

1 **Title:**

2 Dental cementum virtual histology of Neanderthal teeth from Krapina (Croatia, 130-120 ky): an informed estimate
3 of age, sex, and adult stressors.
4

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28

29 **Abstract:**

30

31 The evolution of modern human reproductive scheduling is an aspect of our life history that remains vastly
32 uncomprehended. The present work aims to address this gap by validating a non-destructive cutting-edge
33 methodology to infer adult life history events on modern teeth with known life history and then applying it to fossil
34 specimens. We use phase-contrast synchrotron X-ray microtomography to visualize the dental cementum of 21
35 specimens: nine contemporary humans; ten Neanderthals from Krapina (Croatia, 130-120 ky); one Neolithic *H.*
36 *sapiens* from Ajmana (Serbia); and one Mesolithic *H. sapiens* from Vlasac (Serbia). We were able to correctly detect
37 and time (root mean square error = 2.1 years; $R^2=0.98$) all reproductive (menarche, parturition, menopause) and
38 other physiologically impactful events in the modern sample. Nonetheless, we could not distinguish between the
39 causes of the events detected. For the fossil specimens we estimated age at death and age at occurrence of
40 biologically significant events. Finally, we performed an exploratory analysis regarding possible sexual dimorphism
41 in dental cementum microstructure, which allowed us to correctly infer the sex of the Neolithic specimen, for
42 which the true value was known via DNA analysis.

43

45 The factors that have shaped *Homo sapiens'* life history are largely still to be understood. Given our significant
46 reliance on material culture and on (pro)social behavior for the acquisition of energy, care of offspring and many
47 other correlated aspects, it is likely that our life history, cultural and social evolution are intertwined (Kramer &
48 Otárola-Castillo, 2015). Life history variables (LHVs) are those variables that directly record the timing of the
49 several major life history events, such as gestation length, age at weaning, age at sexual maturity, age at first birth,
50 interbirth intervals, age at menopause and longevity. Nonetheless, tracking the evolution of LHVs in the fossil
51 record has proven to be a significant challenge as it is limited to what can be gleaned and inferred from teeth and
52 bones (Robson & Wood, 2008; Schwartz, 2012). The current knowledge regarding LHVs in the hominin fossil record
53 is very scant, being limited to estimates of age at death (Bromage & Dean, 1985), gestation length, onset of
54 weaning (e.g. Nava et al., 2020), and physiological stress occurrence (e.g. Smith et al., 2018). The present work
55 aims to improve and enrich our understanding of some of the more elusive LHVs using a non-destructive cutting-
56 edge methodology.

57 Human teeth permanently record physiologically impactful events during their formation, enabling the collection
58 of data about stressors experienced during development in addition to the estimation of the age at death (Hogg,
59 2018). In *Recording structures of mammals*, Klevezal (1995) thoroughly describes the different types of growth
60 layers pertaining to the mineralized tissues of bone, dental enamel, dental cementum, horn and other mammalian
61 mineralized tissues. All recording structures are periodically layered, and each layer is the visible result of changing
62 micromorphology which corresponds to changing physiological states of the organism (Bromage et al., 2016).
63 These structures are extremely conserved across mammalian taxa, from cetaceans to cervids to primates.
64 Recording structures can be categorized according to three parameters (Klevezal, 1995): sensitivity, which relates
65 to the frequency with which new layers are added and which can vary across period of registration (Nava et al.,
66 2019); period of registration (when does the structure start and stop recording), and persistence of a record (for
67 how long the recording structure is maintained unchanged) (Table 1).

68 Of the mineralized tissues present in primates, only cementum retains both the lifetime period of registration and
69 lifetime persistence of the record, even if with a low sensitivity due to its yearly periodicity and low growth rate
70 (Bosshardt & Selvig, 1997). Physiological events that are strong enough to produce a disruption of organismal
71 mineral homeostasis or organic matrix synthesis leave marks in the corresponding position of the developing
72 cementum, visible as changes in tissue refractive index (Cerrito et al., 2020; Cipriano, 2002; Kagerer & Grupe,
73 2001; Lieberman, 1993). Previous studies already ascertained the possibility to use phase-contrast synchrotron X-
74 ray microtomography (PC-SR μ CT) for a non-destructive three-dimensional (3D) visualization of yearly incremental
75 cementum bands in both modern human and fossil animal teeth (Le Cabec et al., 2019; Mani-Caplazi et al., 2017;
76 Newham et al., 2020a; Newham et al., 2020b), which provide the basis for age at death estimation. Furthermore,

