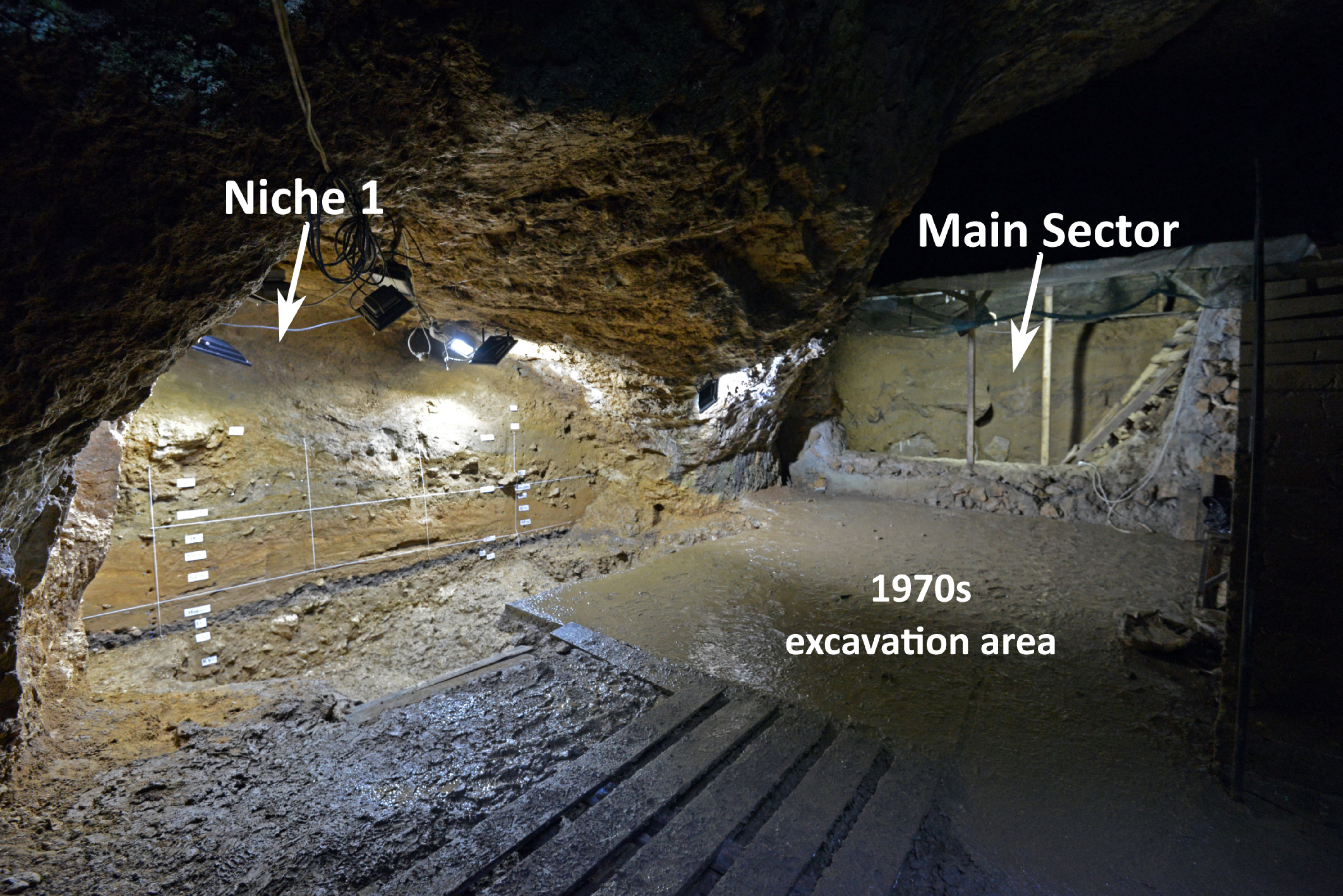
**Niche 1**



**Main