

1 **Cementochronology using synchrotron radiation tomography to determine**
2 **age at death and developmental rate in the holotype of *Homo luzonensis***

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24 **Abstract**

25 *Homo luzonensis*, a fossil hominin from the Philippines, is smaller than modern
26 humans. At present, very little is known about the life history of this species.
27 Cementochronology can answer life history questions, but usually involves
28 destructive sampling. Here, we use synchrotron radiation to count the yearly
29 cement lines of teeth belonging to a single individual. This approach allows us to
30 determine that this individual was likely 31 years old at time of death and
31 apparently had a developmental pattern comparable to chimpanzees. To our
32 knowledge, this is the first time that cementochronology using synchrotron
33 radiation tomography is used for life history and age-at-death estimation.

34

35 **Introduction**

36 Islands provide amazing examples of evolution and have inspired both Charles
37 Darwin and Alfred Russel Wallace. Nonetheless, little is known about insular
38 evolution, often resulting in miniature or giant versions of mainland animals (1).
39 Whether hominins are also subject to the insular dwarfing to the same degree as
40 other taxa, is still a matter of debate (2, 3) and possible examples include the
41 *Homo floresiensis* from Indonesia (4) and *Homo luzonensis* from the Philippines
42 (5).

43 The remains of *Homo luzonensis* were found in Callao Cave on Luzon Island and
44 were described in 2019 (6). The holotype of the species consists, amongst other
45 bones and teeth, of a dental series from upper right P³ to upper right M³, CCH6-a
46 to CCH6-e, (6). The teeth are characterised by their small size and mosaic of
47 primitive and derived features (7). Two *Homo luzonensis* elements, the third
48 metatarsal CCH1 and the upper right M3 CCH6-a, were directly dated to
49 minimum ages of 67 thousand years (kyr) and 50 kyr respectively (5, 8). Whether
50 these hominin remains represent a species that dwarfed under the influence of
51 insular conditions remains to be determined (6), although a descent from *Homo*
52 *erectus* seems likely based on the internal organisation of its teeth (7). This begs
53 the questions: Did the life history parameters of this species differ from that of
54 other hominins? And, how old was this individual when he or she died?

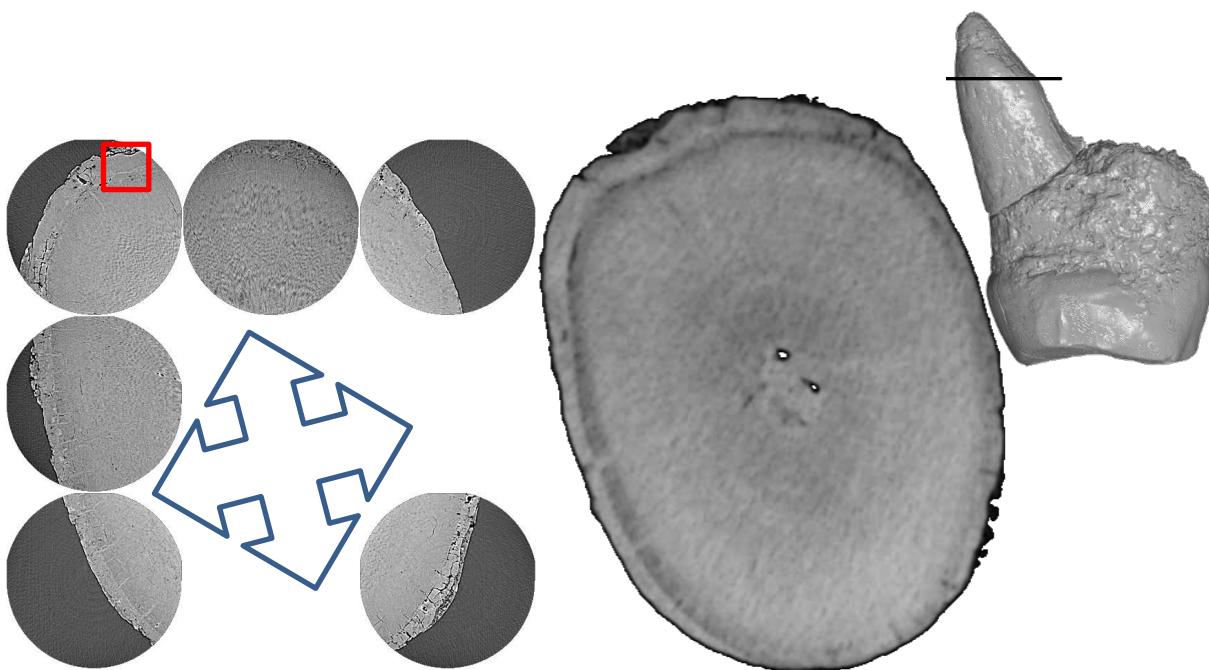
55 In medical terms, the incremental lines in tooth cementum are called “lines of
56 Salter”(9, 10). The morphology of tooth cementum resembles lamellar bone (11).
57 As in the case of lamellar bone (12), there are heavy debates on the nature and
58 the depositional mechanism of the lines of Salter in tooth cementum (13)
59 centering around analogous hypotheses. It is generally agreed upon that the
60 lines of Salter are annual lines representing summer and winter (13).
61 Nevertheless, the study of seasonality in human cementochronology has mostly
62 been performed on samples from the northern hemisphere from areas with four
63 seasons (13). It is still not understood how the colours of the lines of Salter
64 correspond to the seasons in the southern hemisphere or in areas with
65 monsoonal seasonality.
66 There is no evidence for tooth cement abrasion during decomposition, unlike
67 tooth enamel (14), making tooth cement a promising candidate for chronological
68 research. No taphonomical studies on geological time scales have thus far been
69 performed, however. In the present paper, we answer the above research
70 questions using cementochronology, or tooth cementum annulation, derived from
71 synchrotron radiation tomography.

