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Re-examination of the Subalyuk Neanderthal remains uncovers signs of probable TB infection (Subalyuk Cave, Hungary)

György Pálfi, Erika Molnár, Zsolt Bereczki, Hélène Coqueugnot, Olivier Dutour, Anne-Marie Tillier, Wilfried Rosendahl, Antal Sklánitz, Zsolt Mester, Mihály Gasparik, et al.

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4 **Re-examination of the Subalyuk Neanderthal remains uncovers signs of probable TB**
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49 **ABSTRACT**

50 In 1932, skeletal remains of two Neanderthal individuals, a young adult female and a 3–4–year-old
51 child, were discovered in Subalyuk Cave in Northern Hungary [1,2]. Results of the anthropological
52 examination were published some years after this important discovery. Methodological progress
53 encouraged re-examination of the material during the last few years. Radiocarbon dating revealed a
54 chronological age of 39,732–39,076 cal. BP for the adult female and 36,117–35,387 cal. BP for the
55 child [3]. Morphological paleopathological studies of these Neanderthal remains uncovered distinct
56 evidence of skeletal infections. Alterations of the adult individual’s *sacrum* suggest probable early-
57 stage *sacroiliitis*, while several vertebral bodies indicate superficial osseous remodelling of
58 infectious origin. Traces of pathological lesions were observed on the endocranial surface of the
59 child’s skull, reflecting a reaction of meningeal tissues, a consequence of a probable TB-related
60 meningeal infectious process. Results of recent paleomicrobiological examinations – lipid
61 biomarker and aDNA studies – support the morphological diagnosis of probable TB infections [4].

62

63 **KEYWORDS**

64 Neanderthal remains; paleopathology; *tuberculosis*; tuberculous *meningitis*; Subalyuk Cave;
65 Hungary

66

67 INTRODUCTION

68 The paleopathology of human *tuberculosis* (TB) is one of the wealthiest subfields of the science of
69 diseases whose existence can be demonstrated based on ancient human remains. Although there are
70 still difficulties in the way of discovering the exact evolutionary past of *tuberculosis*, human
71 paleopathology provides an abundance of scientific information related to past occurrences of this
72 severe infectious condition. This is partly due to the fact that some skeletal lesions are relatively
73 specific to TB; it is one of the few infectious diseases that can leave behind quite pronounced and
74 rather specific bone alterations. The classical diagnostic criteria of skeletal TB are characteristic
75 lytic lesions with reactive new bone formation that tend to show predilection in hematopoietic red
76 marrow areas, mainly in the *vertebrae*, ribs, and *metaphysis* and *epiphysis* of long bones. Although
77 TB can potentially affect any bone of the human skeleton, vertebral TB (tuberculous *spondylitis*,
78 often occurring in the vertebral bodies of the thoracic and lumbar regions) is the most typical
79 representation of skeletal *tuberculosis*; the second most frequent alteration in TB is *arthritis* of the
80 large, weight-bearing joints [5,6,7,8]. Although until the 1990s, human paleopathology was
81 essentially limited to the identification of typical skeletal lesions of chronic extrapulmonary
82 *tuberculosis*, several hundreds of convincing ancient TB cases were identified and published during
83 the first century of human paleopathology, between the end of the 19th and 20th centuries [9,10]. In
84 the 1990s, it was generally accepted that *tuberculosis*, a disease of zoonotic origin, appeared in the
85 Neolithic period because of the domestication of the aurochs, the wild ancestor of cattle [11]. The
86 last quarter of a century has brought progress in the paleopathological diagnostics of TB, both in
87 morphological [12,13,14,15,16,17,18,19] and biomolecular [20,21,22,23,24,25,26,27,28,29]
88 diagnostical methods, thus opening significant new perspectives for ancient tuberculosis research.

89 According to a recent review of the paleopathological literature, several 20th century
90 paleopathological studies have already proved the existence of Neolithic human tuberculosis. The
91 introduction of new morphological and molecular methods accelerated the progress in this field
92 since the turn of the *millennium*, and many ancient morphological and molecular cases were
93 published in the last decades [30]. Since 2015, we have evident and confirmed paleopathological
94 proof that human TB has predated domestication [31]. However, ancient human skeletal material
95 (especially from the periods before the Early Neolithic) is still scarce, and all probable Paleolithic
96 TB occurrences, either evident, confirmed, or suspicious are of great importance. Therefore, despite
97 all progress in ancient TB paleopathology research, our corresponding paleoepidemiological
98 knowledge needs to be revised in the future.

100 **Material – the Subalyuk hominin remains**

101 The Subalyuk hominin remains were uncovered in 1932 in a cave of the same name (called
102 ‘Mussolini Cave’ at the time of the discovery and the first publications) in the Bükk Mountains,
103 Northern Hungary (Supplementary Figures 1-2). The remains represent two individuals, Subalyuk 1
104 (25-35-year-old adult female, Fig. 1) and Subalyuk 2 (3-4-year-old child, Fig. 2). They are curated
105 in the Hungarian Natural History Museum, Department of Anthropology, Budapest, Hungary. Both
106 sets of skeletal remains are highly incomplete, but they provide substantial anthropological data,
107 and their Neanderthal affiliation has been widely accepted in the literature. Since the first
108 description in 1940 [1], the Subalyuk hominin remains have been the subject of several
109 international anthropological studies [2, 32, 33, 34]. Preliminary paleopathological studies were
110 also carried out during the last couple of years [35, 36].

111 These two individuals’ first direct dating results have been recently published [3]. The radiocarbon
112 dating places them among the last Neanderthals of Central Europe. Dating was carried out at the
113 Curt-Engelhorn-Centre Archaeometry, Mannheim (Germany), and revealed an age of 39,732–
114 39,076 cal. BP (2σ) for the adult female, and 36,117–35,387 cal. BP (2σ) for the child [3]. During
115 the dating process, the issue of an old preservative varnish has also been addressed, which covers all
116 remaining bone elements and somewhat hinders observation. However, most bone surfaces are still
117 fit for examination despite the varnish.

118

119 **Methods**120 **Morphological analysis**

121 In the frame of an international paleopathological research project, detailed macromorphological
122 analyses have been performed on both Neanderthals in 2011-2012. During the identification of
123 pathological conditions, a standard system of categorization was used [7]. In the first step of this
124 project, all the preserved skeletal elements were examined morphologically. This process led to the
125 discovery of different alterations, which previously had remained undetected.

126 In order to obtain a more precise dating of these remains, both specimens were sampled for a
127 radiocarbon analysis [3]. The same sampling process furnished bone powder material for the
128 subsequent paleomicrobiological analysis.

