

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

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## Cementochronology on a fossil hominin using synchrotron radiation

- 1 Cementochronology using synchrotron radiation tomography to determine
- 2 age at death and developmental rate in the holotype of *Homo luzonensis*
- 3
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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
- 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

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 Philippines42 (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 bones and teeth, of a dental series from upper right P<sup>3</sup> to upper right M<sup>3</sup>, CCH6-a 45 to CCH6-e, (6). The teeth are characterised by their small size and mosaic of 46 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?

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- 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
- 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
- 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
- tooth enamel (14), making tooth cement a promising candidate for chronological
- 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

- In 2015, three teeth (CCH6-d: P<sup>4</sup>; CCH6-c: M<sup>1</sup>; CCH6-b: M<sup>2</sup>) 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
- cementochronology to modern human canines using synchrotron radiation (15,
- 16), this is the first instance, to our knowledge, that synchrotron based
- cementochronology is used on 1) a series of teeth belonging to the same
- individual, 2) that are completely fossilized, and 3) for the purpose of estimating
- 81 life history traits through dental development.
- Since transverse sections are advised for human teeth (13),  $P^4$  and  $M^2$  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.
- Each projection required 0.4 s exposure time, and 300 images were acquired

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- 86 over 180° (roughly 2 minutes per tomogram). The reconstructed slices were
- used to provide a preview of the transverse section, in order to select the
- locations with the best-preserved cement structures (Figure 1). The buccal root
- of P<sup>4</sup> is broken off, so the lingual root was mapped. Rather than use a mosaic
- 90 mapping approach, M<sup>1</sup> was scanned, also transversally, at a relatively low
- 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).



Figure 1: Left: Map at 6 mm from the tip of the root of P<sup>4</sup>(CCH6-d). The red square indicates the position of
the detailed scan in Figure 3 at the lingual aspect of the lingual root. Centre: For illustrative purposes, a
slice of a micro-CT scan of P<sup>4</sup> (7) at the level of the map. Right: The line in the distal view of the 3D model
indicates the level where the scan was made.

97

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

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- 105 Although acellular cementum is usually utilised for cementochronology (17), the
- 106 cellular cementum was better preserved in M<sup>1</sup>, and has also been shown to
- 107 consist of yearly lines (18, 19).
- 108



109

Figure 2: Left: Section at 0.93 mm from the buccal tip of the distal root of M<sup>1</sup> (CCH6-c) on a low-resolution
scan. The red square indicates the position of the detailed scan of the distobuccal aspect in Figure 4. Right:
The line in the distal view of the 3D model indicates the level where the scan was made.

113

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

121

## 122 **Results**

123 The cement on the root of M<sup>1</sup> CCH6-c was best preserved out of all three dental

- elements. The right upper P<sup>4</sup> CCH6-d is relatively well preserved and displays 24
- lines (Figure 3), equivalent to 24 years. The right upper M<sup>1</sup> displays 28 lines in
- the tooth root cement (Figure 4), which are equivalent to 28 years. Despite taking

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- 127 detailed scans at 10 positions in M<sup>2</sup>, surface erosion caused the counts to be
- incomplete at each position, although it was possible to count the yearly lines.
- 129 The difference between the number of lines of P<sup>4</sup> and M<sup>1</sup> is four (=28-24) in the
- 130 holotype of Homo luzonensis CCH6.
- 131



## 132

- Figure 3: Detailed scan at 6 mm from the root of P<sup>4</sup>. The red arrows indicate the 24 yearly lines. The orange 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

age at eruption or the age of root completion. The margins of error of these two

- approaches are not significantly different, because the timing of these event is
- 141 equal on a yearly timescale (13).
- 142 In *Homo luzonensis* CCH6, the cement of the root of M1 was very well preserved
- and the cement lines count can be considered reliable. The cement of P4 was
- 144 slightly less well preserved, but was mostly deformed rather than eroded, since
- the cement lines follow the shape of the outer surface and the cracks. (Figure 3,
- 146 orange dotted line).

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In anatomically modern humans (Homo sapiens), a difference of five lines would 147 be expected between these dental elements, since the M<sup>1</sup> erupts at age six and 148 149 the P<sup>4</sup> 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 erupts at age three and P<sup>4</sup> at age seven, so we would expect a difference of four 152 153 lines between the  $M^1$  and the  $P^4$  (22). The mean interval in gorillas (Gorilla gorilla) on the other hand would be three lines, since M<sup>1</sup> erupts in the fourth year of life 154 155 and P<sup>4</sup> 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 guite dissimilar from modern humans. Consequently, a reasonable estimate for the 162 eruption ages of  $M^1$ ,  $M^2$  and  $P^4$  *H. luzonensis* would be three, six and seven 163 164 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 M<sup>1</sup>. The red arrows indicate the 28 yearly lines.

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## 168

169 Assuming a chimpanzee-like developmental pattern based on the M<sup>1</sup>-P<sup>4</sup> eruption 170 interval, the root of M<sup>1</sup> 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 M<sup>1</sup>. P<sup>4</sup>, 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  $P^4$ . A maximum potential age at death for the type specimen of H. luzonensis may be calculated by assuming a human-like developmental pattern 175 176 with estimated eruption ages of  $M^1$ ,  $M^2$  and  $P^4$  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

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

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

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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)

and could provide valuable information on the life of fossil hominins. As shown in

this work, the potential of using fast, low-resolution measurements combined with

slower, high-resolution measurements in selected areas could be important here.

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,

however, this would be problematic. Firstly, it would be extremely difficult, if not

impossible, to ground-truth any such protocol, due to a lack of fossils with known

individual age at death and/or known age at eruption of the respective dental

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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. Theoretically, it should be possible to determine whether an individual died in 231 232 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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