Dental development and age at death of the holotype of *Anapithecus hernyaki* (RUD 9)

using synchrotron virtual histology.

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Abstract

The chronology of dental development and life history of primitive catarrhines provides a crucial comparative framework for understanding the evolution of hominoids and Old World monkeys. Among the extinct groups of catarrhines are the pliopithecoids, with no known descendant. *Anapithecus hernyaki* is a medium-size stem catarrhine known from Austria, Hungary and Germany within a restricted time (~10 Ma) and represents a terminal lineage of a clade predating the divergence of hominoids and cercopithecoids, probably more than 30 Ma. In a previous study, *Anapithecus* was characterized as having fast dental development. Here, we used non-destructive propagation phase contrast synchrotron micro-tomography to image several dental microstructural features in the mixed mandibular dentition of RUD 9, the holotype of *Anapithecus hernyaki*. We estimate its age at death to be 1.9 years and describe the pattern, sequence and timing of tooth mineralization. Our results do not support any simplistic correlation between body mass and striae periodicity since RUD 9 has a 3-day periodicity, which was previously thought unlikely based on body mass estimates in *Anapithecus*. We demonstrate that the teeth in RUD 9 grew even faster and initiated even earlier in development than suggested previously. Permanent first molars and the canine initiated 49 and 38 days prenatally, respectively. These results in *Anapithecus* contribute to a better understanding of dental development in *Anapithecus* and may provide a window into the dental development of the last common ancestor of hominoids and cercopithecoids.

Keywords (max. 6)

Introduction

Much research on dental development has focused on fossil hominoids and other extant primates, while stem catarrhines remain poorly studied. New non-destructive imaging techniques have now made it possible to study the microanatomy of fossil enamel and dentine (Tafforeau et al., 2006; Zabler et al., 2006; Smith et al., 2010a). This in turn enables us to reconstruct the chronology of enamel development and the timing and sequence of tooth initiation in the jaws of fossil specimens that would otherwise be impossible without resorting to destructive techniques (T.M. Smith et al., 2015). Understanding more about dental development in primitive catarrhines, such as the pliopithecoid *Anapithecus*, provides important background for the study of extant and extinct crown taxa. This in turn will eventually contribute to defining the ancestral condition and the polarity of characters that can be used in phylogenetic analyses.

Catarrhines are estimated to have originated during the late Oligocene, 32.1 Ma (95% highest posterior density confidence interval = 29.4–33.8 Ma) based on mtDNA evidence (Pozzi et al., 2014). The divergence of the hominoids (apes) and cercopithecoids (Old World monkeys = OWM) is estimated to be between 23 to 28 Ma (Steiper and Seiffert, 2012; Harrison, 2013; Raaum, 2015). Pliopithecoids have no extant descendants, precluding a divergence date based on DNA. However, since pliopithecoids are catarrhines but lack derived characters shared by OWM and apes, their appearance must predate the divergence of OWM and apes. Therefore, and from the two ranges of time previously cited, their origin can be estimated to have occurred between 23 and 32.1 Ma. Geographically, the pliopithecoids range from Western Europe to China. They first appear in the fossil record at about 18 Ma in China (Harrison et al., 1991; Begun, 2002). So there is a gap in the fossil record of pliopithecoids of at least 5 Ma and as much as 13 Ma.

Anapithecus hernyaki is among the later occurring species of pliopithecoid, dated to about 10 Ma (Kordos and Begun, 2001). It is known from a few specimens recovered from the sites of Salmendingen in Germany (one lower molar) and Götzendorf in Austria (several teeth), but by far the best collection of *Anapithecus* comes from the R. II locality at Rudabánya (Begun, 1989, 2002). While pliopithecoids occur with apes at a number of localities (Sukselainen et al., 2015), the only site where sympatry can be demonstrated taphonomically is Rudabánya, where the stem hominine *Rudapithecus* is found in the same sedimentological layer, elevations and 1×1 m² excavation units as *Anapithecus* (Kordos and Begun, 2001). *Anapithecus* is the largest-known pliopithecoid, with an estimated body mass of between about 8 and 15 kg (Begun, 2002; Nargolwalla et al., 2005; Begun, unpublished data). The depositional environment of the R.II locality at Rudabánya preserves the remains of a late Miocene swamp in an overall mildly seasonal forested setting (Kordos and Begun, 2001, 2002; Eronen and Rook, 2004; Eastham et al., 2016).