77 recent work in classic histology (Cerrito et al., 2020; 2021) has provided evidence, in two distantly related species
78 of primates (*H. sapiens* and *M. mulatta*), of changes in tissue microstructure in relation to pregnancies, cessation
79 of fertility and illnesses. Fertility data derived from human teeth are scarce, constituting an almost unexplored
80 area of research, particularly when dealing with hominin fossil teeth. The combination of age at death estimation
81 provided by the yearly periodicity and the markers associated with physiological stressors can be used to
82 determine their chronology, thus attempting a first informed estimate in fossil remains regarding the timing of
83 possible parturitions (Penezić et al., 2020). Thus far, studies attempting to investigate them (Cerrito et al., 2020;
84 Dean & Elamin, 2014; Penezić et al., 2020) have relied on destructive methods which are substantially prevented in
85 paleoanthropological specimens for obvious preservation reasons. To date, non-destructive virtual histology
86 studies based on the use of PC-SR μ CT techniques are increasing in number (Le Cabec et al., 2017; Mahoney et al.,
87 2021; Nava et al., 2017; Tafforeau et al., 2012) but remain scarce in the exploration of dental cementum and non-
88 existent in the investigation of fossil hominin cementum.

89 Here we report for the first time the application of this approach on the cementum of Neanderthal teeth,
90 specifically from Krapina (Croatia, 130-120 ky (Rink et al., 1995)). Using synchrotron-based high-resolution 3D
91 imaging we acquired data on ten Neanderthal specimens, in addition to one Mesolithic, one Neolithic (both from
92 the Danube Gorges area of Serbia) and nine known life history contemporary humans. In addition to estimating the
93 age at death following previously published methods (Le Cabec et al., 2019; Newham et al., 2020a) we attempt a
94 first informed estimate of sex and reproductive events and/or other types of stressors for the fossil specimens.

95 **1. Methods**

96 **1.1 Sample**

97 A total of 21 permanent teeth (each with an approximate size of 1.5cm x 0.8cm x 0.8cm) were selected for this
98 study (Table 2). All contemporary *H. sapiens* specimens were accompanied with age, sex, medical and life history
99 information. They were acquired by GR and were donated by patients who had them extracted for medical
100 purposes. All patients signed an informed consent. Specimens were fully anonymized by GR and there is no
101 possibility to trace back any identifying information about the participants. The research was carried out under the
102 auspices of the College of Medicine Research and Ethics Committee (COMREC) Protocol Number: P.05/06/373 of
103 the NYU College of Dentistry. All methods were carried out in accordance with relevant guidelines and regulations
104 and all experimental protocols were approved by the NYU College of Dentistry. The Late Mesolithic (Burial H326/7,
105 ca. 50 years old, Vlasac, Serbia: Borić et al., 2014) and Early Neolithic (Burial 6/A2309, Ajmana, Serbia: Borić &
106 Price, 2013; Radosavljević-Krunic, 1986) specimens were known to be females through previously performed DNA
107 analysis (Mathieson et al., 2018). The Neanderthal specimens from Krapina had previously been studied by
108 Wolpoff (1979) who estimated their ages at death based on dental wear. Contemporary samples were chosen so
109 that the following categories were represented: younger and older males, nulliparous and multiparous females,

110 reproductive and post-reproductive females. Neanderthal teeth were chosen based on the dental wear, and the
111 Krapina Dental Person (KDP) assignment made by Wolpoff (1979). We selected the most worn teeth, presumably
112 belonging to older individuals and we chose more than one tooth from the same KDP. Macroscopically, all
113 specimens showed good cementum preservation.

114 1.2 Image acquisition and processing

115 The permanent tooth roots were measured via SR μ CT at the SYRMEP beamline of the Elettra synchrotron facility
116 (Basovizza, Trieste, Italy) in August 2020. The measurements were performed in propagation-based phase-contrast
117 mode using a filtered white X-ray beam (filter = 1.5 mm Si + 1.0 mm Al) with a mean energy of 27 keV. After the
118 alignment of the μ CT set-up, a series of scout scans was first acquired on one of the samples in order to optimize
119 the experimental conditions to be used for the high-resolution measurements. For each tomographic scan 1800
120 sample radiographs were recorded during continuous rotation over a total scan angle of 180°. The sample-to-
121 detector distance was set at 150 mm. Samples were imaged with a water-cooled, 16-bit sCMOS macroscope
122 camera whose effective pixel size was set at $0.9 \times 0.9 \mu\text{m}^2$ yielding a maximum field of view of about 1.85×1.85
123 mm^2 . The detector system is equipped with a motorized quadrupole hosting 4 different scintillator screens. A 17
124 μm -thick GGG: Eu scintillator screen (ESRF, Grenoble, France) was selected for our experiment. The exposure
125 time/projection was of 3.5 sec. For each tooth we imaged at least one Region Of Interest (ROI) located at
126 approximately 1/3 of the tooth root from the cervical enamel. We reconstructed the volumes using the filtered
127 back-projection algorithm provided by the SYRMEP Tomo Project (STP) software (Brun et al., 2015). Prior to slice
128 reconstruction a single-distance phase-retrieval algorithm (Paganin et al., 2002) was applied to the sample
129 projections with a d/b parameter (the ratio between the real and imaginary parts of the complex refraction index
130 of the material) in the range of 10-17 depending on the specific sample features.