129 In 2017, we had the opportunity to examine the Subalyuk 1 vertebral bodies and the Subalyuk 2
130 cranium using digital light microscopy. Morphological changes on the surface of these selected

131 skeletal elements were scanned and analyzed with Keyence VHX-5000 Digital microscope for
132 better understanding and visualization of the structural remodeling. These examinations were
133 carried out in the Central Quality Laboratory, Continental Automotive, Hungary.

134 The detected osteoarticular lesions were analyzed, discussed, and diagnosed using relevant
135 paleopathological and modern medical literature.

136 **Biomolecular analyses**

137 This paper presents the results of the morphological paleopathological investigation carried out on
138 the Subalyuk remains. The methods used for the aDNA and lipid biomarker analyses are detailed in
139 a paper by Lee and collaborators [4]

140 **RESULTS**

141 The adult human remains (Subalyuk 1) are represented by an incomplete mandible (**Fig. 1**), the
142 *manubrium sterni*, the *atlas* (in three parts), three vertebral bodies, one spinous process of a thoracic
143 *vertebra*, an incomplete *sacrum*, the left *patella*, three more or less complete metatarsals (second
144 and third right one, left fourth one) and fragments of a second left metacarpal [2,3,4].

145 The subadult Neanderthal remains (Subalyuk 2) consist of an incomplete *cranium* (**Fig. 2**) (basis
146 not represented), two *maxillae*, an isolated left nasal bone, and a few vertebral bodies [2,3,4].

147 Based on the results of our preliminary paleopathological analysis, we can state that the adult
148 Subalyuk 1 mandible, *atlas*, *patella*, metatarsals, and metacarpal remains were either free of
149 macroscopically detectable pathological changes or the postmortem degradation of the frequently
150 damaged bones had rendered them unrecognizable. However, some lesions of potential pathological
151 origin could be found on the *manubrium sterni*, the *sacrum*, and on the three vertebral bodies. These
152 (in some cases incomplete or fragmentary) bones are presented in **Supplementary Figures 3a-b,**
153 **4a-b-c-d-e, 5.**

154 As for the incomplete subadult material, the Subalyuk 2 facial bones or vertebral remains do not
155 show traces of paleopathological changes. Since in this study, we deal with the skeletal elements in
156 which the paleopathological examination found traces of evident, probable, or at least possible
157 lesions of pathological origin, in the case of the Subalyuk 2 child specimen, the subject of our study
158 was only the partially reconstructed skull (**Supplementary Figure 6**).

159 Despite the incomplete, and for most of the elements fragmentary character of the Subalyuk 1 adult
160 skeleton, changes of possible or probable pathological origin could be recognized during the
161 macromorphological study. Among the observed anomalies, the two circumscribed bone defects of

162 the *manubrium sterni* (**Supplementary Figures 3a-b**) were probably the most difficult to
163 differentiate from taphonomical (*post mortem*) changes. The two, slightly oval, 15-20 mm long, 5-
164 10 mm wide, 5-10 mm deep defects open in the cortical bone of the posterior left side of the
165 *manubrium sterni* (**Fig. 3**). Both lytic *foci* are characterized by loss of cortical and spongy bone
166 material. Even though considerable new bone formation cannot be detected inside the cavities,
167 small-scale remodeling on the upper left margins of the superior defect suggests possible *in vivo*
168 origin of the lesions, or at least in some parts of the lesions. Traces of porosity can be observed in
169 the remnants of the original cortical bone over the superior bone defect. However, some *post*
170 *mortem* degradation is also apparent in and around the lesions. As for the few preserved spinal
171 elements of the adult Neanderthal specimen Subalyuk 1, there is no detectable alteration on the
172 fragmentary *atlas* and the only thoracic spinous process. However, all preserved thoracic and
173 lumbar vertebral bodies show pathological changes. The no. Hb11 thoracic vertebral body has
174 suffered considerable taphonomical damage. The macroscopic picture of the relatively well-
175 preserved anterior surface (**Supplementary Figure 4b**) shows evidence of hypervascularization and
176 superficial bony remodeling. These alterations, especially the new bone formation is clearly
177 confirmed by digital microscopic surface scanning (**Fig. 4, Supplementary Figure 4c**). As for the
178 other partially preserved thoracic vertebral body no. Hb15, a less developed superficial remodeling
179 with discoloration, can be observed on the left side, which became more visible through digital
180 microscopic surface scanning (**Supplementary Figures 4d-e**).

181 The relatively well-preserved upper lumbar vertebral body no. Hb3 carries very similar
182 pathological lesions (hypervascularization, superficial new bone formation), predominantly on the
183 body's left side and medial surfaces. The alterations can be easily recognized macroscopically (**Fig.**
184 **5a**). The digital microscopic surface scanning makes the superficial remodeling more evident (**Fig.**
185 **5b**). As for the observability of the bone surfaces, the *sacrum* is one of the best elements among the
186 Subalyuk 1 skeletal remains. Unfortunately, the lower two segments of bone were broken off and
187 lost *post mortem* in a slightly asymmetrical manner (**Supplementary Figure 5**). Approximately in
188 the middle of the complete right side auricular surface of the *sacrum*, a 4 mm centimeter wide,
189 small, round bone cavity can be observed (**Fig. 6a**). On the left side of the *sacrum*, the lower third
190 of the auricular surface is missing. The macromorphological examination revealed a 4-5 mm wide
191 depression and nine more or less sharply demarcated more minor, round, erosion-like defects in the
192 upper, preserved part of the left auricular surface (**Fig. 6b**). The accurate restoration of the Subalyuk
193 2 subadult skull remains has permitted the paleoanthropological study of the specimen during the
194 last century, but paleopathological alterations were never described. However, in during our
195 investigation we found traces of different alterations in the endocranial surface of the infant skull

196 (Supplementary Figure 6). Several areas of the original endocranial cortex are covered with a
197 widespread, off-white, discolored periosteal new bone layer on the restored and preserved parietal
198 bones, predominantly at both sides of the sagittal suture (Fig. 7a). The most evident lesions can be
199 observed on the relatively well-preserved parietal endocranial surfaces. However, the frontal bone
200 and the occipital region are also slightly involved. Within and around these new bone plaques, a
201 great number of small abnormal blood vessel impressions can be detected (Fig. 7a, Supplementary
202 Figures 7a-b). The microscopic examination supported the macroscopic morphological results
203 (Fig. 7b, Supplementary Figure 7c). In addition to the observed traces of periosteal appositions
204 and abnormal blood vessel impressions, the digital microscopic surface scanning has revealed the
205 presence of several small, 0,5-1 mm large roundish granular impressions with smooth margins (Fig.
206 7c-d, Supplementary Figure 7d). These lesions are usually isolated or sometimes forming clusters,
207 and, even more rarely, are confluent (Supplementary Figure 7e).