Nargolwalla et al. (2005) have previously depicted dental development in *Anapithecus* as fast in comparison to hominoids, with extreme overlap in molar development due to early initiation of sequentially erupting molar teeth.   T.D. Smith et al. (2015) suggested that rates of mineralization of postcanine teeth may be more rapid in folivores compared with frugivores. Godfrey et al. (2005a) have, however, noted that lemurs do not seem to reliably follow this rule. Old World monkeys are reported to lack any significant degree of molar overlap (Nargolwalla et al., 2005). Among anthropoids, growth rates (including dental development) correlate negatively with body mass: larger species have slower development and a relatively early eruption of the replacement teeth (incisors and premolars; Schwartz et al., 2007). In contrast, on the one hand, indriids show an accelerated dental development but sequences of eruption/emergence that are

similar to those of hominoids; and on the other hand, lemurids are characterized by a relatively slow dental eruption whereas this sequence of eruption/emergence would be indicative of a fast process of eruption (molars erupt before premolars; Schwartz et al., 2007). Furthermore, in lemurs, there is only a poor correlation between body mass and the pace of dental development (Schwartz et al., 2007). Given the relatively large body mass estimate of *Anapithecus*, of about 15 kg (Begun, 2002; Nargolwalla et al., 2005; Begun et al., unpublished data), the conclusion of rapid dental development evidenced by closely stacked molar development seemed unusual and has no known equivalent among extant catarrhines.

Multi-scale propagation phase contrast micro-computed tomography now allows a non-destructive visualization of the growth increments in dental tissues. In the present study we employed this non-destructive imaging technique to better characterize dental development in the holotype of *Anapithecus hernyaki*, RUD 9, a juvenile specimen, housed in the collections of the Natural History Museum of Hungary. In addition to determining the age at death of the individual, we aimed to gather information about the dental developmental variables (e.g., long-period striae periodicity, cuspal daily secretion rates) that had been previously estimated (Nargolwalla et al., 2005) and to describe the pattern of tooth cusp initiation and calculate enamel crown formation times.

Materials and Methods

Sample and synchrotron micro-CT imaging

The holotype of *Anapithecus hernyaki,* RUD 9, comprises a mandibular mixed dentition with both isolated teeth and teeth in situ (Kretzoi, 1975; Kordos and Begun, 2001; Nargolwalla et

al., 2005). These include the RM_1 to RM_3 (all isolated), the LC_1 (isolated) to LM_2, as well as both left mandibular deciduous premolars. The roots of the LdP_3 and the LdP_4 are preserved in situ in a mandibular fragment, together with the germ of the LP_3 still in its crypt beneath the dP_3; the isolated germ of LP_4 is also preserved. The LM_1 and LM_2 are both in situ in a small part of the mandible that fits together with the fragment containing the deciduous premolars.

RUD 9 was scanned at the ID 19 beamline at the European Synchrotron (ESRF, Grenoble, France). A first acquisition at 12.61 μm provided an overview of the whole specimen for a morphological description (Fig. 1, SOM Fig. 1). A second set of acquisitions was performed at 3.42 μm to image the dental incremental growth lines in each individual tooth. Finally, the metaconid of the RM_3 was subjected to two high resolution scans at 0.638 μm to image the cross-striations and laminations and determine the long-period line periodicity of the individual: the first scan was acquired at the cusp tip, and the second in the cervical area of the lateral enamel. The details of the scanning parameters are provided in SOM Table 1. Virtual 2D sections were generated from the 3.42 μm scans in full resolution (no binning), as well as 3D models (from the semi-automatic segmentation of the dental tissues) in VGStudio MAX2.2 for the needs of the study. The rendering techniques used here have been previously described in Le Cabec et al. (2015). All calculations of rate and measurements of length are rounded to one decimal place in the main text.

Developmental analysis

Developmental morphology Each tooth was described from virtual 2D sections optimized for visualizing crown and root formation, following the method described in Smith et al (2010a). For crown development, each virtual section passed through the dentine horns of a tooth (for multi-cusped teeth, the tip of one dentine horn was set as the center of rotation of the tooth) and the

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pulp horns beneath. This ensured that the lowest point on the enamel cervical margin was also included in the plane of section. Similarly for imaging root development, the plane of section passed through the pulp horns (for multi-cusped teeth, one tip of the pulp chamber was set as the center of rotation of the tooth), and included the maximum length of root possible.