131 Of the 21 teeth imaged, one contemporary human (ARF36) and six Neanderthal (KR7, KR25, KR176, KR1, KR137
132 and KR176) did not present enough cementum preserved in the region selected to offer consistent LHV estimates.
133 Moreover, PC-SR μ CT data revealed that extensive diagenetic damage had clearly occurred to the cementum of the
134 Mesolithic specimen (H326/7) so no further analyses were carried out on this tooth. Consequently, we carried out
135 data analyses on the remaining 14 teeth: eight contemporary humans, five Neanderthals, and one Neolithic
136 individual (Table 2).

137 Virtual 2D sections were extracted from the reconstructed 3D volumes using the open source FIJI ImageJ
138 (Schindelin et al., 2012). To ensure maximum visibility of the tooth cementum annulations (TCA), using the *reslice*
139 tool in FIJI ImageJ, the virtual sections were made perpendicular to the root surface (Maat et al., 2006), rather than
140 to the tooth axis, as in the latter case the section would cut the cementum layers in an oblique fashion. To
141 minimize the risk of analyzing a non-representative area, and to factually sample in 3D, even if by discrete

142 increments, we made five virtual sections for each tooth (Figures 1 and 2). For each of the 70 2D virtual sections
143 we obtained (Supplementary Script 1) between 15 and 25 linear plots (depending on the length of the cementum
144 segment) of the gray-level value of pixels across the entire thickness of the cementum (see SI Methods for more
145 detailed description). Finally, we saved the data of the plots, with x axis representing the distance (in years) from
146 the cementum-dentine junction (CDJ), which was manually identified, and y axis the gray-level pixel value (Cerrito
147 et al., 2021).

148 1.3 Data analysis

149 1.3.1 Age at death (or tooth extraction in contemporary individuals) estimation

150 We followed previously published methods (Le Cabec et al., 2019; Newham, et al., 2020a) of TCA count on PC-
151 SR μ CT data with the variant of measuring increments on five sections per tooth, rather than just one (see
152 paragraph 2.2). Images were assigned randomized numbers to prevent unconscious bias in increment count. At
153 least 24 hours elapsed between the first and the second time each observer measured the increments on each of
154 the 30 micrographs (five micrographs for each of the five neanderthal and one Neolithic individual). In Table 3 we
155 report the number of increments counted on each micrograph by each of the four independent observers (PC, TB,
156 AN and LB), as well as the average value across the five micrographs.

157 Age at death estimation based on TCA counts is performed by adding the age at which cementum deposition
158 begins, which is the estimated age at eruption of the tooth, to the number of annuli counted. While this constant is
159 debated even for modern human populations (see: Le Cabec et al., 2019), the question is even more complicated
160 when dealing with fossil hominins for which data on tooth formation time is limited (Bayle et al., 2009;
161 Macchiarelli et al., 2006; Smith et al., 2007; 2010) and cementum initiation is poorly documented. Ideally, one
162 would want to add the age at which cementum begins being secreted specifically in the region of the tooth where
163 one is measuring the increments. Since for Neanderthal teeth that information is not available, we added the
164 minimum age at gingival emergence as estimated by Wolpoff (1979). For the *H. sapiens* specimen, we added the
165 age at 1/3 of root completion reported by AlQahtani et al., 2010 for maxillary teeth (males and females are pooled
166 together), Liversidge et al., 2006 for mandibular teeth excluding the third molar (estimates differ for males and
167 females), and Liversidge, 2008 for the mandibular third molar (estimates differ for males and females).

168 All statistical analyses were performed in R (v. 4.0.2) language (R Core Team, 2020). To assess the agreement
169 between observers and between successive measurements made by the same observer (intraobserver error) we
170 used intraclass correlation coefficient (ICC) (Shrout & Fleiss, 1979) as implemented through the R package *psych*
171 (Revelle & Revelle, 2015). For each of the four observers we report (Table S1) the ICC estimated values together
172 with their 95% confidence intervals and p-values using a two-way mixed effects model ICC (3, k) where the first

173 number in brackets refers to the model (1, 2 or 3) and the second number refers to the type, which is either a
174 single rater/measurement (1) or the mean of k raters/measurements (k) (Koo & Li, 2016).