208

209 DISCUSSION AND CONCLUSIONS

210 In all paleopathological studies, the first step has to confirm the *ante mortem* origin of the observed
211 alterations. In the case of the two Subalyuk specimens, for most of the detected lesions, the *post*
212 *mortem* origin is quite easy to rule out because of the presence of evident traces of new bone
213 formation. The superficial new bone formation on the three adult vertebral bodies or the plaque-
214 like, discolored new bone layers (traces of periosteal appositions) in certain zones of the child's
215 endocranial surface are evident proofs of their formation during the life of the individuals. The
216 delineated, circumscribed anomalies with intact newly formed border, that we can observe around
217 the abnormal blood vessel impressions and small granular impressions on the endocranial surface,
218 the hypervascularization of the vertebral bodies, or the quite regular erosive changes on the adult
219 sacral auricular surfaces can be considered as results of *in vivo* processes. The left side sacral
220 auricular surface, or the Hb11 thoracic vertebral body, are broken and incomplete; however, we see
221 enough anomalies on the remains to be sure that these skeletal elements were also subjects of *ante*
222 *mortem* pathological alterations. The only element among the presented bone remains without
223 strong enough evidence concerning the *ante mortem* character of the alterations is the *manubrium*
224 *sterni*. The taphonomic damage here is too substantial at the potential lesions, which does not
225 facilitate an unequivocal conclusion. During earlier investigations of the Subalyuk specimens in the
226 first half of the 20th century, the shape and regular character of these two lesions oriented the
227 paleoanthropologist toward a very special explanation: they were considered as probable bite marks
228 from animals gnawing at the remains [1]. We do not want to completely exclude this type of
229 taphonomic explanation, but we consider it improbable because of the position of the lesions,

230 among other reasons. Moreover, since one of the lesions has a slightly sclerotic border, we prefer to
231 keep the manubrium defects as potential pathological lesions of uncertain origin, contrarily to the
232 other described lesions where there is no doubt concerning their real pathological origin.

233 The paleopathological study of the Subalyuk specimens has been set back both by the very
234 incomplete nature of the remains and the very limited accessibility of this precious
235 paleoanthropological material. Essentially, our data come from two study campaigns: a first
236 macromorphological study carried out in 2011-2012 (followed by sampling for biomolecular tests)
237 and a second occasion for digital microscopy of some selected skeletal elements in 2017.
238 Paleoradiological or other specific studies could not be carried out.

239 The most striking and newly recognized alterations were the endocranial changes observed on the
240 subadult Subalyuk 2 skull. The presented lesions can be divided into three groups: traces of
241 abnormal blood vessel impressions (ABVI), periosteal appositions (PA), and granular impressions
242 (GI). According to the results of the paleopathological research in the last quarter of a century, all
243 these changes are compatible with the consequences of tuberculous meningitis (TBM)
244 [7,13,14,16,17,18,19,37,38,39,40,41,42]. Pioneering works by Schultz [13,38], and later the studies
245 by Spekker and collaborators [17,18,41] have proved essential differences in the diagnostic values
246 of these alterations. Among these alterations, GIs are caused by pressure atrophy of the tubercles
247 formed on the outermost meningeal layer during the later stages of TBM. GIs on the inner skull
248 surface have been considered pathognomonic features of TBM and can also be used as diagnostic
249 criteria for TBM in the paleopathological practice [13,17,38,40,41]. During the first twenty years of
250 our century, the different forms of the endocranial abnormal blood vessel impressions (ABVIs) and
251 endocranial periosteal appositions (PAs) were generally considered as paleopathological diagnostic
252 criteria for TBM [7,14,16,39,42]. However, ABVIs and PAs on the inner surface of the skull are the
253 traces of the inflammatory and/or haemorrhagic processes of the meninges and can be generated by
254 several infectious (including TBM, but also nonspecific infections) and non-infectious conditions
255 (e.g., trauma and scurvy). As solitary symptoms, they cannot be considered pathognomonic features
256 of TBM during a paleopathological study. However, they have a diagnostic value in the
257 identification of TB in human osteoarchaeological material, when they simultaneously occur with
258 other probable TB-related lesions [18,40,41]. Endocranial traces of ABVIs and PAs can reveal
259 previous vasculitides. As tuberculous cerebral vasculitis (a frequent complication of TBM) is
260 among the most frequent secondary cerebral vasculitides [43,44,45], simultaneous ABVIs and PAs
261 occurrence on the inner skull surface can be used as indicators of probable TBM.

262 Among the presented lesions of the Subalyuk 1 adult skeletal remains, the clearest and most striking
263 are the traces of superficial vertebral osseous remodeling. Hypervascularizations accompanied by

264 periosteal new bone formation (PNBF) are seen on the anterior and lateral aspects of the 3
265 preserved thoracic and lumbar vertebral bodies. PNBFs on all vertebral bodies are of remodeled
266 character. Since the end of the last century, vertebral hypervascularization has been considered a
267 paleopathological marker of early-stage skeletal TB [12,46]. The anterior or antero-lateral PNBF
268 lesions of vertebral bodies with hypervascularization, with or without erosive lesions, and without
269 morphological changes of the endplates (due to the preservation of the disk spaces) are most
270 frequently the consequences of subligamentous abscesses related to infectious spondylitis. A
271 subligamentous spread of three or more vertebral segments is more suggestive of tuberculous
272 spondylitis than pyogenic spondylitis [6,8,47,48]. During a macromorphological examination, and
273 especially in the case of such an incomplete skeletal material, we cannot exclude pyogenic or other
274 skeletal infections; TB is only one of the possible etiologies. However, based on the lesions'
275 character and multivertebral spread, the most probable reason would be TB. Recent
276 paleopathological studies from anatomical collections support the possible TB diagnosis, since
277 many documented and confirmed spinal TB cases were analyzed and published during the last two
278 decades with symptoms similar that of Subalyuk 1 [7,16,49,50].

279 Although the erosive lesions observed on the auricular surfaces of the incomplete *sacrum* are less
280 informative, they suggest a possible bilateral inflammatory process of the sacroiliac (SI) joints. We
281 do not exclude the possibility of a non-infectious origin of these changes. However, the lack of any
282 signs of spondyloarthropathy and the presence of vertebral changes of probably infectious origin
283 suggest higher probability for an infectious origin and a possible link between the spinal and SI
284 joint lesions. Among them, one of the plausible explanations could be a TB infection
285 [7,8,16,49,50,51].