Determining cuspal daily secretion rates and periodicity Both enamel cuspal daily secretion rate (CuDSR) and long-period Retzius line periodicity were obtained from the 0.638 μm scan of the RM3 metaconid. Despite the clarity of the daily laminations and/or cross-striations within the cuspal enamel, these could not be counted continuously through the whole cuspal enamel thickness because of the strong enamel decussation present. Instead, and to determine the CuDSR, the total cuspal thickness was divided into six equal zones and ten sets of local measurements were taken across five cross-striations within each zone. Although potentially small differences may exist between tooth types, the average value obtained from the RM3 metaconid was the only one that was possible and so was used throughout this study for calculating the cuspal formation time of all teeth (see next section). Periodicity was determined from counts and measurements of daily enamel increments made between long period Retzius lines. Periodicity is usually determined by counting cross-striations between several pairs of Retzius lines reaching the outer enamel surface (OES). In this individual daily laminations in the outer enamel were used where cross-striations could not be identified and assumed to be equivalent to daily cross-striations.

Dentine extension rates Dentine daily secretion rates (DDSR) and root extension rates in μm/day (DRER) were calculated for as many tooth loci as possible. DDSRs were calculated by measuring a distance along the dentine tubules and then dividing it by the corresponding enamel formation time (SOM Fig. 2 and drawing in SOM Table 2). First, homologous stress lines in the dentine of

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the P_4 and M_2 were matched in both teeth. Then the same stress lines were identified in the

dentine of the LC_1 and matched with enamel developing at the same time to establish a formation

time. DDSR at the developing root apex for both LP_4 and RM_2 (Table 1) were both estimated in

this way. DRERs were calculated by dividing the total root formation time (RFT) by the total root

length (measured between the most cervical point of enamel and the most distal outer aspect of

the root apex of a 2D virtual section). In the canine, the small portion of root formed on the disto-

lingual aspect of the tooth was matched with the corresponding enamel on the mesial side, using

stress lines visible in both areas. Retzius lines counted in the enamel, were then used to calculate

the corresponding root formation time and canine DRER (Table 1). Local DRERs were

calculated in the LP_4 and RM_2, by using stress lines identified in the dentine of the root apex

(SOM Fig. 2 and drawing in SOM Table 2). DRER calculations for LP_4, RM_1 and RM_2 were only

possible across the whole root length and were calculated by dividing the overall root length by

the total root formation time.

Initiation times, and crown and root formation times In teeth that initiate before birth, the

neonatal line is usually visible in the cuspal enamel and so enables the thickness of enamel

formed prenatally to be measured. This thickness can then be divided by the CuDSR to yield the

prenatal formation time, and thus the initiation time of the cusp. Regarding teeth initiating

postnatally, the initiation time may be back-calculated from the age at death. If they are known,

crown and root formation times can be subtracted from age at death to yield the time of initiation.

This method of back-calculation can also be used to provide other missing information such as

CFT (or any of its components: cuspal or lateral enamel formation times) or root formation time.

Stress lines in this specimen that could be matched in 2D and 3D also contributed to cross-

checking tooth initiation times between tooth types (P_4 and M_2).

Calculating crown formation time (CFT) involves summing cuspal and lateral enamel formation times. Since Retzius lines are often difficult to identify in the cuspal area, cuspal formation time was calculated by multiplying the cuspal thickness (measured from the dentine horn to the location of the first perikymata) by the CuDSR. For the LC_1, LP_4 and RM_3 the formation time for lateral enamel was based on counts of Retzius lines made on 2D virtual sections (where variation in slice thickness and orientation often improved the visibility of the incremental growth lines; see Le Cabec et al., 2015 for details). However, for LP_3, LP_4 and RM_2, perikymata counts, made on high quality 3D models, multiplied by the enamel long-period line periodicity were used. Whenever possible (e.g., LP_4 lateral enamel formation time), we checked the consistency of our results by counting both Retzius lines and perikymata. For most of the teeth, the method described by Dean (2012), Dean and Vesey (2008), and Dean and Cole (2013) was used and is briefly described here as used in *Anapithecus* teeth. From the dentine horn, at a point on the EDJ, a line was drawn along the path of enamel prisms to a second point 100 μm into enamel formation. From this second point, an accentuated growth marking (stria of Retzius) was identified running obliquely back to the EDJ within enamel. This stria was then tracked back to intersect the EDJ further along the crown at an end point further along the EDJ. The distance along the EDJ between the start-point at the dentine horn and the end-point represents the length of EDJ formed in the same time it takes to form a 100-μm thickness of enamel. The length of EDJ formed in this time represents the enamel extension rate and can be expressed in μm/day. This procedure was repeated from the cusp tip to the enamel cervix and the data for enamel formation time was cumulated. Since perikymata and Retzius lines were visible and easily made use of most of the time, this technique was only used to double check the reliability of the counts (see SOM Table 2).

