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ABSTRACT

93 **Objectives:** Early evidence for the treatment of dental pathology is found primarily among food-
94 producing societies associated with high levels of oral pathology. However, some Late Pleistocene
95 hunter-gatherers show extensive oral pathology, suggesting that experimentation with therapeutic
96 dental interventions may have greater antiquity. Here we report the second earliest probable evidence
97 for dentistry in a Late Upper Paleolithic hunter-gatherer recovered from Riparo Fredian (Tuscany,
98 Italy).

99 **Materials and Methods:** The Fredian 5 human consists of an associated maxillary anterior dentition
100 with antemortem exposure of both upper first incisor (I¹) pulp chambers. The pulp chambers present
101 probable antemortem modifications that warrant in-depth analyses and direct dating. Scanning
102 electron microscopy (SEM), microCT and residue analyses were used to investigate the purported
103 modifications of external and internal surfaces of each I¹.

104 **Results:** The direct date places Fredian 5 between 13,000-12,740 calendar years ago. Both pulp
105 chambers were circumferentially enlarged prior to the death of this individual. Occlusal dentine
106 flaking on the margin of the cavities and striations on their internal aspects suggest anthropic
107 manipulation. Residue analyses revealed a conglomerate of bitumen, vegetal fibers, and probable
108 hairs adherent to the internal walls of the cavities.

109 **Discussion:** The results are consistent with tool-assisted manipulation to remove necrotic or infected
110 pulp *in vivo* and the subsequent use of a composite, organic filling. Fredian 5 confirms the practice of
111 dentistry – specifically, a pathology-induced intervention – among Late Pleistocene hunter-gatherers.
112 As such, it appears that fundamental perceptions of biomedical knowledge and practice were in place
113 long before the socioeconomic changes associated with the transition to food production in the
114 Neolithic.

115

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117 To date, the earliest examples of definitive prehistoric dentistry come from Neolithic
118 contexts. A Neolithic graveyard (MR3) at Mehrgarh in Pakistan contained 11 drilled teeth, belonging
119 to nine individuals, of which at least four of the teeth had associated decay (Coppa et al., 2006). It is

120 not possible determinate whether the lack of decay in the remaining seven teeth was due to successful
121 removal of infected dental tissue. An individual from a Danish Neolithic passage grave at Hulbjerg
122 exhibits drilling near the bifurcation of the right M² roots (Bennike and Alexandersen, 2003; Bennike
123 and Fredebo, 1986). The individual also exhibits periodontal disease and caries suggesting that
124 drilling was related to pathological intervention (Bennike and Alexandersen, 2003). A final example
125 of an early dental intervention concerns a ‘beeswax’ filling from Neolithic Slovenia, which was
126 probably used to seal an antemortem/perimortem crown fracture for palliative purposes (Bernardini
127 et al., 2012). While many more chronologically-recent cases of pathology-induced dental
128 interventions are well-documented among both food-producers and hunter-gatherers from Old and
129 New World contexts (Bennike and Alexandersen, 2003; Ortiz et al., 2016, Schwartz et al., 1995;
130 Seidel et al., 2005; Turner, 2004, White et al., 1997), there is little evidence for similar pathological
131 interventions preceding the Neolithic.

132 An exception is a Late Upper Paleolithic specimen from Villabruna (Sovramonte – Belluno,
133 Italy, directly dated to 14,160-13,820 calendar years ago [cal BP]) (Vercellotti et al., 2008). The
134 Villabruna 1 individual exhibits caries on the right M₃ that was clearly manipulated with a lithic tool
135 *in vivo* in an effort to partially clean decay through scraping and levering actions (Oxilia et al., 2015).
136 However, the location of the caries in the distal-most portion of the mouth would have made it very
137 difficult to fully clean and may explain why this manipulation was less extensive than many of the
138 more obvious drilling interventions in later prehistoric and historic examples (e.g. Bennike and
139 Alexandersen, 2003; Coppa et al., 2006; Ortiz et al., 2016; Schwartz et al., 1995; Seidel et al., 2005;
140 Turner, 2004; White et al., 1997).

141 Other evidence for the palliative treatment of inflamed gingiva among Pleistocene hunter-
142 gatherers derives from interproximal grooves caused by dental probing or “toothpicking” (Lozano et
143 al., 2013; Ungar et al., 2001). However, these features are also documented throughout the Holocene
144 and are not always clearly associated with pathology (Brown and Molnar, 1990, Lukacs and Pastor,
145 1988; Molnar, 2008; Molnar, 1971). By contrast, for Late Upper Paleolithic tooth extractions (i.e.,
146 avulsion or ablation) that were likely related to cultural modification of the dentition as an expression

147 of social identities (Bocquentin, 2011; De Groote and Humphrey, 2016; Humphrey and Bocaege,
148 2008; Stojanowski et al., 2014; Willman et al., 2016). While not related to the treatment of
149 pathology, ablation does offer evidence of invasive dental modifications in Late Upper Paleolithic
150 contexts. Thus, toothpicking, caries manipulation, and ablation among Late Pleistocene hunter-
151 gatherers experiencing high rates of dentognathic pathology (e.g., Capasso, 2011; Frayer, 1989;
152 Humphrey et al., 2014; Lacy, 2014, 2015; Willman et al., 2016), suggest that the prerequisite stimuli
153 (i.e., pathological affliction) and cultural practices for developing early dentistry practices may have
154 much greater antiquity than currently documented.

155 Here we analyze two upper central incisors from a modern human recovered from the Late
156 Upper Paleolithic site of Riparo Fredian (Molazzana, Lucca, Italy) (Boschian et al., 1995). Both I¹s
157 exhibit antemortem modification to their pulp chambers in the form of striations and the presence of
158 a composite material (bitumen and organic fibers) on the walls of the pulp cavities (Fig. 1). We
159 provide a differential diagnosis for these features, and suggest that the modifications are intentional
160 anthropogenic by-products of a pathology-induced therapeutic dental intervention.