72

73 **Materials and Methods**

74 In 2015, three teeth (CCH6-d: P⁴; CCH6-c: M¹; CCH6-b: M²) of the holotype of
75 *Homo luzonensis* were scanned in Synchrotron SOLEIL in France at the PSICHE
76 beamline. Although other researchers have, in the meantime, also applied
77 cementochronology to modern human canines using synchrotron radiation (15,
78 16), this is the first instance, to our knowledge, that synchrotron based
79 cementochronology is used on 1) a series of teeth belonging to the same
80 individual, 2) that are completely fossilized, and 3) for the purpose of estimating
81 life history traits through dental development.
82 Since transverse sections are advised for human teeth (13), P⁴ and M² were
83 mapped transversally by performing a mosaic of quick acquisitions, for which the
84 detector image was binned 4x4 to create an effective pixel size of 2.6 microns.
85 Each projection required 0.4 s exposure time, and 300 images were acquired

86 over 180° (roughly 2 minutes per tomogram). The reconstructed slices were
87 used to provide a preview of the transverse section, in order to select the
88 locations with the best-preserved cement structures (Figure 1). The buccal root
89 of P⁴ is broken off, so the lingual root was mapped. Rather than use a mosaic
90 mapping approach, M¹ was scanned, also transversally, at a relatively low
91 resolution with 2.6 micron pixel size, 1500 projections over 180° with 170 mm
92 propagation distance and 0.15s exposure time at 40 keV (Figure 2).