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Root formation time (RFT) was calculated using one of four different methods (according to their feasibility). First, root length was measured on a slice optimized for visualizing maximum root development (as described for SOM Fig. 2), and the length divided by the DRER. Second, in some cases matching stress events across teeth between the dentine and/or the enamel of 2D sections and 3D models (EDJ, OES, or root surface) enabled a direct calculation of RFT (see later for LP_4 and RM_2). This technique has been previously been described in Le Cabec et al. (2015). 3D images of the dentine cap and enamel surface, each containing matching stress lines, were perfectly scaled side by side with the 2D image of the longitudinal slice through the dentine horn (see Fig. 9 in Le Cabec et al., 2015). A horizontal line draw across all three images then passed through a matching stress line in the dentine at the EDJ (on the 2D section) and its homologous stress marking on the dentine cap surface. This point could then be tracked up from the EDJ along a matching enamel stria to the corresponding perikymata on the enamel surface (see the red lines where the 2D section was recorded on the OES and dentine surface on Fig. 9 in Le Cabec et al., 2015). Fig. 2B illustrates how approach how this was applied on RUD 9 to estimate RFT. Third, when there was no incremental record of root formation at all but when other data for CFT, tooth initiation time and age at death were known, RFT was back-calculated from these data. Fourth, the method described by Dean (2012), Dean and Vesey (2008), and Dean and Cole (2013) and briefly described above was applied (see drawing in SOM Table 2).

Age-at-death Ideally age at death is best calculated from a single tooth that registered both birth (neonatal line) and death. The sum of the postnatal cuspal enamel and the lateral enamel formation times and any additional root formation time equals the age at death of RUD 9. In the case of RUD 9, based on preservation, the canine was determined to be the best tooth for this

calculation, but the very last formed enamel was counted in the third molar (as the cervix of the canine appears to be slightly damaged) and so both teeth were used in the final calculation.

<u>Building a developmental chart</u> This chart (see Fig. 4 in Results section) provides an overview of the sequence of dental development of RUD 9. Cuspal enamel, lateral enamel and root formation are indicated using different colors, and any uncertainty in the timing of these events is represented by using color-fading at the transition between them. Birth and death are indicated as well as three additional stress events that were visible as stress lines in dentine and enamel on 2D virtual sections and as linear enamel hypoplasia in 3D. These stress events were important throughout for checking the congruence of matching developmental stages of all the teeth.

Results

Tooth formation stages

The developmental stage of each tooth is illustrated in 3D in SOM Fig. 1, and in 2D in SOM Fig. 3. Both deciduous premolars and the LP_3 are preserved in their anatomical position in one fragment of the jaw, while the LM_1 and LM_2 are in situ in a second fragment of the jaw.

<u>LdP_3</u> The crown and the cervical part of the mesial and distal roots are broken off. Only the roots are preserved in situ. In cross section, the mesial root is triangular in shape while the distal root is 'figure-of-eight-shaped'. At about half of the root length, both roots divide into two root canals, with the buccal root being broader. The distal root, the tip of which is broken off, has caused a localized resorption area on the LdP_4 mesial root. This is most likely due to space competition in the lingual aspect of the jaw, as there is no indication of pathology. None of the LdP_3 roots seem to be affected by any significant root resorption, despite the presence of the well-developed LP_3

germ beneath them. A significant amount of cementum is deposited on the LdP_3 roots: a ~175 μm-thick (sagittally) layer on the distal aspect of the mesial root, and a ~240 μm-thick layer on the mesial aspect of the distal root.