161

162 **[Figure 1 here].**

163

164

Archaeological context

165 The Riparo Fredian is a mountainous area in northern Tuscany situated between the Alpi
166 Apuane ridge to the west and the Apennines to the east. The site is located within the valley of the
167 Turrite Secca River (in the territory of Molazzana, near Lucca), a tributary of the Serchio River (Fig.
168 2).

169

170 **[Figure 2 here].**

171

172 Thorough archaeological surveys carried out within the area brought to light several
173 prehistoric settlements ascribed to the Late Upper Paleolithic (Late Epigravettian) and Mesolithic

174 (Sauveterrian and Castelnovian) (Biagi et al., 1981; Guidi, 1989; Tozzi, 1995). The results reveal that
175 the area was completely abandoned during the Late Alpine Glacial, when the glacial fronts expanded
176 downward to an elevation of about 700-800 m. The first groups re-entered the area during the Late
177 Glacial Interstadial, and occupied sites at the bottom or on the lower sides of the valleys, whereas
178 sites at higher elevation were not colonized until the Early Holocene. Riparo Fredian was found
179 during these surveys, and systematic excavations were carried out from 1987 to 1990. It is situated on
180 a river terrace about 2-3 m above the bottom of the valley, at about 360 m above sea level, and
181 includes a habitation area of a few square meters.

182 The stratigraphic sequence (Fig. 2) is rather thin (1.60 m). The bottom of the sequence
183 includes sandy river deposits (layers 8, 7 and 6), overlain by an archaeological sequence that includes
184 Late Epigravettian (layer 5) and Mesolithic (layers 4 and 3) lithic industries. The sequence is
185 terminated by thin lenses (layers 2 and 1) containing a few minute fragments of coarse pottery
186 (Boschian et al., 1995). A cobble pavement of limited size was found at the top of layer 5 in the
187 innermost area of the rock shelter (Fig. 3). This pavement included several large river cobbles that
188 were irregularly distributed on a surface of about 2 m² and slightly protruded upwards into layer 4.
189 Layer 4 also overlies layer 5 in the other areas of the shelter, where the two layers are in direct
190 contact, and lack the cobble pavement. Most of the teeth found in the outer part of the cobble
191 pavement were included in layer 4, whereas those found in the inside of the pavement were included
192 mostly in layer 5. The following processes explaining the stratigraphic position of the human remains
193 can be reconstructed by observing the architecture of the stratigraphic unit and the characteristics of
194 the sediments.

195

196 **[Figure 3 here].**

197

198 An erosion process, subsequent to the formation of layer 5 but preceding the deposition of
199 layer 4, eroded layer 5 on the outer side of the shelter and excavated a shallow trough. The cobbles of
200 the outer part of the pavement slid into the trough and rotated towards the outside of the shelter and

201 were found leaning slightly outwards. Sediments of layer 5, reworked by the erosion, accumulated
202 into the trough together with the cobbles and formed the foundation for the outer part of layer 4. This
203 process operated less intensely inside the rockshelter and reworked only the topmost part of layer 5,
204 leaving the cobbles *in situ* and originating the inner part of layer 4, which is much thinner than the
205 outer one. Consequently, layer 4 is thicker in the outer area of the shelter, whereas layer 5 is thicker
206 in the inside area. As a result, layer 4 is largely composed of reworked parts of layer 5. Thus, it
207 appears that the teeth were all originally embedded in layer 5, but those within the outer part of the
208 cobble pavement were incorporated within layer 4 after reworking; conversely, those found in the
209 inner part, where reworking was limited, remained *in situ* and hence were mostly associated with
210 layer 5. Layer 5 was ¹⁴C AMS dated on charcoal to 10,870±119 BP (AA10952, 13040 - 12600 cal BP
211 for ±2σ calibrated range), and layer 4 to 9,458±91 BP (AA10951, 11106 - 10500 cal BP for ±2σ
212 calibrated range).

213 The human remains from Riparo Fredian mostly consist of isolated teeth and these teeth have
214 been attributed to six individuals (three subadults, three adults) based on dental anatomical features
215 and levels of macroscopic wear (Boschian et al., 1995; Vierin, 2012). All of the teeth attributed to
216 individual Fredian 5 (Fig. 4) were recovered from layer 5 next to an cobblestone artificially placed at
217 its top (Boschian et al., 1995), which is attributed to the Final Epigravettian and dated by ¹⁴C on
218 charcoal between 13,040-12,600 cal BP (Boschian et al., 1995; D'Errico et al., 2011).

219

220 **[Figure 4 here].**

221

222 MATERIALS AND METHODS

223

224

The dental remains of Fredian 5.

225 Teeth 133 and 161 are right and left maxillary canines (C¹s), respectively. The occlusal cross-
226 sections are asymmetrically oval, broad anteriorly, tapered distally, and the roots are long. Both C¹s
227 have wear scores of 7 (Smith, 1984), but wear is slightly more advanced on the left C¹. Teeth 31 and
228 134, the subjects of the present study, are right and left maxillary central incisors (I¹s), respectively.

229 Siding is based primarily on the distolateral projection of the root apices. The right I¹ preserves a
230 hairline rim of enamel on its anterior face (stage 7: Smith, 1984). The left I¹ is more circular in cross-
231 section due to its greater degree of occlusal wear (stage 8: Smith, 1984). Both I¹ roots are
232 mediolaterally and anteroposteriorly broad, a characteristic of maxillary central incisors that
233 distinguishes them from the heavily worn C¹s and the maxillary second incisors (I²s). Teeth 5 and 21
234 have been identified as right and left I²s, respectively. The occlusal cross-sections are relatively
235 round (compared to the canines and central incisors) and small in size. Siding is based primarily on
236 wear associations between adjacent teeth. Each left tooth (134, 21, and 161) has a total length (root
237 apex to occlusal surface) that is several millimetres less than that of their right-side anteriors.