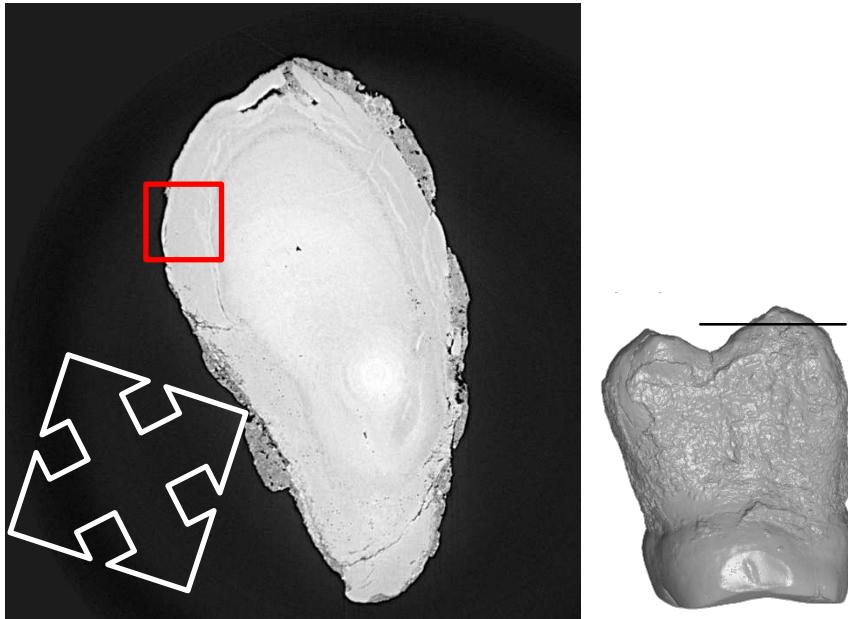


93 *Figure 1: Left: Map at 6 mm from the tip of the root of P⁴(CCH6-d). The red square indicates the position of
94 the detailed scan in Figure 3 at the lingual aspect of the lingual root. Centre: For illustrative purposes, a
95 slice of a micro-CT scan of P⁴ (7) at the level of the map. Right: The line in the distal view of the 3D model
96 indicates the level where the scan was made.*

97
98 Based on the maps, 10 appropriate positions for the detailed transverse scans
99 were chosen on M² and 4 on P⁴ in areas where acellular cementum was present.
100 Additionally, five suitable positions for the high-resolution transverse scans were
101 chosen on M¹. All detailed transverse scans on the three teeth were scanned at
102 40 keV, 70 mm propagation distance, with 3x 0.4 s accumulated images and
103 3000 projections over 360° (roughly 1 hour per tomogram). In all cases, 40 keV
104 monochromatic radiation was used, with a flux of around 2E11 photons/s/mm².

105 Although acellular cementum is usually utilised for cementochronology (17), the
106 cellular cementum was better preserved in M¹, and has also been shown to
107 consist of yearly lines (18, 19).

108



109

110 *Figure 2: Left: Section at 0.93 mm from the buccal tip of the distal root of M¹ (CCH6-c) on a low-resolution*
111 *scan. The red square indicates the position of the detailed scan of the distobuccal aspect in Figure 4. Right:*
112 *The line in the distal view of the 3D model indicates the level where the scan was made.*

113

114 After the detailed scans were completed, we chose the clearest images from the
115 dataset and counted the growth lines manually, since automated counts have
116 been found to underestimate the total number of increments (20). Growth lines
117 were counted on multiple images to assure the maximum number of lines was
118 counted. The lines of Salter were counted multiple times (21) in 2015 and in
119 2022. In both case the same numbers of lines were found. Representative
120 images are shown here.

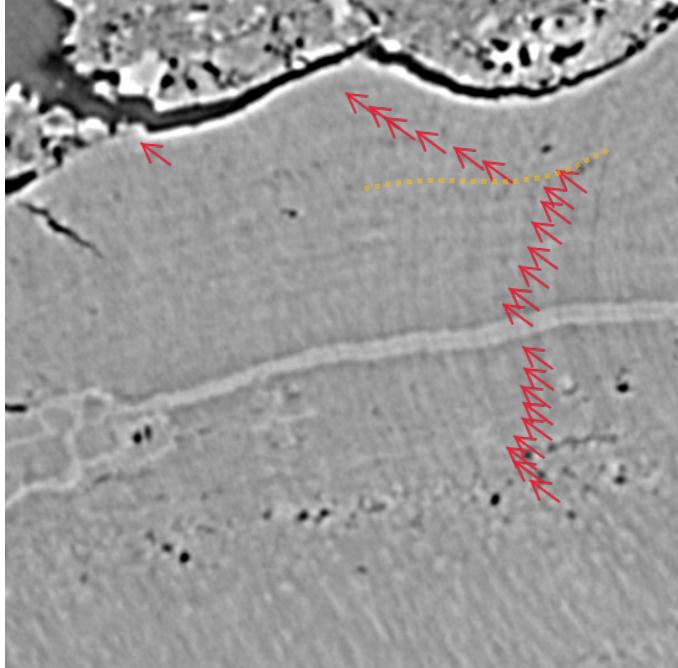
121

122 **Results**

123 The cement on the root of M¹ CCH6-c was best preserved out of all three dental
124 elements. The right upper P⁴ CCH6-d is relatively well preserved and displays 24
125 lines (Figure 3), equivalent to 24 years. The right upper M¹ displays 28 lines in
126 the tooth root cement (Figure 4), which are equivalent to 28 years. Despite taking

127 detailed scans at 10 positions in M^2 , surface erosion caused the counts to be
128 incomplete at each position, although it was possible to count the yearly lines.
129 The difference between the number of lines of P^4 and M^1 is four (=28-24) in the
130 holotype of *Homo luzonensis* CCH6.