Sector**

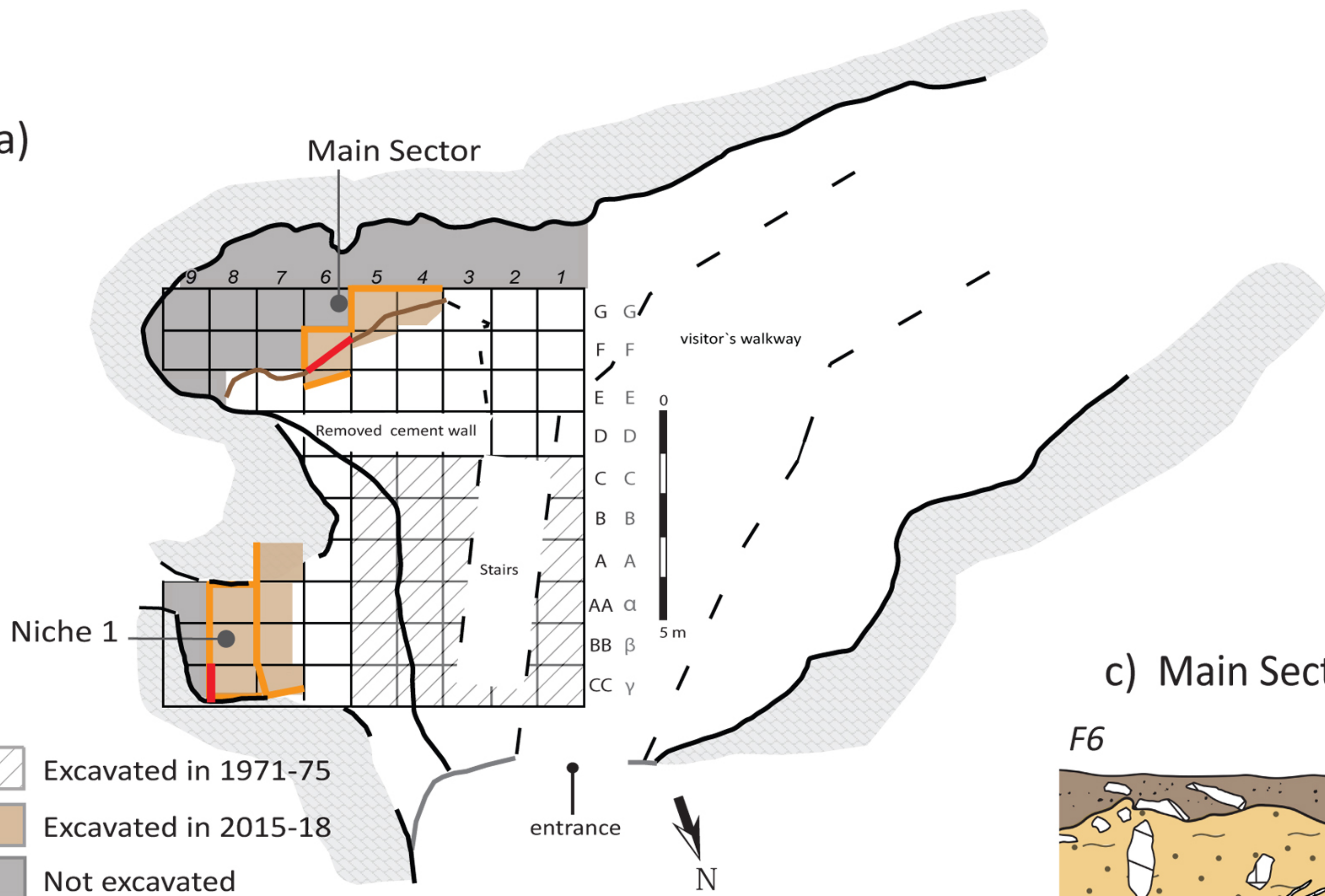


**1970s  