238 Further evidence for tooth siding is provided through wear pattern associations. For instance,
239 there is continuity in the wear planes and edge-rounding by side, which suggest that the behaviors
240 resulting in wear differed between right and left sides of the mouth. The differential wear suggests
241 that the left-side anterior teeth were used more extensively for masticatory and paramasticatory
242 behaviors given that compensatory hypereruption would have kept the teeth in the same occlusal
243 plane as the right-side anterior teeth despite progressive occlusal wear. However, the cause of
244 differential wear is not immediately apparent. One possibility is that the anterior dental wear
245 asymmetries may relate to the handedness of Fredian 5 during masticatory and non-masticatory
246 behaviors. Another possibility is related to the timing of pulp exposure, infection, and subsequent
247 antemortem modification of the pulp chambers. These explanations need not be mutually exclusive
248 but are difficult to disentangle.

249 The subsequent analyses will focus on the pathological nature of the teeth as well as
250 purported antemortem modifications indicative of probable dentistry.

251

252 **MicroCT and digital reconstruction**

253 High-resolution MicroCT images of the two upper central incisors were obtained with a Xalt
254 MicroCT scanner (Panetta et al., 2012). All teeth were scanned at 50 kVp, 2 mm Al filtration, 960
255 projections over 360°, 0.9 mAs/projection for a total scan time of 50 minutes per sample. All the

256 tomographic images were reconstructed using a modified Feldkamp algorithm (Feldkamp et al.,
257 1984) with embedded compensation for mechanical misalignments and raw data pre-correction for
258 beam-hardening and reduction of ring artifacts in the digital images. All images were reconstructed
259 on a volume dataset of 600x600x1000 cubic voxels, each with a size of 18.4 μm . The image stacks
260 were segmented using a semiautomatic threshold-based approach in Avizo 7 (Visualization Sciences
261 Group Inc.) to distinguish between the dental tissues and the residue filling the pulp chamber as well
262 as to reconstruct 3D digital models of the teeth.

263

264 **Scanning electron microscope (SEM) and energy dispersion X-ray spectroscopy (EDS)**

265 Back-scattered electron images and EDS spectra were collected on a low-vacuum ESEM FEI
266 Quanta 200, equipped with an Oxford energy dispersive spectrometer. The analyses were conducted
267 using an acceleration voltage up to 30 kV and EDS analyses performed at a working distance of 10
268 mm for 100 seconds. No sample preparation was required.

269

270 **3D digital microscope**

271 Multifocal images of anthropic cavities (up to 160X) were obtained using a Hirox KH-7700
272 Digital Microscope equipped with MX(G)-5040Z lens and an AD-5040LOWS adapter. Multifocal
273 images of vegetal fibers as well as 3D images of microstriations (up to 7000X) were captured using a
274 MX(G)-10C lens equipped with a OL-140II and OL-700II adapters and an AD-10S Directional
275 Lighting Adapter. Multifocal and 3D images were created by overlapping a series of 120 photographs
276 taken at different focus levels (Crezzini et al., 2014; Moretti et al., 2015). This procedure enables the
277 observation of analyzed surfaces from different points of view, creation of cross-sections of the
278 microstriations, and allows collection of linear, angular, and areal measurements (Boschin and
279 Crezzini, 2012; Crezzini et al., 2014).

280

281 **Fourier-Transformed Infrared Spectroscopy (FTIR)**

282 FTIR spectroscopy was chosen because its sensitivity allows information to be gained from
283 the small amount of material extracted from the teeth, which is otherwise insufficient for
284 chromatographic analyses. Moreover, the advantages of FTIR (i.e., speed, economical and permits
285 sample size) are added to the Attenuated Total Reflection (ATR) mode, which does not require
286 sample preparation because the powdered sample is placed directly on the ATR prism. In this way,
287 the impact preparation in KBr pellet and chemical alterations that may occur with chromatography
288 are avoided (Hollund et al., 2013).

289 Once the incrustation of secondary dentine and matrix adhering at the bottom of the cavity
290 was removed, FTIR-ATR was performed on the black film found inside the pulp cavities of both
291 teeth. The samples were obtained with a scalpel scraping the inner surface subsequent to analysis of
292 surface striae. Samples were also collected from the soil in which the teeth were embedded to control
293 for possible contamination from exogenous materials.

294 FTIR analyses were performed in ATR mode with a Tensor 27 FTIR Spectrometer equipped
295 with a diamond crystal. Spectra were recorded in the range of 4000-400 cm^{-1} at a spectral resolution
296 of 4 cm^{-1} and 128 scans. Data acquisition was carried out using OPUS 7.2 software, the spectra were
297 baseline corrected, the CO_2 was removed and a smooth performed.

298

299

Raman microscopy

300 A small amount of material containing the black patina encrusted on the internal surface of
301 the teeth was investigated by Raman microscopy. The Raman spectra were collected with a Bruker
302 Senterra Microscope interfaced with an Olympus microscope (20x-50x objective lens) fitted with a
303 785nm laser. The analyses were carried out with a 10mW laser power in the 50-2600 cm^{-1} spectral
304 region and a resolution of 3 cm^{-1} .