286 Finally, the most uncertain but possibly paleopathological alterations observed on the Subalyuk
287 skeletal remains are that of the *manubrium sterni*. We do not exclude their taphonomical origin.
288 However, the remnants of some sclerotic border at the upper rim of one of the holes may support
289 the idea of a possible *in vivo* pathological process. If that case is valid, an eventual sternal
290 osteomyelitis may be a possible explanation [52,53].

291 The morphological examination of the two Subalyuk specimens has furnished rich
292 paleopathological results despite the skeletal elements's very incomplete and frequently
293 fragmentary nature. As detailed above, the diagnostic value of the observed pathological alterations
294 is diverse. As for the skull of the Subalyuk 2 child, the 3 observed types of endocranial lesions are
295 all compatible with TBM. GIs are pathognomonic for TBM, and their simultaneous occurrence with
296 the ABVIs and PAs strengthen the TBM-related origin of the latter lesions, too. The incomplete
297 adult skeletal material Subalyuk 1 exhibited lesions in 3 locations.

298 On all preserved thoracic and lumbar vertebral bodies, traces of hypervascularization and
299 superficial remodeling were detected. Although these lesions are much less pathognomonic for TB
300 than the previously mentioned endocranial changes of Subalyuk 2, they were very probably related
301 to some chronic infectious disease, and they can be seen relatively frequently in pre-antibiotic
302 modern skeletal TB cases. The small, erosive superficial changes of probable pathological origin
303 observed in both auricular surfaces of the incomplete *sacrum* may reveal a possible bilateral
304 sacroiliitis. The lesions of the *manubrium sterni* might be the consequences of an eventual sternal
305 osteomyelitis. Unfortunately, most of the adult skeleton is missing; therefore, a hypothetical
306 association of lesions in separate locations cannot be recognized.

307 After evaluating of all morphological changes of the two specimens, we can conclude that about 39
308 thousand years ago in the Carpathian Basin, a 25-35-year-old adult Neanderthal female suffered
309 from some chronic infection. The traces of this disease are compatible with a few non-
310 pathognomonic symptoms of TB. Approximately 3 thousand years later, the remains of a 3-4-year-
311 old child accumulated in the same Subalyuk Cave who had suffered from TBM, which eventually
312 could have played a role in their early death.

313 Because of the apparent 3 thousand years gap between the two specimens proved by the
314 radiocarbon dating, we do not want to look for a direct link between the two individuals. However,
315 a potential link concerning this supposedly common disease was recently supported by the
316 biomolecular analysis of the Subalyuk samples. Application of PCR amplification to the subadult
317 Subalyuk 2 *cranium* and a *vertebra* gave a positive result (IS6110) for tuberculosis, backed up by
318 spoligotyping. Lipid biomarker analyses of the same two specimens revealed definitive signals for
319 C₃₂ mycoserosate, a very characteristic fatty acid found in the *Mycobacterium tuberculosis* complex
320 (MTBC). A *vertebra* from the adult Subalyuk 1 provided weak evidence for mycocerosate
321 biomarkers [4]. These new results complete and confirm the morphological data. Based on both the
322 morphological observations and their biomolecular support, we can conclude that tuberculosis was
323 present in Central Europe during the Late Pleistocene, approximately 36-39 thousand years ago.
324 Several earlier paleopathological data have already supported the presence of the disease in the
325 prehistoric Carpathian Basin; but until now the oldest cases were found in different, approximately
326 7 thousand years old Neolithic anthropological series [54,55,56,57,58,59,60]. Our new results
327 confirm an at least 36–39-thousand-year-old ancestry of TB in Europe. The occurrence of TB in late
328 Neanderthal remains is compatible with the theory by Comas and coworkers that TB and humans
329 co-evolved from 70 ka onwards and emerged “Out of Africa”. Our results support the concept by
330 David E. Minnikin, who has recently proposed the possibility of an important contribution of
331 *tuberculosis* to the rapid extinction of Neanderthals [4,36,61,62].

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338

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340 **REFERENCES**

341

- 342 [1] Bartucz L (1940). Der Urmensch der Mussolini-Höhle. In L.Bartucz, J. Dancza, F.
 343 Hollendonner, O. Kadić, M. Mottl, V. Pataki, E. Pálosi, J. Szabó, & A. Vendl, *Die Mussolini-*
 344 *Höhle (Subalyuk) bei Cserépfalu* (pp. 49–105). Geologica Hungarica Series Palaeontologica
 345 14, Budapest: Editio Instituti Regii Hungarici Geologici.
- 346 [2] Pap I, Tillier A-m, Arensburg B, Chech M (1996). The Subalyuk Neanderthal remains
 347 (Hungary): A re-examination. *Annales historico-naturales Musei nationalis hungarici*, 88,
 348 233–270.
- 349 [3] Mester Z, Coqueugniot H, Tillier A-m, Rosendahl W, Friedrich R, Zink A, Maixner F, Dutour
 350 O, Bereczki Z, Gasparik M, Pap I, Pálfi G. (2023). First direct dating of the Late Neanderthal
 351 remains from Subalyuk Cave in Northern Hungary. *Anthropologischer Anzeiger*,
 352 DOI: [10.1127/anthranz/2023/1716](https://doi.org/10.1127/anthranz/2023/1716)
- 353 [4] Lee OY-C, Wu HHT, Besra GS, Minnikin DE, Jäger HY, Maixner F, Zink A, Gasparik M,
 354 Pap I, Bereczki Z, Pálfi G (2023). Lipid biomarker detection for tuberculosis in Late
 355 Neanderthal skeletons from Subalyuk Cave, Hungary. *Tuberculosis (Edinb)*. (under review)
- 356 [5] Sorrel É, Sorrel-Dejerine Y 1932. *Tuberculose osseuse et ostéo-articulaire 1-3*, Masson, Paris
- 357 [6] Resnick D, Niwayama G. 1988. *Diagnosis of bone and joint disorders*. Saunders,
 358 Philadelphia.
- 359 [7] Ortner DJ. 2003. *Identification of pathological conditions in human skeletal remains*. San
 360 Diego, CA, USA: Academic Press.
- 361 [8] Tuli S.M. 2016. *Tuberculosis of the Skeletal System: Bone, Joints, Spine and Bursal Sheaths*.
 362 3rd Edition, Jaypee Brothers Medical Publishers, New Delhi.
- 363 [9] Pálfi, G., Dutour, O., Deák, J., Hutás, I. (Eds.), 1999. *Tuberculosis: Past and Present*. Golden
 364 Book Publisher – Tuberculosis Foundation, Budapest, Szeged.