175 1.3.2 Sexual dimorphism

176 We undertook exploratory research on possible sexual dimorphism in cementum layering and collected several
177 variables from the line profiles of each virtual section of the individuals for which we knew the sex. The same
178 variables were also collected on the fossil specimens: five Neanderthals and one Neolithic individual, which was
179 treated as sex=NA even though the sex was known for the Neolithic specimen (see 1.1). First, each line profile was
180 smoothed according to two different denoising thresholds, thus producing two datasets that were analyzed
181 independently. The variables we measured were: the standard deviation of the gray-level value of pixels; the
182 average amplitude of the peaks; the frequency of both positive and negative peaks; the standard deviation of the
183 area of the peaks; the average width of both positive and negative peaks. The values were then averaged across
184 the several datafiles of each micrograph. On this data from the modern samples we used the *rpart* function
185 (Therneau et al., 2010) in R to construct a classification tree with sex as the grouping variable. We used it with
186 *method = "class"* and *rpart.control* at default settings. Additionally, we conducted two-tailed (Bonferroni corrected)
187 t-tests to determine whether there was a significant difference between males and females. Subsequently, we
188 applied the parameter of the splitting criteria obtained from the supervised classification on the training datasets
189 (modern specimens) to the fossil specimens. Finally, for the variables that showed a statistically significant
190 difference between sexes in the modern specimens we observed and reported their values for the fossil
191 specimens. Methods are described in greater detail in the Supplementary Information file.

192 1.3.3 Age at physiologically significant event occurrence

193 To determine the age at which physiologically significant events occurred (event detection is described below) we
194 proceeded under the verified assumption (Cerrito et al., 2020; 2021) that cementum growth rate is roughly
195 constant and that therefore proportional relationships can be made in the form of: total cementum thickness /
196 total years of cementum deposition = x-value (in pixels, or micrometers) of event detected / age at event
197 occurrence (in years).

198 For each line profile of each of the 70 virtual sections we used proprietary algorithms (Supplementary Scripts 4 and
199 5) to: (a) denoise the signal; (b) identify peaks as local maxima and minima (Figures S1 and S2); (c) detect peaks
200 that were consistent amongst most (>40%) of the line profiles of a same virtual section; (d) identify those that
201 were present at highest frequencies when grouping the output peaks of the several virtual sections of a same
202 individual together. Step (d) was carried out imposing a cut-off threshold at either 30% or 40% of the maximum
203 frequency to minimize false positives (30% threshold) and to minimize false negatives (40%). Results are reported

204 for both settings. The value in years of each peak thus obtained was summed to the age at cementum initiation to
205 produce the final estimate of age at event occurrence. Methods are described in greater detail in the
206 Supplementary Information file.

207 For the modern teeth we calculated the average absolute difference between known and inferred age, the
208 percentage error of the inferred age, the root mean square error (RMSE), the coefficient of determination (R^2)
209 between the two, the false positive rate and the false negative rate.

210 **2. Results**

211 **2.1 Age at death**

212 Krapina neanderthals: the cementum based estimated ages at death are reported in Table 3, together with the
213 estimates made by Wolpoff (1979) based on dental wear. For KR27 and KR162 cementum based estimates are very
214 close to those based on wear. However, our results do not match the individual assignment to a KDP made by
215 Wolpoff, according to whom KR27 and KR85 belong to KDP23 while KR162 and KR172 belong to KDP25. Our age
216 estimation is discordant between KR27 and KR85 (9.35 years difference) and between KR162 and KR172 (5.05
217 years difference), which could derive from the margin of error regarding age estimates based on TCA counts (Le
218 Cabec et al., 2019).

219 The results of the ICC (Table S1) indicate that the reliability is excellent for both observers (see: Koo & Li, 2016 for
220 ICC results interpretation), meaning that the intraobserver error is very low. Conversely, the results of the ICC on
221 the four different observers indicate that inter-observer agreement is lower (ICC = 0.71; 95% CI: 0.56 < ICC < 0.83).
222 Specifically, the agreement between AN and LB is very good (ICC = 0.88; 95% ICC: 0.76 < ICC < 0.94), while PC
223 consistently overestimates AN and LB by an average of 0.8 years, while TG consistently underestimates them by an
224 average of 1.5 years. We took the arithmetic average between the four observers as our final age estimate.

225 **2.2 Sexual dimorphism**

226 The results of the recursive partitioning (Figure S3) on the known-sex sample indicate that the average width of
227 positive peaks is the best discriminating variable between the sexes regardless of noise threshold (0.3*Std or
228 1*Std). Based on this parameter the misclassification rate for females is 4%, while for males it is 40% (Figure S4).
229 This difference in misclassification rate is likely due to the larger number of female than male specimen. For this
230 variable the range for females is 22.7 to 32.15, while for males it is 18.28 to 26.29 (Figure 3). In addition, several
231 other variables (Table S2) appear to be significantly different between the two sexes based on the results of the t-
232 tests. Since some of the variables that were significant at 0.3*Std were not at 1*Std, we only use the ones
233 significant in both settings to make our informed suggestions regarding the sex of the fossil specimens. We
234 therefore used the areas of non-overlap in values between the male and female ranges (thus excluding the range of

235 22.7 to 26.29) to suggest it as either male or female. The misclassification rate on the modern specimens based on
236 non-overlapping ranges is 0% for both males and females, with 54.3% of the cases resulting in no estimate being
237 made (Figure S5). By suggesting an estimation of sex only if the value fell within the exclusive (non-overlapped)
238 range of either sexes our results indicate that the neanderthal fossils KR172 and KR179 might be females, the
239 Neolithic specimen A2309 is female, while for the remaining three Neanderthal specimens we make no informed
240 suggestion (Table 4). Our analyses correctly predicted the sex of A2309.