305

306

Identification of the fibers

307 The samples were stained with the fluorochrome Calcofluor White M2R (Fluorescent
308 Brightener 28, Sigma) that readily binds to cellulose and chitin. A working stock solution of 10 mg

309 ml⁻¹ of Calcofluor white M2R was made in distilled water and then filtered through a 0.22 µm filter.
310 The samples, mounted between slides and glass coverslips in distilled water, were treated with one
311 drop of the Calcofluor solution. After removing the excess water, the presence of lignin was analyzed
312 through acid Phloroglucinol staining (Phloroglucinol Sigma). The samples mounted between slides
313 were treated with the stain (1% in ethanol) and then acidified with a drop of concentrated
314 hydrochloric acid. The stained samples were observed under an inverted epifluorescence microscope
315 Zeiss Axiovert 100, equipped with an UV filter (BP 365, FT 395, LP 397). The microscope was
316 equipped with a Nikon color video camera Digital Sight DS-Fi2 with a DS-U3 control unit for image
317 capture and Nis Elements-3 software was used for image analysis.

318

319 **Radiocarbon dating**

320 *Fourier-Transform Infrared Spectroscopy*

321 Both dentine and enamel from the Fredian 5 canine were analyzed with FTIR analysis to
322 determine the state of preservation. A few dozen micrograms of dentine and enamel were separately
323 powdered and homogenized in an agate mortar and pestle, mixed with a few milligrams of anhydrous
324 KBr (Aldrich), and formed into a pellet. Infrared spectra were obtained at 4 cm⁻¹ resolution Nicolet
325 380 FT-IR in transmission mode. The infrared splitting factors were calculated from the spectra
326 following the method of Weiner and Bar-Yosef (Weiner and Bar-Yosef, 1990). The splitting factor
327 for the enamel and dentine were 4.0 and 3.1, respectively. These values are in the range of well-
328 preserved enamel and dentine (Asscher et al., 2011a,b). The FTIR spectrum of dentine mineral also
329 showed absorption peaks at 1,651 cm⁻¹ (amide I) and 1,556 cm⁻¹ (amide II), indicating the presence
330 of collagen clearly.

331

332 *Dentine Collagen Extraction, Purification and Characterization*

333 Some 193 mg of dentine was dissolved in 1N HCl to remove the mineral phase, centrifuged
334 and rinsed three times in deionized water by centrifugation (6000 rpm for 2 min), and resuspension of
335 the pellet. The pre-treatment procedure (Boaretto et al., 2009) for radiocarbon dating uses the acid-

336 alkali- acid (AAA) technique and filtration, after gelatinization, with Eezi filter and ultrafiltration
337 (Yizhaq et al., 2005). Prior to the AMS (Accelerator Mass Spectrometry) target preparation the
338 extracted collagen was analyzed with FTIR (Asscher et al., 2011a) The spectrum showed the three
339 aminoacid peaks of amide I, II and hydroxyproline at 1650, 1550 and 1450 cm^{-1} , respectively. No
340 other minerals were detected.

341

342 *Target Preparation and AMS Analysis*

343 The extracted collagen sample RTD-8546 was combusted to CO_2 in vacuum sealed quartz
344 tubes containing approximately 200 mg of copper oxide (Merck) and heated to 900°C for 200
345 minutes. The CO_2 was divided into 3 aliquots and then each was reduced to graphite using cobalt
346 (Fluka) (approximately 1mg) as a catalyst and hydrogen, and heated to 700°C for 20 hours. The
347 graphite produced was analyzed for ^{14}C content at the D-REAMS Radiocarbon Laboratory at the
348 Weizmann Institute. Calibrated ranges in calendar years have been obtained from calibration tables
349 (Reimer et al. 2013) by means of OxCal v4.2.4 (Bronk Ramsey and Lee, 2013).

350

351

RESULTS

352

353 Both upper central incisors are heavily worn with occlusal exposure of each pulp chamber
354 (RI^1 : mesio-distal =2.82 mm; labio-lingual=3.08 mm; LI^1 : mesio-distal=2.77 mm; labio-lingual=2.84
355 mm). The pulp chambers show a rounded perforation (hereafter called “cavity”) that appear to be
356 circumferentially (albeit unevenly) enlarged (Fig. 1a, d) and extend into the root for 4.82 mm (RI^1)
357 and 4.25 mm (LI^1), with a sudden transition with the preserved portion of the pulp canal, which is
358 partially filled with organic residue (Fig. 1c, f). Scanning Electron Microscopy (SEM) analysis
359 showed microwear in the form of small scratches on the polished incisal surface and occlusal margins
360 of the cavities (Fig. 5).

361

362 **[Figure 5 here].**

363

364 Additional SEM analysis revealed striations in the internal cavity surface (Fig. 6), which
365 differ from the typical dental microwear pattern, along with two dentine chips on the lingual (RI¹)
366 and labial (LI¹) margins, respectively (Fig. 1a, d). The margins of the chipped dentine exhibit smooth
367 and rounded edges, similar to antemortem enamel chipping (Bonfiglioli et al., 2004; Scott and Winn,
368 2011), which indicates some degree of *in vivo* occlusal wear and tool-use following exposure of the
369 pulp cavity and the chipping of the dentine. Together, the scratches and rounding of the dental chips
370 on the margins of the cavities suggest that Fredian 5 survived initial pulp exposure and continued to
371 use their anterior teeth for daily activities prior to death.

372

373 **[Figure 6 here].**

374

375 The striations on the internal surfaces of the pulp cavities are distinguished from the scratches
376 on the occlusal surface by a difference in orientation and by a distinct morphological appearance.
377 The shape and cross-section of the striations are diagnostic of the instrument used to produce them
378 and the activities involved. Some are “V” shaped in transverse section and have a combination of
379 attributes similar to the recognition criteria of slicing cut marks (Fig. 6d) (morphological categories
380 2, 4 and 5 [Boschin and Crezzini, 2012]) produced by stone tools, while others are shallower with
381 more rounded cross-sections (Fig. 6b, f). The latter resemble those produced during experimental
382 tests in dentine with bone tool (Oxilia et al., 2015).