- 365 [10] Roberts CA, Buikstra JE. The bioarchaeology of tuberculosis: a global view on a reemerging
366 disease. University Press of Florida, Gainesville, 2003.
- 367 [11] Ortner DJ. Paleopathology: Implications for the history and evolution of tuberculosis. In:
368 Pálfi G, Dutour O, Deák J, Hutás I, editors. Tuberculosis: Past and present, Szeged, Hungary:
369 TB Foundation & Budapest, Hungary: Golden Book Publisher; 1999, pp. 253-262.
- 370 [12] Baker BJ. Early manifestations of tuberculosis in the skeleton. In: Pálfi G, Dutour O, Deák J,
371 Hutás I, editors. Tuberculosis: Past and present, Szeged, Hungary: TB Foundation &
372 Budapest, Hungary: Golden Book Publisher; 1999, pp. 301–307.
- 373 [13] Schultz M. The role of tuberculosis in infancy and childhood in prehistoric and historic
374 populations. In: Pálfi G, Dutour O, Deák J, Hutás I, editors. Tuberculosis: Past and present,
375 Szeged, Hungary: TB Foundation & Budapest, Hungary: Golden Book Publisher; 1999, pp.
376 503–507.
- 377 [14] Hershkovitz I, Greenwald CM, Latimer B, Jellema LM, Wish-Baratz S, Eshed V, et al.
378 *Serpens endocrania symmetrica* (SES): A new term and a possible clue for identifying
379 intrathoracic disease in skeletal populations. *Am J Phys Anthropol* 2002;118(3):201–216.
380 <https://doi.org/10.1002/ajpa.10077>
- 381 [15] Santos, A. L.; Roberts, C. 2006. Anatomy of a serial killer: differential diagnosis of
382 tuberculosis based on rib lesions of adult individuals from the Coimbra Identified Skeletal
383 Collection, Portugal. *American Journal of Physical Anthropology*, 130 (1): 38-49.
- 384 [16] Pálfi, G., Bereczki, Z., Ortner, D.J., Dutour, O, 2012. Juvenile cases of skeletal tuberculosis
385 from the Terry Anatomical Collection (Smithsonian Institution, Washington DC, USA). *Acta*
386 *Biologica Szeged.*, 56 (1), 1-12.
- 387 [17] Spekker O, Hunt DR, Paja L, Molnár E, Pálfi G, Schultz M. Tracking down the White Plague:
388 The skeletal evidence of tuberculous meningitis in the Robert J. Terry Anatomical Skeletal
389 Collection. *PLOS ONE* 2020;15(3):e0230418. <https://doi.org/10.1371/journal.pone.0230418>
- 390 [18] Spekker O, Schultz M, Paja L, Váradi OA, Molnár E, Pálfi G, , et al. Tracking down the
391 White Plague. Chapter two: The role of endocranial abnormal blood vessel impressions and
392 periosteal appositions in the paleopathological diagnosis of tuberculous meningitis. *PLOS*
393 *ONE* 2020;15(9):e0238444. <https://doi.org/10.1371/journal.pone.0238444>
- 394 [19] Spekker O, Hunt DR, Berthon W, Paja L, Molnár E, Pálfi G, et al. Tracking down the White
395 Plague. Chapter three: Revision of endocranial abnormally pronounced digital impressions as
396 paleopathological diagnostic criteria for tuberculous meningitis. *PLOS ONE*
397 2021;16(3):e0249020. <https://doi.org/10.1371/journal.pone.0249020>

- 398 [20] Spigelman, M., Lemma, E., 1993, The Use of Polymerase Chain Reaction (PCR) to Detect
399 Mycobacterium tuberculosis in Ancient Skeletons. *International Journal of Osteoarchaeology*
400 3: 137-143. <https://doi.org/10.1002/oa.1390030211>
- 401 [21] Salo, W., Aufderheide, A., Buikstra, J., Holcomb, T., 1994, Identification of Mycobacterium
402 tuberculosis DNA in a pre-Columbian Peruvian mummy. *Proc. Natl. Acad. Sci. USA* , 91:
403 2091-2094. Smith NH, Crawshaw T, Parry J, Birtles RJ. 2009. *Mycobacterium microti*: More
404 diverse than previously thought. *J Clin Microbiol.* 47, 2551-2559. [doi: 10.1128/JCM.00638-](https://doi.org/10.1128/JCM.00638-09)
405 [09](https://doi.org/10.1128/JCM.00638-09)
- 406 [22] Donoghue, H.D., Spigelman, M., Zias, J., Gernaey-Child, A.M., Minnikin, D.E., 1998.
407 *Mycobacterium tuberculosis* complex DNA in calcified pleura from remains 1400 years old.
408 *Lett Appl Microbiol*, 27: 265-269. <https://doi.org/10.1046/j.1472-765X.1998.00436.x>
- 409 [23] Gernaey AM, Minnikin DE, Copley MS, Power JJ, Ahmed AMS, Dixon RA, Roberts CA,
410 Robertson JD, Nolan J, Chamberlain A. Detecting ancient tuberculosis. *Internet Archaeol.*
411 1998; http://intarch.ac.uk/journal/issue5/gernaey_index.html.
- 412 [24] Zink A, Haas CJ, Reischl U, Szeimies U, Nerlich AG. 2001. Molecular analysis of skeletal
413 tuberculosis in an ancient Egyptian population. *J. Med. Microbiol.*, 50: 355-366.
414 <https://doi.org/10.1099/0022-1317-50-4-355>
- 415 [25] Donoghue, H.D., 2009. Human tuberculosis - an ancient disease, as elucidated by ancient
416 microbial biomolecules. *Microbes Infect* 11: 1156-62.
417 <https://doi.org/10.1016/j.micinf.2009.08.008>
- 418 [26] Minnikin DE, Lee OY-C, Wu HHT, Besra G, Donoghue H, 2012. Molecular biomarkers for
419 ancient tuberculosis. In: Cardona, P-J. (ed.) *Understanding tuberculosis - deciphering the*
420 *secret life of the bacilli*. Rijeka, Croatia: InTech Open Access Publisher, p. 3–36.
- 421 [27] Chan JZ, Sergeant MJ, Lee OY, Minnikin DE, Besra GS, Pap I, Spigelman M, Donoghue
422 HD, Pallen MJ. (2013): Metagenomic analysis of tuberculosis in a mummy. *Comment in N*
423 *Engl J Med.* 2013 Oct 17; 369 (16): 1572.
- 424 [28] Donoghue HD, Taylor GM, Stewart GR, Lee OY-C, Wu HHT, Besra GS, Minnikin DE.
425 Positive Diagnosis of Ancient Leprosy and Tuberculosis Using Ancient DNA and Lipid
426 Biomarkers. *Diversity.* 2017; 9(4):46. <https://doi.org/10.3390/d9040046>
- 427 [29] Jäger HY, Maixner F, Pap I, Szikossy I, Pálfi G, Zink AR. Metagenomic analysis reveals
428 mixed Mycobacterium tuberculosis infection in a 18th century Hungarian midwife.
429 *Tuberculosis (Edinb).* 2022 Dec;137:102181. doi: 10.1016/j.tube.2022.102181. Epub 2022
430 Feb 21. PMID: 35210171.