241 2.3 Age at event occurrence

242 We found that there is a strong and statistically significant positive correlation ($R^2 = 0.9874$; $p < 0.05$) between
243 known and inferred ages at physiological stressor (pregnancy, illness, etc) in the modern reference sample. The
244 inferred ages together with the known ages at event occurrence are reported in Table S3. The intercept value ($y =$
245 0.1063) of the equation of the regression line reported in Figure 4 ($y = 1.0034x + 0.1063$) indicates that there is no
246 systematic error towards over or underestimating the ages at event occurrence. The average absolute difference
247 between known and inferred ages in the estimates of all known events is 1.9 years; the RMSE is 2.1 years. The false
248 positive and false negative rates differ considerably depending on the final parameter used in the analysis. With
249 cut-off threshold at 40% of the maximum frequency the false negative rate is 0%, meaning that all known events
250 are detected, however the false positive rate is extremely high at 55.2%. When implementing a stricter cut on the
251 final output (30% of the maximum frequency), the false negative rate increases to 10.3% while the false positive
252 one logically decreases, to 34.5% (Table 5). The inferred ages at event occurrence for the fossil specimens are
253 reported in Table 6. Only one event was detected for each specimen.

254 3. Discussion

255 Our age estimates match fairly well with those based on dental wear, even though there are some potential
256 differences from the KDP assignment made by Wolpoff (1979). Nonetheless, as previously reported (Le Cabec et
257 al., 2019) there is a non negligible margin of error regarding age estimates based on TCA counts. The difference
258 between known and inferred age, for individuals <50 years of age, is reported to be between 6.8 and 6.5 years (Le
259 Cabec et al., 2019). Future works could benefit from recently-developed semi-automatic TCA count methods
260 (Newham et al., 2021; Tanner et al., 2021) in order to eliminate inter- and intra-observer error. Additionally,
261 because of the inhomogenous nature of cementum, tracking the growth increments via 3D virtual reconstruction
262 could help reduce error in age estimates. The age at which cementum begins formation is another source of error,
263 which in the future could be addressed by acquiring scans of whole teeth to enable precise measures of crown and
264 root formation times. Hence, the results of our study are not conclusive enough to add meaningful information to
265 the KDP assignment. It is worth noting that, although our sample size is small, there does not appear to be any
266 systematic over- or underestimation of age, compared to the one derived from dental wear.

267 The exploratory research regarding sexual dimorphism in cementum microstructure has provided insights into a
268 potentially novel area of research. Based on our small sample and preliminary observations it appears that the
269 width of peaks in the gray-level profile of cementum transects might be sexually dimorphic, with males presenting
270 on average narrower event oscillations than females. This could reflect the rapidity (frequency of peaks also seems
271 to be sexually dimorphic) in changes in tissues deposition and could be caused by underlying metabolic differences
272 between the sexes, which in turn are likely correlated with body size sexual dimorphism since metabolic rate scales
273 with body size (West et al., 2003). Indeed, research has shown that inner fundamental metabolic rhythms
274 reconcile and regulate tissue formation and organ masses across primates (Bromage et al., 2009; Bromage & Janal,
275 2014). This is reflected, for example in the sexually dimorphic periodicity of repeat intervals (RI) in enamel
276 formation such that the average Havers-Halberg Oscillation RI values are lower (hence frequency is higher) for
277 males than for females (Karaaslan et al., 2020). Accordingly, other works have demonstrated the presence of
278 sexual dimorphism in bone accretion with an increased turnover in males which suggests a higher metabolic
279 activity in this sex (Gracia-Marco et al., 2010; Van Coeverden et al., 2002). Although our study has a small sample
280 size, we were able to correctly estimate the sex of a Neolithic fossil tooth. Our preliminary results seem to confirm
281 recent research (Newham et al., 2020a) which has reported evidence of sexual dimorphism in the cementum of
282 another primate species (*M. mulatta*). It is therefore likely that there is sexual dimorphism in the microstructure of
283 this tissue, but systematic exploration on a larger sample is necessary in order to better understand it, especially
284 across different taxa, which in turn have differing degrees and forms of sexual dimorphism (e.g. macaques have
285 sexually dimorphic canines while humans do not).