131



132

133 *Figure 3: Detailed scan at 6 mm from the root of P^4 . The red arrows indicate the 24 yearly lines. The orange*
134 *dotted line follows the curvature of the cement lines.*

135

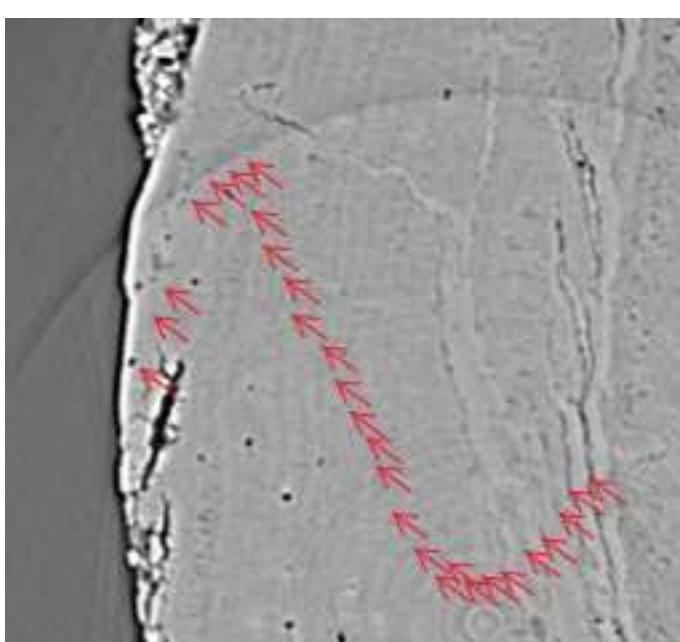
136 **Discussion**

137 Development and Age at Death

138 To assess age at death, the number of lines of Salter need to be added to the
139 age at eruption or the age of root completion. The margins of error of these two
140 approaches are not significantly different, because the timing of these events is
141 equal on a yearly timescale (13).

142 In *Homo luzonensis* CCH6, the cement of the root of M^1 was very well preserved
143 and the cement lines count can be considered reliable. The cement of P^4 was
144 slightly less well preserved, but was mostly deformed rather than eroded, since
145 the cement lines follow the shape of the outer surface and the cracks. (Figure 3,
146 orange dotted line).

147 In anatomically modern humans (*Homo sapiens*), a difference of five lines would
148 be expected between these dental elements, since the M¹ erupts at age six and
149 the P⁴ at age 11 on average (22). Nevertheless, there is some intraspecific
150 variation and a difference of four lines may be expected in approximately 5% of
151 the population (23). In chimpanzees (*Pan troglodytes*) on the other hand M¹
152 erupts at age three and P⁴ at age seven, so we would expect a difference of four
153 lines between the M¹ and the P⁴ (22). The mean interval in gorillas (*Gorilla gorilla*)
154 on the other hand would be three lines, since M¹ erupts in the fourth year of life
155 and P⁴ in the seventh (22). Although individual variation needs to be taken into
156 account (24), cementochronology is the most accurate method for age-at-death
157 estimation (25), and it is possible to speculate that the dental development
158 pattern of *H. luzonensis* might have been more similar to that of extant
159 chimpanzees than to that of anatomically modern humans, although this is not
160 necessarily a reflection of overall development. Even if an unknown number of
161 lines would be missing from P⁴, this would imply a developmental strategy quite
162 dissimilar from modern humans. Consequently, a reasonable estimate for the
163 eruption ages of M¹, M² and P⁴ *H. luzonensis* would be three, six and seven
164 years old respectively, based on a chimpanzee-like dental development pattern.
165



166

167 *Figure 4: Detailed scan at 0.93 mm from the root of M¹. The red arrows indicate the 28 yearly lines.*

168

169 Assuming a chimpanzee-like developmental pattern based on the M¹-P⁴ eruption
170 interval, the root of M¹ indicates that CCH6 died at the age of 31, calculated from
171 28 years of root cement formation and three years of postnatal life before the
172 eruption of M¹. P⁴, also points to an age at death of 31 years, consisting of 24
173 years of root cement formation and seven years of postnatal life before the
174 eruption of P⁴. A maximum potential age at death for the type specimen of *H.*
175 *luzonensis* may be calculated by assuming a human-like developmental pattern
176 with estimated eruption ages of M¹, M² and P⁴ of six, 12 and 11 years old. This
177 would result in an age at death of 34 or 35 years old. Since the ages calculated
178 assuming a chimpanzee-like developmental pattern correspond perfectly with
179 each other, we think it is likely that the type specimen of *H. luzonensis* was
180 approximately 31 years old at time of death but might have been a few years
181 older.