383 The residue filling the pulp canals was removed and analyzed by SEM and stereomicroscopy.
384 SEM analysis shows the presence of dentinal tubules, suggesting the residue has extensive dentine
385 adhering to it postmortem (Supporting Information Figure S1). Moreover, a number of microscopic
386 materials with a fibrous-like morphology were found; however, only a few could be isolated due to
387 their small dimensions and fragmented state. The fibers were observed using an optical microscope
388 and examined by means of histochemical methods. Two main morphological classes were
389 documented. The Type 1 fiber had a length of 51.56 μm and an irregular width with a mean diameter
390 of 24.4 μm (Fig. 7a). It was flexible with some distinct folds and reacted with the staining specific for

391 cellulose and chitin (Fig. 7b), but not with the one specific for lignin. Due to the size and
392 morphology, this fiber type was more consistent with a plant fiber classification rather than fungi.
393 The Type 2 fiber had a light brown pigmentation, a round morphology with a diameter of
394 approximately 60 μm , and was also flexible but seemingly hollow (Fig. 7c). This fiber did not react
395 with either cellulose (Fig. 7d) or lignin (Fig. 7e) stains. The size, morphology and histochemical
396 results obtained from this fiber suggest it should be classified as hair.

397

398 **[Figure 7 here].**

399

400 Fourier transform infrared spectroscopy (FTIR) analysis was carried out on the black patina
401 adhering to the inner walls of the cavities and on the soil from the deposit from which the teeth were
402 retrieved. First, it was possible to discard external contamination as the soil analyses showed a
403 composition of calcite, silicates and quartz (Supporting Information Figure S2). The FTIR spectra
404 obtained on the black patina are similar in both samples (Fig. 8). The peaks at 1022, 600 and 562 cm^{-1}
405 ¹ (stretching and bending modes of PO_4) are related to hydroxyapatite, due to the contamination of
406 dentine adhering to the black patina. Furthermore, the sharp and strong peaks at 2922 (CH_3 bending
407 bond) and 2850 cm^{-1} (CH_2 bending bond) and the weak peak at 2956 cm^{-1} show the presence of
408 organic matter with strong absorption of aliphatic CH. The lack of a defined peak in the 1750-1650
409 cm^{-1} region suggests the organic material does not have a carbonyl group, thereby excluding the
410 presence of oil, wax, gums, natural resin or proteinaceous material, such as egg or animal glue (Daher
411 et al., 2010; Derrick et al., 1999). According to previous studies (Cârciumaru et al., 2012; Hassan et
412 al., 2013; Lamontagne et al., 2001), the two characteristic peaks at 1472 and 1382 cm^{-1} could indicate
413 the presence of CH_2 and CH_3 bending bonds, respectively. The closest spectral match is with a
414 reference spectrum gained from the IRUG online database (Harvard University Database, 2016) and
415 is ascribable to bitumen.

416

417 **[Figure 8 here].**

418

419 A Raman spectrum was additionally acquired on the internal surface of the pulp cavities to
420 distinguish the characteristic peaks of hydroxyapatite at 962 cm^{-1} (Supporting Information Figure
421 S3). The spectrum of interest on the black patina, instead, shows broad peaks around 1305 and 1595
422 cm^{-1} , which can be associated with amorphous carbon, probably attributable to bitumen.

423 Bitumen is an organic material with a very complex chemistry (Vandenabeele et al., 2007)
424 because it is a mixture mostly of hydrocarbons with a small number of heterocyclic species and
425 functional groups containing sulphur, nitrogen and oxygen. Accordingly, the energy dispersion X-ray
426 spectroscopy (EDS) spectra were acquired on a small grain of material containing the black patina
427 encrusted on the tooth's inner surface. An increasing degree of carbon (C) and the presence of
428 sulphur (S) and nitrogen (N) were found in addition to the elements related to the chemical
429 composition (Ca, P, O) of the teeth (Supporting Information Figure S4). This result can therefore be
430 explained by the presence of sulphur, nitrogen and oxygen in the bitumen composition as
431 heterocyclic atoms (McNally, 2011).

432 A direct radiocarbon date for Fredian 5 was obtained from the dentine of the right canine
433 (RTD 8546) (Supporting Information Figure S5; Supporting Information Table S1). The new
434 radiocarbon date, $11,000\pm 40\text{ }^{14}\text{C}$ year BP is well in the range of the Epigravettian period with a
435 95.4% probability calibrated range of 13,000-12,740 cal BP.

436

437

DISCUSSION

438

439

439 Fredian 5 exhibits occlusal pulp exposure of both I¹s, but this affliction is not an unusual
440 occurrence among Late Upper Paleolithic hunter-gatherers (e.g., Capasso, 2001; Da-Gloria and
441 Larsen, 2014; Lieverse et al., 2007; Lukacs, 1988; Porr and Alt, 2006) that warrants further
442 explanation. However, the internal surface modifications to the pulp cavities, in addition to the
443 presence of bitumen and organic fibers, is an unusual occurrence among Late Upper Paleolithic
444 hunter-gatherers that begs further explanation. Caries manipulation was previously recorded in the
445 penecontemporaneous (Epipaleolithic) individual Villabruna 1 individual (Oxilia et al., 2015),

446 suggesting that the presence of the above features could be the result of similar pathology
447 manipulation in Fredian 5. Thus, we offer a differential diagnosis for the suite of characteristics
448 associated with the pulp cavity modifications documented for Fredian 5. We have identified four
449 possible diagnoses: 1) Postmortem/Taphonomic Modifications; 2) Ingestive Behaviors and Teeth-as-
450 Tools; 3) Cultural Modification for Social Expression; and 4) Therapeutic Dentistry. We explore
451 each diagnosis in detail below and discuss potential overlap between them.