286 While previous research has confirmed that physiologically impactful events, such as those relating to
287 reproduction, are permanently recorded in cementum microstructure and can be accurately timed (Cerrito et al.,
288 2020, 2021; Cipriano, 2002; Kagerer & Grupe, 2001), this is the first study to demonstrate that it is possible to
289 obtain such information in a non-destructive manner. This is of tremendous importance for its applicability to fossil
290 specimens. Indeed, the results of our analyses support the hypothesis that physiologically impactful events are
291 detectable using PC-SR μ CT technique. However, the high false positive rates of the known-event sample indicate
292 that most events are detected but not all detected events are necessarily real. Nonetheless, it is important to note
293 that we cannot be completely certain that false positives are not actually real events, which the anonymous
294 participant omitted or forgot to include in their questionnaire, as documented by previous research that has
295 addressed the problem of retrospective reporting (Berney & Blane, 1997; Gillespie et al., 2006). Additionally, it is
296 possible that events subjectively deemed insignificant and not directly related to health and reproduction (which is
297 what our questionnaire assessed) actually have significant effects on cementum deposition. Indeed, recent
298 research (Lemmers et al., 2021) has shown that in Mandrills (*Mandrillus sphinx*) events such as changes of alpha
299 male within a group correlate with an accentuated line in enamel.

300 When applying our event-detection algorithms to our sample of fossils, our results (Table 6) indicate only one
301 physiologically impactful event per individual. While our known-event specimens had a high false positive rate,
302 they also had a 0% false negative one, meaning that we should expect all known events to be detected. It is likely
303 that the physiology of modern urban-dwelling people is more perturbed and less tightly correlated to seasonal
304 rhythms than that of Neanderthals, leading to an increased amount of perturbations in the cementum that are not
305 correlated to any known stressors. Additionally, the results of our sex, age and event estimations combined could
306 suggest that the physiologically impactful events reported for A2309 (15.5 years) and KR172 (16.6 years) could
307 reasonably relate to menarche, which in contemporary hunter-gatherer populations has a mean of 16.6 and a
308 median of 17.1 years (Konner, 2017), and is expected to be earlier for Neanderthals who had an overall accelerated
309 life history scheduling comparing to *H. sapiens*. As for KR179, for whom we report only one physiologically
310 significant event at 23.6 years, we are inclined to suggest the possibility of it relating to a reproductive event or
311 severe illness. However, we lack an explanation for the absence of a menarche signal, if this is indeed a female.

312 However, it is necessary to acknowledge that all inferred events are between 0.7 and 5.4 years before death,
313 making it possible that the physiologically stressful events detected close to death were possibly correlated to it.
314 Notwithstanding, as already acknowledged by several recent works (Cerrito et al., 2020; Penezić et al., 2020) it is
315 not possible to infer the cause of stressor from the histological analyses of cementum alone. It is likely that
316 elemental analyses would provide more event-specific information, in that each different type of stressor alters
317 the mineral homeostatic balance in a different way. However, that type of data is not yet available using
318 nondestructive methods. Future research aiming at collecting elemental data and teasing apart the cause of the
319 stressor is necessary, such as synchrotron X-ray fluorescence mapping (Dean et al., 2018) on the cementum of
320 fossil teeth having natural broken roots.

321 In conclusion, the present work provides tentative evidence that sex can be recovered from cementum
322 microstructure, and robust evidence that the chronology of physiologically impactful events is both preserved in
323 cementum microstructure and detectable using non-destructive 3D imaging methods. Future research will
324 hopefully build on the present work and advance further our knowledge.

325
326 **Ethics.** The research was carried out under the auspices of the College of Medicine Research and Ethics Committee
327 (COMREC) Protocol Number: P.05/06/373. All methods were carried out in accordance with relevant guidelines
328 and regulations and all experimental protocols were approved by the NYU College of Dentistry.

329
330 **Author Contributions.** PC initiated the study. PC, AN, DR, LB and TGB designed the study. PC, AN, DR, LM and APK
331 collected the data. PC, LC, TB, LB and TGB analyzed the data. DR, GR, DB and DWF contributed theoretical context
332 and materials. PC wrote the manuscript with critical input from all the authors.

333 **Data Accessibility.** All data supporting the present work can be found at:
334 https://drive.google.com/drive/folders/1mBgL-NiCB0Tu4yxxZx2R_M13X4SFMI17?usp=sharing

335 **Competing Interest Statement.** The authors declare no competing interests.

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492

493 **Figures captions and Tables**

494 **Figure 1** | Schematic representation of the workflow (from left to right): five 2D virtual sections of approximately
495 1.85mm² were made for each tooth; from each section we obtained between 14 and 25 (depending on the
496 extension of the cementum) 1-pixel-thick linear plots of the gray-level value of pixels across the entire width of the
497 cementum, starting from the CDJ outwards. Each line plot was acquired at a distance of three pixels from the
498 previous one.

499

500 **Figure 2** | 2D Virtual section of: **A**) specimen ARF77 (a mandibular central incisor), contemporary *H. sapiens*; **B**) the
501 fossil specimen KR172 (a mandibular second molar), *H. neanderthalensis*. For each specimen: small square on top-
502 left: zoom-in on the region of interest. Top-right: plot of gray-value of pixels (y-axis) along the cementum segment
503 indicated by the solid yellow line. Bottom-right: 3D surface plot of the thicker cementum segment indicated by
504 shaded yellow rectangle. The 3D surface plot is for illustrative purposes only, data was collected on a series of line
505 scans as shown by the plot on top-right.