182 Implications for the Life History of *H. luzonensis*

183 In primates, dental eruption pattern is correlated with brain size (26). The
184 diminutive size of *H. luzonensis* has already hypothesised (8), so, similar to *H.*
185 *floresiensis*(27), its brain might also have been small, which would not be
186 discordant with the dental eruption pattern found in this study.

187 In primates, various life history traits, such as life expectancy, are also correlated
188 to the dental eruption pattern (28, 29). In anatomically modern humans, life
189 expectancy has changed over the course of (pre-)history (30). In the Mesolithic,
190 life expectancy was between 21 and 40 years at birth, whereas in the Neolithic
191 life expectancy was 17 to 25 years (31). In the Copper, Bronze and Iron ages, life
192 expectancy at birth was between 28 and 44 years (30). CCH6 died in his or her
193 early thirties. This seems relatively young for current standards but is in line with
194 Mesolithic modern humans, as well as corresponding to the potentially fast
195 development of *H. luzonensis*.

196 Possible Evolutionary Implications

197 *Homo luzonensis*, a small hominin from the Philippines, displays a dental
198 eruption pattern which could be similar to that of modern chimpanzees, but also

199 occurs in a small portion of the modern human population (23). Consequently, its
200 life history traits might to follow a more ape-like pattern than a modern human
201 pattern. This could have implications for human evolution, but more evidence is
202 clearly needed. It had already been established with *H. floresiensis* that relatively
203 recent hominins could have brain sizes in the range of chimpanzees (27).

204 **Perspectives**

205 Here, we used a new methodology to calculate age at death using synchrotron
206 radiation. This method is non-destructive and can be used on valuable fossils,
207 such as those of Callao Man. The methodology described herein is a proof of
208 concept and holds great potential for the study of fossil hominins and other
209 valuable dental remains that are not suitable for destructive sampling. Although it
210 is difficult to supply conclusive evidence of life history parameters based on two
211 teeth, the methodology could be applied to more complete tooth rows with the
212 prospect of deciphering an individual's complete dental development. Comparing
213 fossil taxa through time will provide direct evidence for the evolution of dental
214 development with implications for the associated life history traits. Furthermore,
215 this method allows for an assessment of age at death and, when applied to
216 multiple individuals of the same population, might provide an estimate of average
217 life expectancy. As suggested by von Jackowski et al. (15), studying the yearly
218 cementum lines over the whole tooth root cross-section might allow for the
219 detection of potential interruptions or irregularities of the individual layers. These
220 features may be associated with childbirth in females or certain diseases (32-34)
221 and could provide valuable information on the life of fossil hominins. As shown in
222 this work, the potential of using fast, low-resolution measurements combined with
223 slower, high-resolution measurements in selected areas could be important here.
224 To apply cementochronology to the lines of Salter, a standardised protocol for
225 each species, or even each dental element, would be ideal (13). In fossils,
226 however, this would be problematic. Firstly, it would be extremely difficult, if not
227 impossible, to ground-truth any such protocol, due to a lack of fossils with known
228 individual age at death and/or known age at eruption of the respective dental

229 elements. Secondly, due to taphonomical processes, the researcher will have to
230 work with those parts of the tooth cementum that are sufficiently well-preserved.
231 Theoretically, it should be possible to determine whether an individual died in
232 fall/winter or spring/summer. These seasons are characterised by dark or light
233 lines respectively in light microscopy (13). More research on modern humans is
234 needed, however, to determine how the cement layers are formed in a
235 monsoonal climate. It will also be challenging to extrapolate this analysis to
236 synchrotron radiation tomography. It is not currently known how the dark and
237 light lines correspond to the dark and light lines in light microscopy, since the
238 light and dark lines in tomography are caused by differences in density, whereas
239 the cause of the light and dark lines in normal microscopy is still under debate
240 (13) and differs according to the type of microscope used (35, 36).
241 Furthermore, the strong contrast between cementum and air at the surface
242 generates an intense phase contrast fringe, which interferes with interpretation of
243 near surface lines. This could be partially improved by surrounding the tooth with
244 a medium closer to the electron density of cementum, as in Lak et al. (37).
245

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257

258 **References**

259 1. van der Geer AAE, Lyras G, Vos Jd, Dermitzakis M. Evolution of island
260 mammals: Adaptation and extinction of placental mammals on islands. 1 ed.
261 Chichester: Wiley-Blackwell; 2010. 496 p.