452

453 *1. Postmortem/Taphonomic Modifications*

454 The exposed pulp chambers are undoubtedly antemortem, but the extent to which the
455 markings on the internal surface of the pulp cavities are of antemortem versus postmortem origin
456 must be explored further. Dental drilling tends to produce parallel striations or microgrooves around
457 the circumference of the drilled cavity (e.g., Bennike and Alexandersen, 2003; Coppa et al., 2006;
458 Ortiz et al., 2016; Schwartz et al., 1995; Seidel et al., 2005; Turner, 2004; White et al., 1997). The
459 case of Fredian 5 shows less-intensive markings than documented in chronologically more recent
460 examples of dental drilling and the striations are parallel to the horizontal axis of the tooth. These
461 markings would be consistent with the twisting of a hard implement (e.g., bone or lithic) placed
462 inside the pulp cavities, and are similar to the striations created by the scraping and levering actions
463 during caries manipulation in Villabruna 1 (Oxilia et al., 2015). The same forms of striations are not
464 found on the occlusal external root surfaces of the Fredian 5 anterior teeth. If the markings on the
465 internal surface are the product of postmortem damage caused by cleaning, we would expect to see
466 similar marking on the occlusal surfaces, but there are no such markings. Therefore, we find it
467 difficult to explain how a postmortem process could preferentially leave marking on internal surfaces
468 while leaving the external surfaces unmarked.

469 Moreover, bitumen is known to have been used as hafting compound by Pleistocene foragers
470 from Middle and Upper Paleolithic contexts (Boëda et al. 1996, 2008; Cârciumaru et al., 2012).
471 Bitumen, along with other hafting materials (e.g., pitch and resin), have been documented in museum
472 collections derived from decades old excavations (Cârciumaru et al., 2012; Dinnis et al., 2009),

473 which attests to the possibility of long-term preservation of such residues following excavation,
474 repeated handling, and curation. It is difficult to explain how a postmortem processes that would
475 cause an organic substance such as bitumen to be preferentially deposited (and preserved) only inside
476 the two pulp cavities, but be absent on the external surfaces of the teeth and surrounding
477 archaeological matrix from the site (see Results). Consequently, we view a scenario in which
478 postmortem, taphonomic processes caused the modifications to the Fredian 5 pulp cavities as
479 unlikely.

480

481 ***2. Ingestive Behaviors and Teeth-as-Tools***

482 The exposure of pulp chambers through attrition in hunter-gatherers is not uncommon, for it
483 is often found among foragers with extensive anterior tooth wear caused by a combination of
484 ingestive food processing behaviors and non-masticatory uses of the “teeth-as-tools”. In the case of
485 Fredian 5 it is evident from the presence of fine occlusal striations and rounding of the dentin chips
486 around the exposed pulp cavities that the IIs continued to be used after pulp exposure for ingestive
487 and/or non-masticatory behaviors.

488 Given the presence of occlusal wear following antemortem pulp exposure there is a possibility
489 that the striations inside the pulp cavities could have been caused by continued anterior tooth-use. For
490 instance, some hunter-gatherers retouch the working-edge of lithic implements with their anterior
491 teeth (Gould, 1968), a process that could introduce microflakes into exposed pulp cavities. Grit, bone
492 fragments, and other abrasive materials from food or various materials worked between the anterior
493 teeth (e.g., wood, hide, plant and animal fibers) could also have entered the exposed pulp cavities of
494 Fredian 5 unintentionally. With this scenario, the foreign materials or debris entering the pulp
495 cavities would had to have moved along a horizontal plane to produce the striations documented in
496 the Fredian pulp cavities, but such movements are unlikely to be produced by the vertical motions
497 and compressive forces of the teeth and jaws during ingestive and/or non-masticatory behaviors.
498 Rather, such striations are more likely to have been induced by movements that involved twisting and
499 scraping an implement along a horizontal axis within the pulp cavity. A lack of dietary microwear

500 within the cavities also suggests that mastication was unlikely to have contributed greatly to the
501 expansion of the cavities. The most parsimonious explanation is that Fredian 5, perhaps with the
502 assistance of another individual, intentionally manipulated an object that produced horizontal
503 striations on the internal walls of the cavity.

504 Support for this interpretation comes from Villabruna 1, which also lacked dietary microwear
505 deep within the manipulated caries but does present distinctive, tool-induced striations within the
506 margins of the caries (Oxilia et al., 2015). Furthermore, experiments show that striations similar to
507 those in the present (in shape, cross-section, and orientation [horizontal]) are produced through
508 levering and twisting actions (Oxilia, et al., 2015 SOM).

509 If Fredian 5 used their anterior dentition to manipulate implements covered in bitumen (e.g.,
510 items waterproofed with bitumen or hafted objects), then it is also likely that the occlusal surfaces of
511 the I¹s would be more extensively impregnated with bitumen. Instead, only the edges of the exposed
512 pulp cavities, the internal surfaces, and the deep recesses within the pulp canal are infilled with
513 bitumen. Furthermore, there are no traces of bitumen on the occlusal surfaces of the other four
514 anterior teeth of Fredian 5 despite their similar states of wear. If the bitumen in the pulp cavities
515 entered unintentionally, we expect that traces of bitumen on the occlusal surfaces to be present on all
516 six anterior teeth, not just within the pulp cavities of the I¹s. The majority of the residue is found deep
517 in the pulp canal rather than distributed throughout the entire pulp chamber/cavity, and it is notable
518 that no bitumen is found embedded recesses of the antemortem enamel and dentin chips on either
519 tooth. We expect that the accumulation of residue through unintentional causes would not limit the
520 majority of bitumen accumulation to the pulp canals, and that the occlusal recesses caused by
521 chipping would be more likely to retain remnants of bitumen even after continued dietary and non-
522 masticatory tooth-use. Given neither circumstance is recorded in Fredian 5, we find the presence,
523 location, and preservation of bitumen in the pulp canals difficult to explain without invoking explicit
524 anthropogenic intentions.