- 431 [30] Buzic I, Giuffra V. The paleopathological evidence on the origins of human tuberculosis: A
432 review. *J Prev Med Hyg* 2020;61(Suppl. 1):e3–38. [https://doi.org/10.15167/2421-](https://doi.org/10.15167/2421-4248/jpmh2020.61.1s1.1379)
433 [4248/jpmh2020.61.1s1.1379](https://doi.org/10.15167/2421-4248/jpmh2020.61.1s1.1379)
- 434 [31] Baker O, Lee OY, Wu HT, Besra GS, Minnikin DE, Llewellyn G, Williams CM, Maixner F,
435 O’Sullivan N, Zink A, Chamel B, Khawam R, Coqueugniot E, Helmer D, Le Mort F, Perrin
436 P, Gourichon L, Dutailly B, Pálfi G, Coqueugniot H, Dutour O. Human tuberculosis predates
437 domestication in ancient Syria. *Tuberculosis (Edinb)* 2015;95:S4-S12.
438 <https://doi.org/10.1016/j.tube.2015.02.001>
- 439 [32] Thoma, A. (1963). The dentition of the Subalyuk Neanderthal child. *Zeitschrift für*
440 *Morphologie und Anthropologie*, 54, 127–150. <https://www.jstor.org/stable/25754835>
- 441 [33] Pap, I., Tillier, A.-m., Arensburg, B., Weiner, S., & Chech, M. (1995). First scanning Electron
442 Microscope Analysis of Dental Calculus from European Neanderthals: Subalyuk (Middle
443 Paleolithic, Hungary). Preliminary report. *Bulletins et Mémoires de la Société*
444 *d’Anthropologie de Paris*, 7, 69–72. <https://doi.org/10.3406/bmsap.1995.2409>
- 445 [34] Coqueugniot, H., Tillier, A.-m., Pálfi, G., Dutour, O. J., Dutailly, B., Desbarats, P., Palkó, A.,
446 & Pap, I. (2014). Contribution of virtual 3D reconstruction and printing (VIRCOPAL®) to
447 paleoanthropology: The case of the Neanderthal Subalyuk 2 child skull (Bukk Mountains,
448 Hungary). *American Journal of Physical Anthropology*, 153 S58, 97.
449 <https://hdl.handle.net/11858/00-001M-0000-0019-8CED-9>
- 450 [35] Pálfi, G., Pap, I., Tillier, A.-m., Szigeti, K., Molnár, E., Rosendhal, W., Krause, J., Posth, C.,
451 Maixner, F., Zink, A. (2020). Re-examination of the Subalyuk Neanderthal Remains
452 (Subalyuk Cave, Hungary). In *26th EAA Virtual Annual Meeting – Abstract Book* (p. 393).
453 Prague: European Association of Archaeologists.
- 454 [36] Minnikin, D., Lee, O., Wu, H., Besra, G., Llewellyn, G., Williams, C., Pap, I., Pálfi, G.,
455 Maixner, F., Zink, A., Jaeger, H. (2020). Lipid biomarkers for tuberculosis are present in
456 Neanderthal skeletal remains from Subalyuk, Hungary. In *26th EAA Virtual Annual Meeting –*
457 *Abstract Book* (p. 394). Prague: European Association of Archaeologists.
- 458 [37] Maczel M. 2003. „On the traces of tuberculosis” *Diagnostic criteria of tuberculosis affection*
459 *of the human skeleton and their application in Hungarian and French anthropological series.*
460 PhD Dissertation, University of La Méditerranée – Aix Marseille II Faculty of Medicine:
461 Marseille, France & University of Szeged, Faculty of Science: Szeged, Hungary.
- 462 [38] Schultz M, Schmidt-Schultz TH. 2015. Is it possible to diagnose TB in ancient bone using
463 microscopy?. *Tuberculosis* 95 (Suppl. 1): S80–S86. [DOI: 10.1016/j.tube.2015.02.035](https://doi.org/10.1016/j.tube.2015.02.035)
- 464 [39] Teschler-Nicola M, Novotny F, Spannagl-Steiner M, Stadler P, Prohaska T, Irrgeher J, Zitek
465 A, Däubel B, Haring E, Rumpelmayr K, Wild EM. 2015. The early medieval manorial estate of