262 2. Argue D, Groves CP, Lee MSY, Jungers WL. The affinities of *Homo*
263 *floresiensis* based on phylogenetic analyses of cranial, dental, and postcranial
264 characters. *Journal of Human Evolution*. 2017;107:107-33.

265 3. van Heteren AH. Is LB1 diseased or modern? A review of the proposed
266 pathologies. *Gene*. 2013;528:12-20.

267 4. Brown P, Sutikna T, Morwood MJ, Soejono RP, Jatmiko, Saptomo EW, et
268 al. A new small-bodied hominin from the Late Pleistocene of Flores, Indonesia.
269 *Nature*. 2004;431(7012):1055-61.

270 5. Mijares AS, Detroit F, Piper P, Grün R, Bellwood P, Aubert M, et al. New
271 evidence for a 67,000-year-old human presence at Callao Cave, Luzon,
272 Philippines. *Journal of Human Evolution*. 2010;59(1):123-32.

273 6. Detroit F, Mijares AS, Corny J, Daver G, Zanolli C, Dizon E, et al. A new
274 species of *Homo* from the Late Pleistocene of the Philippines. *Nature*.
275 2019;568(7751):181-6.

276 7. Zanolli C, Kaifu Y, Pan L, Xing S, Mijares AS, Kullmer O, et al. Further
277 analyses of the structural organization of *Homo luzonensis* teeth: Evolutionary
278 implications. *Journal of Human Evolution*. 2022;163:103124.

279 8. Grün R, Eggins S, Kinsley L, Moseley H, Cambridge M. Laser ablation U-
280 series analysis of fossil bones and teeth. *Palaeogeography, Palaeoclimatology,*
281 *Palaeoecology*. 2014;416:150-67.

282 9. Berkovitz BK, Holland GR, Moxham BJ. Oral anatomy, histology and
283 embryology E-book: Elsevier Health Sciences; 2017.

284 10. Swetha G, Kattappagari KK, Poosarla CS, Chandra LP, Gontu SR, Badam
285 VRR. Quantitative analysis of dental age estimation by incremental line of
286 cementum. *Journal of oral and maxillofacial pathology: JOMFP*. 2018;22(1):138.

287 11. Jones SJ, Boyde A. A study of human root cementum surfaces as
288 prepared for and examined in the scanning electron microscope. *Zeitschrift für*
289 *Zellforschung und Mikroskopische Anatomie*. 1972;130(3):318-37.

290 12. Mitchell J, van Heteren AH. A literature review of the spatial organization
291 of lamellar bone. *Comptes Rendus Palevol*. 2016;15(1):23-31.

292 13. Perrone V, Gocha TP, Randolph-Quinney P, Procopio N. Tooth cementum
293 annulation: A literature review. *Forensic Sciences*. 2022;2(3):516-50.

294 14. Akbulut N, Çetin S, Bilecenoglu B, Altan A, Akbulut S, Ocak M, et al. The
295 micro-CT evaluation of enamel-cement thickness, abrasion, and mineral density
296 in teeth in the postmortem interval (PMI): new parameters for the determination
297 of PMI. *International Journal of Legal Medicine*. 2020;134(2):645-53.

298 15. Von Jackowski J, Müller B, Schulz G, Hotz G, Weitkamp T, Wittwer-
299 Backofen U, et al. Tomographic imaging of unique objects: annual layers in tooth
300 cementum of Anna Catharina Bischoff born 1719: SPIE; 2022.

301 16. Le Cabec A, Tang NK, Ruano Rubio V, Hillson S. Nondestructive adult
302 age at death estimation: Visualizing cementum annulations in a known age
303 historical human assemblage using synchrotron X-ray microtomography.
304 *American Journal of Physical Anthropology*. 2019;168(1):25-44.

305 17. Robbins Schug G, Brandt ET, Lukacs JR. Cementum annulations, age
306 estimation, and demographic dynamics in Mid-Holocene foragers of North India.
307 *HOMO*. 2012;63(2):94-109.