525 The orientation of the striations inside the pulp cavities suggests intentional movements of an
526 extraneous implement, while the presence of bitumen inside the pulp cavities, but no other surfaces,

527 also suggest intentional placement of the bitumen. However, it is much more difficult to rule out an
528 unintentional origin of the vegetal and hair fibers in the pulp cavities. These materials could have
529 been unintentionally adherent to the bitumen when it was placed in the cavities, regardless of whether
530 bitumen was entered through dietary or non-dietary behaviors, or intentionally placed inside the
531 cavities. Given the degraded characteristics of the fibers, and the low number recovered, we cannot
532 rule out their presence as an unintentional result of dietary and/or non-masticatory behavior.
533 Therefore, we suggest intentional behaviors produced the internal pulp cavity striations and presence
534 of bitumen, but we cannot determine intentionality for the presence of organic fibers in the pulp
535 cavities definitively.

536

537 ***3. Cultural Modification for Social Expression***

538 Regional traditions of intentional dental modification for purposes of cultural expression of
539 social identities are well documented in the Late Upper Paleolithic and are best represented by the
540 practice of dental ablation throughout North Africa, Southwest and Southeast Asia, and Australia
541 during the Late Pleistocene (see review in Willman et al., 2016). However, ablation generally leaves
542 large gaps in the dental arcade due to the tooth removal that disrupt for patterns of occlusion and
543 dental wear (Humphrey and Bocaage, 2008). Occlusal wear is relatively even and extensive across all
544 six of the maxillary anterior teeth of Fredian 5, which suggests that the individual's mandibular
545 isomeres were present (i.e., not ablated) and in occlusion. Using the same logic, we can rule out
546 ablation through "tooth-knocking" (i.e., breaking the crown off at the cervix: Pietruszewsky and
547 Douglas, 1993), and add that there are no signs of root resorption (Fig. 4) typical of traumatic
548 fracture (Lukacs, 2007).

549 The filing of anterior dental crowns into specific shapes to express aspects of social identity is
550 well-documented from prehistory into the ethnographic present (Alt and Pichler, 1998; Fastlicht,
551 1976; Milner and Larsen, 1991; Stojanowski et al., 2016; Tiesler, 2011), and provides an alternative
552 for the dental modification found in Fredian 5. However, to date there is only one case of abrasive
553 wear from a Late Upper Paleolithic context that resembles filing (Bocquentin et al., 2013). The case

554 concerns flattened and polished labial enamel on the upper central incisors of an Early Natufian
555 individual from Jordan, but the wear cannot be definitively attributed to the use of teeth-as-tools or an
556 intentional marker of social identity (Bocquentin et al., 2013). Filing generally involves shaping of
557 the crown without removal of the entire crown (e.g., Alt and Pichler, 1998; Fastlicht, 1976; Milner
558 and Larsen, 1991; Stojanowski et al., 2016; Tiesler, 2011), which is inconsistent with the complete
559 loss of crowns in Fredian 5.

560 A last possibility for cultural modification of social expression would be that the pulp
561 chambers were modified, drilled, or otherwise expanded for the inclusion of a foreign object (e.g.,
562 inlays), although this is unlikely for a number of reasons. First, inlays are generally associated with
563 drilling into the labial surfaces of teeth to prepare for the placement of decorative inlays as this would
564 be readily visible (Alt and Pichler, 1998; Fastlicht, 1976; Milner and Larsen, 1991; Tiesler, 2011),
565 and there are no documented cases of decorative inlays being placed in modified pulp
566 chambers/cavities in the archaeological or ethnohistorical literature to our knowledge. Second, while
567 drilling to prepare inlays shares technological attributes with the drilling procedures used for
568 therapeutic purposes (Bennike and Alexandersen, 2003; Coppa et al., 2006; Ortiz et al., 2016;
569 Schwartz et al., 1995; Seidel et al., 2005; Turner, 2004; White et al., 1997), there is no evidence for
570 this form of extensive drilling prior to the Neolithic (Coppa et al., 2006). Moreover, an exposed and
571 modified pulp cavity would have been sensitive to non-therapeutic inclusions such as inlays or other
572 decorative objects, when subjected to any compressive forces during masticatory and/or non-
573 masticatory behaviors. Lastly, the presence of occlusal wear and rounded edges of dentine chips
574 provides evidence for the continued use of the well-worn roots as a functional occlusal surfaces
575 before death. If foreign objects were placed in the pulp cavities for cultural/aesthetic purposes one
576 would not expect to see microwear related to normal tooth-use on the occlusal surfaces. We therefore
577 suggest that an antemortem/cultural expression scenario is unlikely to explain the modifications to
578 the I¹ teeth of Fredian 5.

579

580 ***4. Therapeutic Dentistry***

581 A final possibility for the presence of a suite of antemortem modifications of the I¹ pulp
582 cavities of Fredian 5 dentition may be through a therapeutic dental intervention. Pulp exposure is
583 commonly associated with severe anterior dental attrition among foragers (Da-Gloria and Larsen,
584 2014; Lieverse et al., 2007; Lukacs, 1988; Porr and Alt, 2006), and high rates of oral pathology have
585 recently become well documented among Terminal Pleistocene foragers (e.g., Capasso, 2001; Frayer,
586 1989; Humphrey et al., 2014; Lacy, 2014, 2015; Willman et al., 2016). These rates of pathology
587 suggest a precedent for exploring pathology-induced dental interventions was present among Late
588 Upper Paleolithic foraging groups. Similarly, the recent documentation of dental manipulation
589 associated with pathology (caries) in the Late Upper Paleolithic Villabruna 1 fossil (Oxilia et al.,
590 2015) suggests that other early cases of dental intervention may yet be waiting to be documented.