- 466 Gars/Thunau, Lower Austria: an enclave of endemic tuberculosis?. *Tuberculosis* **95**(Suppl. 1):
 467 S51–S59. [DOI: 10.1016/j.tube.2015.02.017](https://doi.org/10.1016/j.tube.2015.02.017)
- 468 [40] Spekker O. 2018. *Evaluation of endocranial bony changes in relation to tuberculosis in the*
 469 *Robert J. Terry Anatomical Skeletal Collection (Washington, DC, USA)*. PhD Dissertation,
 470 University of Szeged: Szeged, Hungary. [DOI: 10.14232/phd.9714](https://doi.org/10.14232/phd.9714)
- 471 [41] Spekker O, Hunt DR, Berthon W, Molnár E, Pálfi G. Insights into the diagnostic efficacy and
 472 macroscopic appearance of endocranial bony changes indicative of tuberculous meningitis:
 473 Three example cases from the Robert J. Terry Anatomical Skeletal Collection. *Int J*
 474 *Osteoarchaeol* 2022;32(2):444–456. <https://doi.org/10.1002/oa.3079>
- 475 [42] Hershkovitz, I., Donoghue, H.D., Minnikin, D.E., Besra, G.S., Lee, O.Y-C., Gernaey, A.M.,
 476 Galili, E., Eshed, V., Greenblatt, C.L, Lemma, E., Bar-Gal, G.K., Spigelman M., 2008.
 477 Detection and Molecular Characterization of 9000-Year-Old Mycobacterium tuberculosis
 478 from a Neolithic Settlement in the Eastern Mediterranean. *PLoS One*, 3(10): e3426.
 479 <https://doi.org/10.1371/journal.pone.0003426>
- 480 [43] Soni N, Kumar S, Shimle A, Ora M, Bathla G, Mishra P. Cerebrovascular complications in
 481 tuberculous meningitis. A magnetic resonance imaging study in 90 patients from a tertiary
 482 care hospital. *The Neuroradiology Journal*, 2020, Vol. 33(1) 3–16.
 483 <https://doi.org/10.1177/1971400919881188>
- 484 [44] Siva, A. Vasculitis of the nervous system. *J Neurol* 248, 451–468 (2001).
 485 <https://doi.org/10.1007/s004150170154>
- 486 [45] Tai, ML., Viswanathan, S., Rahmat, K. et al. Cerebral infarction pattern in tuberculous
 487 meningitis. *Sci Rep* 6, 38802 (2016). <https://doi.org/10.1038/srep38802>
- 488 [46] Haas, C.J., Zink, A., Molnar, E., Szeimes U., Reisch, I U., Marcsik, A., Ardagna, Y., Dutour,
 489 O., Palfi, Gy., Nerlich, A.G. (2000): Molecular evidence for different stages of tuberculosis in
 490 ancient bone samples from Hungary. - *American Journal of Physical Anthropology*, 113: 293-
 491 304. [https://doi.org/10.1002/1096-8644\(200011\)113:3<293::AID-AJPA2>3.0.CO;2-6](https://doi.org/10.1002/1096-8644(200011)113:3<293::AID-AJPA2>3.0.CO;2-6)
- 492 [47] Moorthy S, Prabhu NK, Spectrum of MR Imaging Findings in Spinal Tuberculosis. *American*
 493 *Journal of Roentgenology*, 2002, Vol 179 (4), <https://doi.org/10.2214/ajr.179.4.1790979>
- 494 [48] Jung N-Y, Jee W-H, Ha K-Y, Park C-K, Byun J-Y, Discrimination of Tuberculous
 495 Spondylitis from Pyogenic Spondylitis on MRI. *American Journal of Roentgenology*, 2004,
 496 Vol 182 (6), <https://doi.org/10.2214/ajr.182.6.182140>
- 497 [49] Spekker, O., Hunt, D.R., Váradi, O.A., Berthon, W., Molnár, E., Pálfi, Gy., 2018. Rare
 498 manifestations of spinal tuberculosis in the Robert J. Terry Anatomical Skeletal Collection
 499 (National Museum of Natural History, Smithsonian Institution, Washington, DC, USA). *Int. J.*
 500 *Osteoarch.* 28. 343-353. <https://doi.org/10.1002/oa.2658>

- 501 [50] Spekker O, Hunt DR, Király K, Kis L, Madai Á, Szalontai C, Molnár E, Pálfi G. Lumbosacral
502 tuberculosis, a rare manifestation of Pott's disease - How identified human skeletons from the
503 pre-antibiotic era can be used as reference cases to establish a palaeopathological diagnosis of
504 tuberculosis. *Tuberculosis (Edinb)*. 2023 <https://doi.org/10.1016/j.tube.2022.102287>.
- 505 [51] Ramlakan RJS, Govender S. Sacroiliac joint tuberculosis. *International Orthopaedics*, 2007,
506 31: 121–124, <https://doi.org/10.1007/s00264-006-0132-9>
- 507 [52] Sachdeva, R., Sachdeva, S., Arora, S., 2013. Sternal Tuberculosis. *Ann. Med. Health. Sci.*
508 *Res.* 3 (1), 21-23, <https://doi.org/10.4103/2141-9248.121213>
- 509 [53] Libor C, Hajdu T, Szeniczey T, Kovács LO, Kunos L, Mateovics-László O, "Tuberculosis on
510 the spot" – Discussion of a probable sternal tuberculosis from a late medieval cemetery from
511 Hungary. *Tuberculosis (Edinb)*. 2023 (accepted)
- 512 [54] Spekker, O., Pálfi, G., Kozocsay, G., Pósa, A., Bereczki, Z., Molnár, E., 2012. New cases of
513 probable skeletal tuberculosis from the Neolithic period in Hungary – A morphological study.
514 *Acta Biologica Szegediensis* 56(2). 115-123.
- 515 [55] Masson M, Molnár E, Donoghue HD, Besra GS, Minnikin DE, Wu HHT, Lee, OYC, Bull ID,
516 Pálfi G (2013) Osteological and Biomolecular Evidence of a 7000-Year-Old Case of
517 Hypertrophic Pulmonary Osteopathy Secondary to Tuberculosis from Neolithic Hungary.
518 *PLoS ONE* 8(10): e78252. <https://doi.org/10.1371/journal.pone.0078252>
- 519 [56] Masson, M., Bereczki, Z., Molnár, M., Donoghue, HD., Minnikin, DE., Lee, OYC., Wu,
520 HHT., Besra, GS., Bull, ID., Pálfi, G., 2015. 7000 year-old tuberculosis cases from Hungary –
521 Osteological and biomolecular evidence. *Tuberculosis*; 95, S1:S13-17.
522 <https://doi.org/10.1016/j.tube.2015.02.007>
- 523 [57] Pósa A, Maixner F, Mende BG, Köhler K, Sola C, Dutour O, Masson M, Molnár E, Pálfi G,
524 Zink A (2015): Tuberculosis in Late Neolithic-Early Copper Age human skeletal remains
525 from Hungary. *Tuberculosis* 95(Suppl 1):S18- S22. <https://doi.org/10.1016/j.tube.2015.02.011>
- 526 [58] Gémes A, Mateovics-László O, Anders A, Raczky P, Szabó G, Somogyi K, Keszi T,
527 Gyenesei KÉ, Kovács LO, Marcsik A, Szabó LD, Kiss K, Köhler K, Zoffmann ZK,
528 Szeniczey T, Hajdu T, Tuberculosis in Prehistory in Eastern Central Europe (Hungary) -
529 Chronological and geographical distribution. *Tuberculosis (Edinb)*. (accepted).
- 530 [59] Köhler K, Pálfi G, Molnár E, Zalai-Gaál I, Oszás A, Bánffy E, Kirinó K, Kiss K, Mende BG,
531 2012. A Late Neolithic Case of Pott's Disease from Hungary. *Int J Osteoarchaeol*, First
532 published online: 28 May 2012. <https://doi.org/10.1002/oa.2254>
- 533 [60] Köhler K, 2015. A Starčevo kultúra embertani leletei Alsónyék-Bátaszék lelőhelyről -
534 Anthropological examination of the Starčevo culture graves excavated at the site of Alsónyék-
535 Bátaszék. *Anthropologiai Közlemények* 2015;56, 3-26. <http://real.mtak.hu/32935/>