308 18. Azorit C, López-Montoya AJ, Mateo-Calahorro BP, Analla M. Seasonal
309 investigation of fallow deer cellular cementum from Mediterranean ecosystems
310 and its implications for reliable cementochronology applications. *Quaternary*
311 *International*. 2022;610:133-43.

312 19. Takahashi S, Yamamoto T, Takahashi T, Yawaka Y. Incremental lines in
313 human cellular cementum: A histological study. *Journal of Oral Biosciences*.
314 2022.

315 20. Czermak A, Czermak A, Ernst H, Grupe G. A new method for the
316 automated age-at-death evaluation by tooth-cementum annulation (TCA).
317 *Anthropologischer Anzeiger*. 2006;25-40.

318 21. Bertrand B, Cunha E, Bécart A, Gosset D, Hédouin V. Age at death
319 estimation by cementochronology: Too precise to be true or too precise to be
320 accurate? *American journal of physical anthropology*. 2019;169(3):464-81.

321 22. Dean MC, Wood BA. Developing pongid dentition and its use for ageing
322 individual crania in comparative cross-sectional growth studies. *Folia Primatol*
323 (Basel). 1981;36(1-2):111-27.

324 23. Savara BS, Steen JC. Timing and sequence of eruption of permanent
325 teeth in a longitudinal sample of children from Oregon. *The Journal of the*
326 *American Dental Association*. 1978;97(2):209-14.

327 24. Machanda Z, Brazeau NF, Bernard AB, Donovan RM, Papakyrikos AM,
328 Wrangham R, et al. Dental eruption in East African wild chimpanzees. *Journal of*
329 *Human Evolution*. 2015;82:137-44.

330 25. Meinl A, Huber CD, Tangl S, Gruber GM, Teschler-Nicola M, Watzek G.
331 Comparison of the validity of three dental methods for the estimation of age at
332 death. *Forensic Science International*. 2008;178(2):96-105.

333 26. Godfrey LR, Samonds KE, Jungers WL, Sutherland MR. Teeth, brains,
334 and primate life histories. *Am J Phys Anthropol*. 2001;114(3):192-214.

335 27. Falk D, Hildebolt C, Smith K, Morwood MJ, Sutikna T, Brown P, et al. The
336 brain of LB1, *Homo floresiensis*. *Science*. 2005;308(5719):242-5.

337 28. Smith BH. DENTAL DEVELOPMENT AS A MEASURE OF LIFE
338 HISTORY IN PRIMATES. *Evolution*. 1989;43(3):683-8.

339 29. Harvey PH, Clutton-Brock TH. Life history variation in primates. *Evolution*.
340 1985;39(3):559-81.

341 30. Galor O, Moav O. The neolithic origins of contemporary variations in life
342 expectancy. Available at SSRN 1012650. 2007.

343 31. Hassan FA. Demographic archaeology. *Advances in archaeological*
344 *method and theory*: Elsevier; 1981. p. 225-79.

345 32. Kagerer P, Grupe G. Age-at-death diagnosis and determination of life-
346 history parameters by incremental lines in human dental cementum as an
347 identification aid. *Forensic Science International*. 2001;118(1):75-82.

348 33. Cerrito P, Cerrito L, Hu B, Bailey SE, Kalisher R, Bromage TG. Weaning,
349 parturitions and illnesses are recorded in rhesus macaque (*Macaca mulatta*)

350 dental cementum microstructure. American Journal of Primatology.
351 2021;83(3):e23235.

352 34. Penezić K, Porčić M, Urban PK, Wittwer-Backofen U, Stefanović S.
353 Stressful times for women-Increased physiological stress in Neolithic females
354 detected in tooth cementum. Journal of Archaeological Science.
355 2020;122:105217.

356 35. Burke A, Castanet J. Histological observations of cementum growth in
357 horse teeth and their application to archaeology. Journal of Archaeological
358 Science. 1995;22(4):479-93.

359 36. Hillson S. Archaeology and the study of teeth. Endeavour.
360 1986;10(3):145-9.

361 37. Lak M, Néraudeau D, Nel A, Cloetens P, Perrichot V, Tafforeau P. Phase
362 contrast X-ray synchrotron imaging: opening access to fossil inclusions in
363 opaque amber. Microscopy and microanalysis. 2008;14(3):251-9.

364