591 Villabruna 1 exhibits striations consistent with scraping, levering, and probing an occlusal
592 surface caries on a mandibular third molar – remnants of behaviors that partially removed the caries
593 (Oxilia et al., 2015). Fredian 5 exhibits a degree of intentional modification that is similar to that of
594 Villabruna I. The Fredian 5 pulp cavities exhibit horizontal striations produced by scraping and
595 twisting actions of a sharp, hand-held implement that resulted in circumferential enlargement of the
596 cavities (in comparison to recent dental drilling interventions). The similarities between the striations
597 in the two specimens suggests intentional, manually-implemented, behaviors rather than
598 unintentional byproducts of tooth-using behaviors, aesthetic modifications, or taphonomy.

599 Crediting a motive to the intentional dental modifications in Fredian 5 is made more difficult
600 by the considerable differences in the form of the modifications compared to other documented cases
601 from the Holocene – namely those that involved drilling for probable therapeutic purposes (Bennike
602 and Alexandersen, 2003; Coppa et al., 2006; Ortiz et al., 2016; Schwartz et al., 1995; Seidel et al.,
603 2005; Turner, 2004; White et al., 1997). The use of levering and scraping in Villabruna 1, rather than
604 drilling, can be explained by the distal position of the carious lesion in the oral cavity (of the right
605 M₃). This is noteworthy because there are no documented cases of third molar dental drilling in more
606 recent contexts (Bennike and Alexandersen, 2003; Coppa et al., 2006; Ortiz et al., 2016; Schwartz et
607 al., 1995; Seidel et al., 2005; Turner, 2004; White et al., 1997).

608 In contrast to Villabruna 1, access to the I¹s of Fredian 5 would not preclude a more invasive
609 drilling intervention like those found in many Holocene context. Nevertheless, the subtle horizontal
610 striations and circumferential enlargement of the cavities do show clear evidence intentional
611 manipulation. However, additional concentric striations may be obscured by remnants of bitumen.
612 Indeed, no bitumen was associated with the Villabruna 1 caries and the striations associated with
613 caries manipulated are much clearer (Oxilia et al. 2015).

614 Numerous other explanations could account for the subtle nature of the horizontal striations in
615 the Fredian 5 pulp cavities (e.g., some striations were erased through later abrasive wear – from
616 removing and reapplying an organic filling, or from food and other debris entering the cavity
617 following the initial use of bitumen). Although it is also probable that the intervention was simple
618 less-invasive than those documented from more recent contexts.

619 The subtle markings from Fredian 5 (and to some extent, Villabruna 1) are infrequently
620 documented compared to the obvious drill-induced modifications from the Holocene, but this
621 infrequency may be biased due to the ease of identification in the latter cases. Indeed, the subtle
622 modifications to the pulp chambers of Fredian 5 and caries manipulation of Villabruna 1 were
623 difficult to observe macroscopically, and required extensive microscopic, microCT, and residue
624 analyses to completely characterize. Consequently, the subtle manipulation of pathologies in the two
625 cases from the Italian Epigravettian suggest that Holocene case studies of purposeful drilling should
626 not be used as baseline characteristics for all pathology-induced dental interventions. It is probable
627 that additional cases have gone undocumented given no reference for identifying the subtle
628 modifications of Fredian 5 and Villabruna 1 existed until recently.

629 The presence of bitumen in the pulp cavities of Fredian 5 is an additional unique finding that
630 is most likely explained by a therapeutic diagnosis. The lack of bitumen on any surface other than the
631 inside of the pulp cavities is suggestive of intentional placement. Uses of bitumen are not unknown in
632 the Paleolithic (Boëda et al. 1996, 2008; Cârciumaru et al., 2012), but have not been documented on
633 dental surfaces prior to this study. However, residue and microfossil studies of dental surfaces are
634 relatively recent innovations in paleoanthropology and unique discoveries have been made in most

635 studies to date (Hardy et al., 2012, 2016; Henry et al., 2011; Radini et al., 2016). The presence of
636 bitumen (and horizontal striations) inside the pulp cavity but not on other surfaces of the teeth
637 suggests intentionality in their placement in the cavities. Therefore, the bitumen and pathological
638 exposure of the pulp chambers through attrition may likely have been therapeutic.

639 While it is speculative in the present study, the use of bitumen could have been used as an
640 antiseptic or to provide an anti-microbial barrier between the body and the environment (Bourée et
641 al., 2011; Connan, 1999). A similar suggestion has been made for a Neolithic beeswax filling
642 (Bernardini et al., 2013). Furthermore, the presence of hair and plant fibers could indicate the use of a
643 composite filling material, but there is no way to be certain that the hair and vegetal fibers were
644 purposefully placed in the cavities like the bitumen likely was. However, the probable use of
645 medicinal plants is not without precedence in the Pleistocene (Hardy et al., 2012) and early Holocene
646 (Aveling and Heron, 1999). There is also ample ethnographic documentation of plants used for the
647 treatment of toothaches, caries, pulpitis, and other ailments (Buckley et al., 2014; Elvin-Lewis, 1982,
648 1986; Moerman, 1998; Willey and Hofmann, 1994).

649

650

CONCLUSIONS

651

652 Given the evidence for probable dentistry in Fredian 5 it is now possible to suggest that the
653 caries manipulation found in Villabruna (Oxilia et al., 2015) may be part of a broader trend, or
654 tradition, of pathology-induced dental interventions among Late Upper Paleolithic Italian foragers.
655 Both Fredian 5 and Villabruna 1 represent cases where implements were used to manipulate dental
656 pathologies. The Late Pleistocene is a period of increasingly diverse and broad spectrum
657 socioeconomic activities. The concomitant increase in dentognathic pathology likely called for novel
658 strategies to cope with changing morbidity profiles. Thus, this discovery marks a much earlier
659 instance of pathology-induced therapeutic dental interventions than previously known.

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661

662

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663
664
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673
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675

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