506 **Figure 3** | Boxplot of the average positive peak width for the contemporary known-sex females (F), contemporary
507 known-sex males (M) and the six fossil specimens of which 5 Neanderthals with unknown sex (gray boxes) and one
508 Neolithic *H. sapiens* (A2390) which is known to be female from DNA analysis. Each datapoint represents the
509 average positive peak width value for a single 2D virtual section, five 2D virtual sections were made per each
510 specimen. The boxes show the interquartile range (IQR); the line shows the median of the data; the whiskers add
511 1.5 times the IQR from the 75th percentile and subtract 1.5 times the IQR from the 25th percentile; the dots
512 represent outliers. The sex of A2390 (Neolithic specimen) is known via DNA analysis and it was then correctly
513 estimated via our analyses.

514 **Figure 4** | Plot of the inferred vs known age at event occurrence for all the known-event individuals included. The
515 superimposed gray line represents the regression line with equation $y = 1.0034x + 0.1063$ ($R^2 = 0.98$ $p < 0.05$). The
516 data used for this plot are available in Table S3.

517

518 **Table 1** | The three fundamental parameters of the four different “recording structures” present in primates
 519 (Klevezal, 1995). These parameters determine the suitability of each structure to the different questions in
 520 anthropological and primatological research.

Structure	Sensitivity	Period of registration	Persistence of a record
Bone	multidien	8th week after conception to end of life	several years, but variable depending on bone and population
Enamel	circadian and multidien	tooth initiation to tooth crown completion	lifetime
Dentine	circadian and multidien	tooth initiation to tooth completion	lifetime
Cementum	annual	tooth completion to death	lifetime

521

522

523 **Table 2** | Specimens analyzed in the present study. For each species/population we report whether sex, and
 524 and/or Life History Variables (LHVs) were known. *Sex was estimated via DNA analysis (Mathieson et al., 2018).
 525 *Estimates based on wear are from Wolpoff (1979) for the Neanderthal specimens, Borić & Price (2013) for the
 526 Neolithic individual and from Borić et al. (2014) for the Mesolithic one. ARF and ARM = contemporary specimens
 527 with known life history.

Specimen	Tooth	Age	Sex	Species	Reliable cementum imaging
ARF36	URP3	36	F	<i>H. sapiens</i> (contemporary)	No
ARF45	ULP4	45	F	<i>H. sapiens</i> (contemporary)	Yes
ARF69	URM2	69	F	<i>H. sapiens</i> (contemporary)	Yes
ARF77_LLI2	LLI2	77	F	<i>H. sapiens</i> (contemporary)	Yes
ARF77_LLI1	LLI1	77	F	<i>H. sapiens</i> (contemporary)	Yes
ARF78	URC	78	F	<i>H. sapiens</i> (contemporary)	Yes
ARF67_UI2	UI2	67	F	<i>H. sapiens</i> (contemporary)	Yes
ARM63	URM3	63	M	<i>H. sapiens</i> (contemporary)	Yes
ARM47	LRC	47	M	<i>H. sapiens</i> (contemporary)	Yes
H327	LLM3	50*	F ⁺	<i>H. sapiens</i> (Mesolithic)	No
A2309	LRM1		F ⁺	<i>H. sapiens</i> (Neolithic)	Yes
KR1	LRM2	16*	N/A	<i>H. neanderthalensis</i>	No
KR7	LRM3	16*	N/A	<i>H. neanderthalensis</i>	No
KR25	LRP3	16*	N/A	<i>H. neanderthalensis</i>	No
KR27	LLP3	16*	N/A	<i>H. neanderthalensis</i>	Yes
KR85	LLM3	16*	N/A	<i>H. neanderthalensis</i>	Yes
KR176	ULM2	24*	N/A	<i>H. neanderthalensis</i>	No
KR137	URM3	24*	N/A	<i>H. neanderthalensis</i>	No
KR179	ULM3	19*	N/A	<i>H. neanderthalensis</i>	Yes
KR162	URM3	25*	N/A	<i>H. neanderthalensis</i>	Yes
KR172	URM2	25*	N/A	<i>H. neanderthalensis</i>	Yes

528

529

530 **Table 3** | Number of cementum annuli counted by each of the two observers (PC and TB) on each of the 30 virtual
531 sections. *The constant added to calculate the estimated age at death is: for Neanderthals (KR specimens) the
532 minimum age at gingival eruption reported by Wolpoff, 1979; for the Neolithic human (A239) the values reported
533 by AlQatani et al., 2010 for age at 2/3 of root completion. *Age estimates based on wear are from: Wolpoff, 1979.