excavation area**

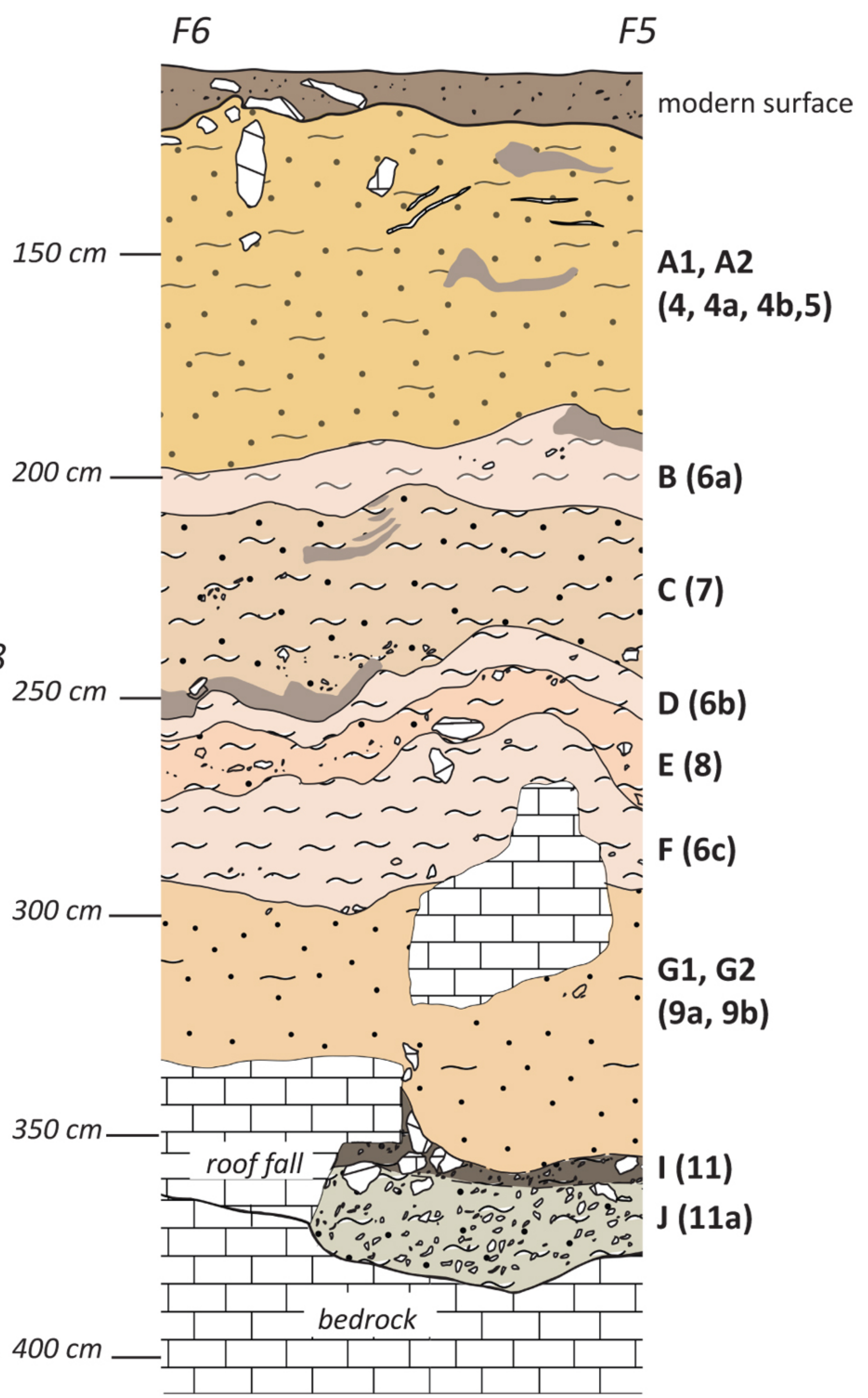




a)