- 536 [61] Lee OY, Wu HH, Donoghue HD, Spigelman M, Greenblatt CL, Bull ID, Rothschild BM,
537 Martin LD, Minnikin DE, Besra GS (2012) *Mycobacterium tuberculosis* complex lipid
538 virulence factors preserved in the 17,000-year-old skeleton of an extinct bison, *Bison*
539 *antiquus*. PLoS One 7(7):e41923 <https://doi.org/10.1371/journal.pone.0041923>
- 540 [62] Comas, I., Coscolla, M., Luo, T., Borrell, S., Holt, K.E., Kato-Maeda, M., Parkhill, J., Malla,
541 B., Berg, S., Thwaites, G., Yeboah-Manu, D., Bothamley, G., Mei, J., Wei, L., Bentley, S.,
542 Harris, S.R., Niemann, S., Diel, R., Aseffa, A., Gao, Q., Young, D. and Gagneux, S. 2013.
543 Out-of-Africa migration and Neolithic co-expansion of *Mycobacterium tuberculosis* with
544 modern humans. Nature Genetics, 45: 1176–1182. <https://doi.org/10.1038/ng.2744>

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547 FIGURE LEGENDS

- 548 **Figure 1:** Lateral view of the adult Subalyuk 1 mandible (68.140.2.) (photo courtesy of Ildikó Pap).
- 549 **Figure 2:** Lateral view of the partially restored subadult Subalyuk 2 *calvaria* (68.146.1.) (photo
550 courtesy of Ildikó Pap).
- 551 **Figure 3:** Circumscribed bone defects in the left side of the adult Subalyuk 1 *manubrium sterni*
552 (68.140.8.) (posterior view, photo courtesy of Ildikó Pap).
- 553 **Figure 4:** Hypervascularization and superficial osseous remodeling on the remnants of the broken
554 thoracic vertebral body no. Hb11 (68.140.11.) (Subalyuk 1 adult specimen, Keyence VHX-5000
555 Digital microscope, 50x magnification, photo courtesy of Antal Sklánitz).
- 556 **Figure 5a:** The adult Subalyuk 1 upper lumbar vertebral body no. Hb3 (68.140.3.) (photo courtesy
557 of György Pálfi).
- 558 **Figure 5b:** Close-up picture showing superficial osseous remodeling on the vertebral body
559 (Subalyuk 1 adult specimen, no. Hb3 (68.140.3.), Keyence VHX-5000 Digital microscope, 20x
560 magnification, photo courtesy of Antal Sklánitz).
- 561 **Figure 6a:** Small, round bone defect in the right auricular surface of the adult Subalyuk 1 *sacrum*
562 (68.140.1.) (photo courtesy of György Pálfi).
- 563 **Figure 6b:** Small erosions in the upper part of the left auricular surface of the adult Subalyuk 1
564 *sacrum* (68.140.1.) (photo courtesy of György Pálfi).
- 565 **Figure 7a:** Endocranial changes on the subadult Subalyuk 2 parietal bones (68.146.1.) (photo
566 courtesy of György Pálfi).

567 **Figure 7b:** Endocranial changes – traces of periosteal plaques, abnormal blood vessel impressions
568 (Subalyuk 2 subadult specimen (68.146.1.), Keyence VHX-5000 Digital microscope, 20x
569 magnification, photo courtesy of Antal Sklánitz).

570 **Figures 7c-d:** Endocranial changes – traces of periosteal appositions, abnormal blood vessel
571 impressions and granular impressions (Subalyuk 2 subadult specimen (68.146.1.), Keyence VHX-
572 5000 Digital microscope, 20x magnification, photos courtesy of Antal Sklánitz).

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576 **Supplementary Figure 1:** Map of Central Europe, detail showing the location of Subalyuk Cave
577 (map courtesy of Zsolt Mester).

578 **Supplementary Figure 2:** Entrance of Subalyuk Cave in the Bükk Mountains, Northern Hungary
579 (photo courtesy of György Pálfi).

580 **Supplementary Figure 3a:** The adult Subalyuk 1 *manubrium sterni* (68.140.8.) in anterior view
581 (photo courtesy of Ildikó Pap).

582 **Supplementary Figure 3b:** The adult Subalyuk 1 *manubrium sterni* (68.140.8.) in posterior view
583 (photo courtesy of Ildikó Pap).

584 **Supplementary Figure 4a:** Adult Subalyuk 1 vertebral remains in the original boxes from 1932
585 (photo courtesy of György Pálfi).

586 **Supplementary Figure 4b:** Remains of the adult Subalyuk 1 thoracic vertebral body no. Hb11
587 (68.140.11.) (photo courtesy of György Pálfi).

588 **Supplementary Figure 4c:** Superficial osseous remodeling on the left side of the thoracic vertebral
589 body no. Hb15 (68.140.15.) (Subalyuk 1 adult specimen, Keyence VHX-5000 Digital microscope,
590 30x magnification, photo courtesy of Antal Sklánitz).

591 **Supplementary Figure 4d:** The adult Subalyuk 1 thoracic vertebral body no. Hb15 (68.140.15.)
592 (photo courtesy of Ildikó Pap).

593 **Supplementary Figure 5:** The adult Subalyuk 1 incomplete *sacrum* (68.140.1.) in anterior view
594 (photo courtesy of Ildikó Pap).

595 **Supplementary Figure 6:** The partially restored subadult Subalyuk 2 *calvaria* (68.146.1.), inferior
596 view (photo courtesy of György Pálfi).

597 **Supplementary Figures 7a-b:** Endocranial changes on the subadult Subalyuk 2 parietal bones
598 (68.146.1.) (photos courtesy of György Pálfi).

599 **Supplementary Figure 7c:** Endocranial changes – traces of periosteal appositions, abnormal blood
600 vessel impressions (Subalyuk 2 subadult specimen (68.146.1.), Keyence VHX-5000 Digital
601 microscope, 20x magnification, photo courtesy of Antal Sklánitz).

602 **Supplementary Figure 7d:** Endocranial changes – traces of periosteal appositions, abnormal blood
603 vessel impressions and granular impressions (Subalyuk 2 subadult specimen (68.146.1.), Keyence
604 VHX-5000 Digital microscope, 20x magnification, photo courtesy of Antal Sklánitz).

605 **Supplementary Figure 7e:** Endocranial changes – traces of periosteal appositions, granular
606 impressions (Subalyuk 2 subadult specimen (68.146.1.), Keyence VHX-5000 Digital microscope,
607 20x magnification, photo courtesy of Antal Sklánitz).

608

609 **AUTHOR CONTRIBUTION STATEMENT**

610 **György Pálfi:** Conceptualisation, Methodology, Investigation, Data curation, Writing – Original
611 draft, Visualisation, Project administration, Funding acquisition

612 **Erika Molnár:** Investigation, Data curation

613 **Zsolt Bereczki:** Investigation, Data curation, Writing

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