Specimen	Tooth	image #	PC1 counts	PC2 counts	TB1 counts	TB2 counts	AN1 counts	AN2 counts	LB1 counts	LB2 counts	Constant added	PC, TB, AN, LB avg age	Age estimate from wear*
KR27	LP3	305	6	7	5	6	5	5	5	4			
KR27	LP3	557	5	6	5	3	4	5	5	4			
KR27	LP3	782	9	8	5	6	8	7	7	5			
KR27	LP3	811	10	9	5	6	9	8	8	6			
KR27	LP3	2035	6	7	3	3	6	5	6	5			
KR27	LP3	avg	7.2	7.4	4.6	4.8	6.4	6	6.2	4.8	9.3	17.025	16
KR85	LM3	917	10	9	7	7	8	10	11	8			
KR85	LM3	921	9	9	7	7	9	8	11	8			
KR85	LM3	950	10	10	6	7	8	7	9	5			
KR85	LM3	1080	9	8	6	8	7	7	10	7			
KR85	LM3	1093	11	12	8	7	9	10	10	8			
KR85	LM3	avg	9.8	9.6	6.8	7.2	8.2	8.4	10.2	7.2	16.3	24.725	16
KR162	UM3	206	9	12	10	8	11	10	9	10			
KR162	UM3	363	11	9	7	7	10	8	9	9			
KR162	UM3	796	9	8	6	6	9	8	9	10			
KR162	UM3	907	13	11	12	11	11	13	10	10			
KR162	UM3	935	10	13	7	9	10	12	11	9			
KR162	UM3	avg	10.4	10.6	8.4	8.2	10.2	10.2	9.6	9.6	16.3	25.95	25
KR172	LM2	1714	15	15	14	14	14	14	13	13			
KR172	LM2	1730	11	10	5	7	12	11	14	12			
KR172	LM2	1772	14	13	11	10	13	14	14	12			
KR172	LM2	1778	9	7	7	8	9	8	14	9			
KR172	LM2	2048	12	13	11	11	13	13	13	11			
KR172	LM2	avg	12.2	11.6	9.6	10	12.2	12	13.6	11.4	9.8	21.375	25
KR179	UM3	6	9	10	8	9	9	10	13	11			
KR179	UM3	22	14	12	10	9	10	11	13	12			
KR179	UM3	302	10	12	9	7	10	10	10	10			
KR179	UM3	332	8	7	5	8	9	7	10	12			
KR179	UM3	464	11	13	10	9	10	10	11	10			
KR179	UM3	avg	10.4	10.8	8.4	8.4	9.6	9.6	11.4	11	16.3	26.25	19
A2309	UM2	224	7	8	5	5	6	6	5	6			
A2309	UM2	664	8	7	5	5	6	5	6	6			
A2309	UM2	965	9	9	9	8	8	7	7	7			
A2309	UM2	1228	8	9	4	5	6	5	6	6			
A2309	UM2	1339	9	9	7	7	7	7	8	7			
A2309	UM2	avg	8.2	8.4	6	6	6.6	6	6.4	6.4	9.5	16.25	

534 **Table 4** | Suggestions of sex of the fossil specimens based on average width of positive peaks. We report the value
 535 for each specimen at two different noise thresholds. The partitioning value derived from recursive partition is
 536 23.13; the range for known-sex females is 22.70 to 32.15, while for males it is 18.28 to 26.29. The ranges of no
 537 overlap are 18.28 to 22.69 for males and 26.30 to 32.15 for females. For the Neolithic specimen sex was known
 538 through DNA analysis and our results correctly predict it. For specimens that had a value falling within the overall
 539 male and female range no suggestion is made.

Species	Specimen	Average width of positive peaks at 0.3*Std	Average width of positive peaks at 1*Std	Sex estimated based on range	Known sex
<i>H. neanderthalensis</i>	KR162	25.834	25.834	/	/
<i>H. neanderthalensis</i>	KR172	27.394	27.394	F	/
<i>H. neanderthalensis</i>	KR179	34.486	34.486	F	/
<i>H. neanderthalensis</i>	KR27	25.872	25.872	/	/
<i>H. neanderthalensis</i>	KR85	25.268	25.268	/	/
<i>H. sapiens</i> (Neolithic)	A2309	27.038	27.038	F	F

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542 **Table 5** | For each of the two settings of the final event detection analysis (see methods for details): the
543 associated average absolute difference between known age at event occurrence; the root mean square error
544 (RMSE); the false negative and false positive rates.

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Setting	Avg absolute difference	RMSE	False negative	False positive
30% of mm	1.93	2.13	0%	55.17%
40% of mm	1.91	2.1	10.34%	34.48%

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548 **Table 6** | Results of the inferred ages at event occurrence for the fossil specimens (KR are the Krapina
549 Neanderthals). Ages at cementum initiation and age at death are estimated (see Age estimation section) and
550 reported in Table 3. Suggested sex is reported as resulting from our analyses (Table 4).

Specimen	Age at event	Sex	Age at death
KR27	13.94	/	17.03
KR85	24.04	/	24.73
KR162	23.84	/	25.95
KR172	15.61	F	21.38
KR179	23.55	F	26.25
A2309	15.52	F	16.25

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