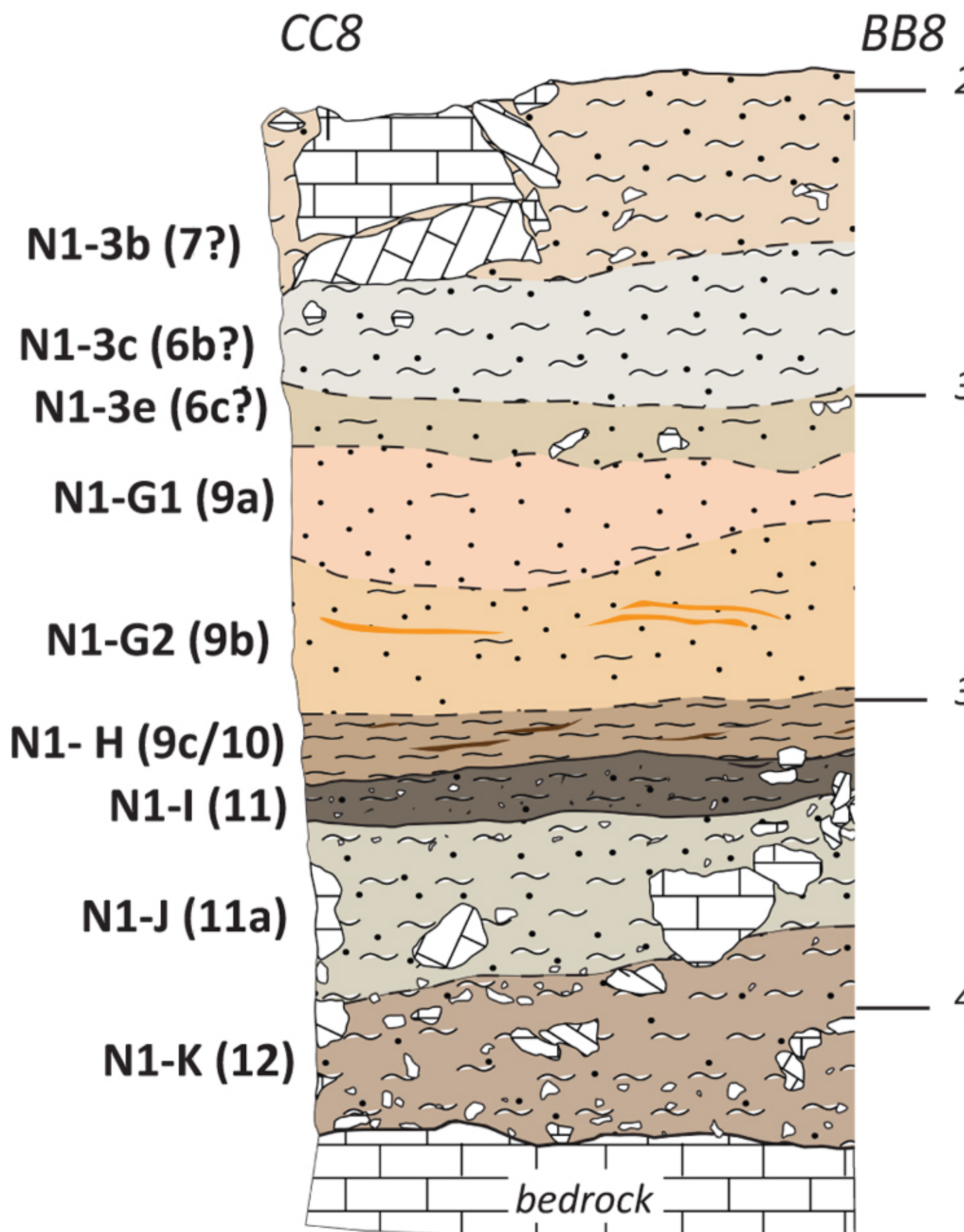


c) Main Sector

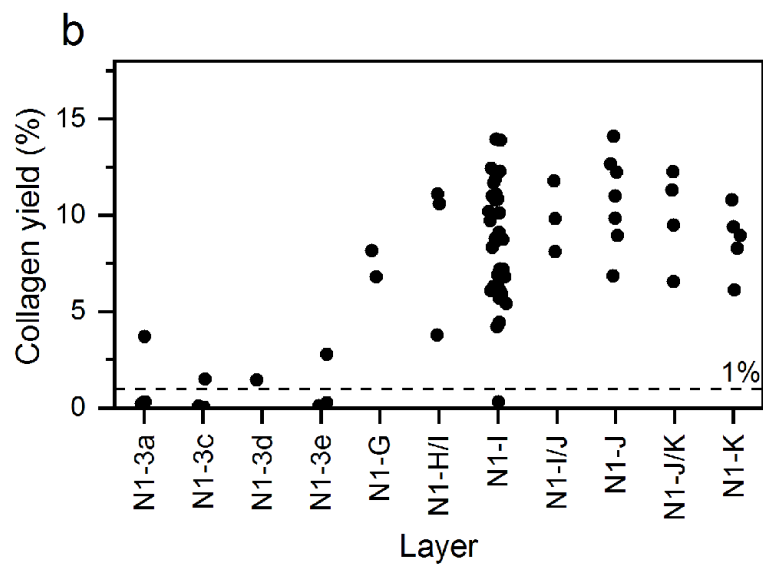
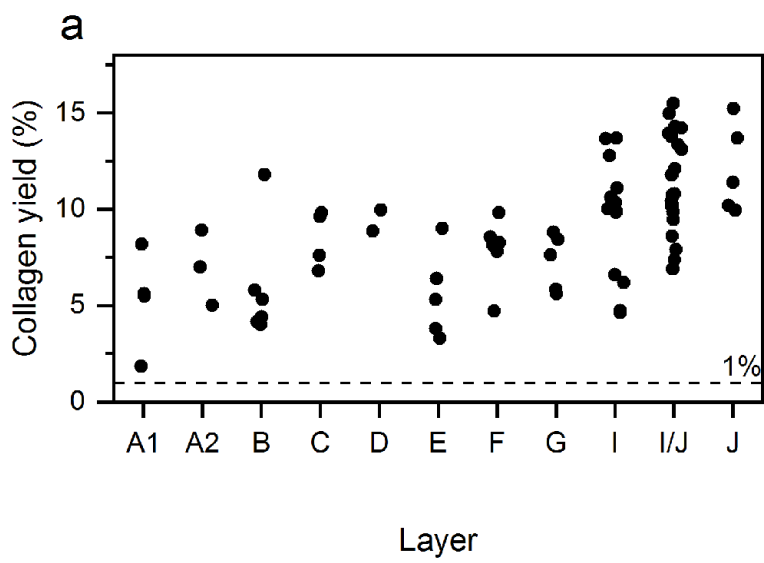


b) Niche 1

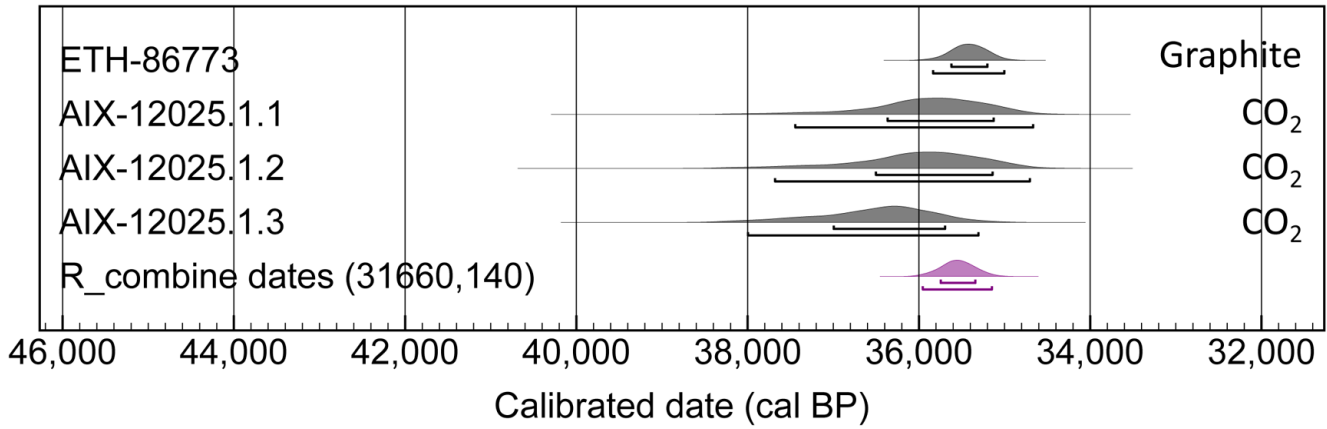
- sandy clay
- loamy sand
- compact clay
- clay
- combustion feature
- clay lenses
- dark lenses
- limestone blocks







**a**



**b**