LdP_4 All cusps are worn down further than a third of the dentine horn heights. Both the mesial and the distal roots change from C-shaped cross section cervically into a 'figure-of-eight-shaped' cross section apically, where the root canals divide into two major branches with accessory canals spreading out laterally. On its lateral aspect the mesial root is affected by a localized resorption, presumably due to the crowding of the LdP_3, and again apically due to contact with the LP_3 germ positioned in its crypt beneath. The amount of root resorption, however, is negligible with respect to the stage of development of the LP_4 germ underneath. There is, however, a considerable accumulation of cementum over the whole length of the Ldp_3 and Ldp_4 roots. The mesio-lingual component of the mesial root of the LdP_4 has a ~ 400 μm-thick layer of cementum in the apical fifth on its distal aspect, and a ~575 μm layer of cementum at the root apex. The cementum deposition follows the same pattern in the distal root although it is somewhat more bulbous in some places. For both deciduous premolars, several accessory canals of variable diameter (~150 μm maximum) branch in a complex ramification involving the apical root pulp canal although without reaching the root surface.

LC_1 The crown of this unerupted tooth is complete or nearly so, although slightly broken at the cervix on the mesial side. On the disto-lingual aspect of the tooth, the crown is complete and 1.3 mm of root are already formed. The crown is 8807 μm high in the developmental section from the dentine horn to most cervical point of enamel (mesial side).

LP_3 and LP_4 Both premolars were still in their crypts (only LP_3 is preserved in situ), and both are crown complete. Less than a third of the LP_3 root is formed overall (based on comparison with

another *Anapithecus* specimen with complete and undamaged roots, RUD 222 which P_3 root is 10.5 mm long), the lingual aspect being 2138 μm long. The LP_4 root is 2274 μm long on its mesiolingual aspect, with a linear root hypoplasia at 592 μm from the cervix that corresponds to crown completion on the buccal side.

RM_1 At the time of RUD 9's death, this tooth had been in functional occlusion for long enough to produce wear facets on all cusps. Although their roots have not yet achieved apical closure, both M_1s already show a noticeable narrow layer of cementum on their apical fifth (approximate maximum thickness measured on 2D sections: 125 μm on the mesial root, and ~205 μm on the mesial aspect of the distal root).

RM_2 Although the roots are almost half formed (estimated from comparisons with the fully formed M_2 roots of RUD 61, 112 and 222 that are overall 9.9 mm ± 0.5 mm long), and so similar to the LP_4, the M_2s are advanced in their stage of eruption when compared to the LP_4. The level of the cusp tips of the LM_2 is half way up the M_1 crown (Fig. 1). Two strong linear hypoplastic markings are visible on the surface of all roots, while another hypoplastic marker-line can be seen on the surface at about the cervical quarter of the EDJ (see the red asterisk on Fig. 2).

RM_3 About three quarters of the unerupted RM_3 crown is mineralized. The enamel has recorded several stresses also visible as hypoplastic lines on the dentine surface.

Developmental histology

Periodicity and daily secretion rate in cuspal enamel In the metaconid of the RM_3, the enamel long-period line periodicity was observed to be 3 days (Fig. 3). The average CuDSR is 4.4 μm/d (Table 2).

Daily root extension rates (DRER) and dentine daily secretion rates (DDSR) Results are reported in detail in Table 1 and SOM Table 2. A root extension rate of 12.7 µm/d was calculated from the small portion of root preserved on the LC_1 (disto-lingual side).

Stress events identified in the RM_3, RM_2 and LP_4 were employed to calculate DRER and DDSR in the P_4 and M_2 . Three of the most accentuated stress lines observed in the RM_3 enamel were indeed also visible as linear hypoplastic markings on the 3D models of the P_4 and M_2 EDJ and root surface (Fig. 2). The earlier stress (i.e., the closest accentuated line to the dentine horn tip of the RM_3 on Fig. 2A) corresponds to the protoconid completion in the RM_2 and is represented in blue in Figs. 2 and 4 and SOM Fig. 4. This blue stress event occurred at the ~384th day of the life of RUD 9. It should be noted that determining the time of occurrence of a stress marking cannot be done with precision to a single day since rounding of the various calculations involved inevitably leads to variation of a few days. The second stress event, represented by a green line (Figs. 2 and 4, and SOM Fig. 4), coincides with the end of the formation of the cuspal enamel in the RM_3 and occurred at the ~510th day of life of RUD 9. The last stress event of interest is figured as a pink line (Figs. 2 and 4, and SOM Fig. 4), and took place at the ~552rd day of RUD 9's life. This stress event that matches across teeth provided a means to calculate overall DRER. The LP_4 DRER on the buccal side is 11.4 µm/d. This value has been supported by another measurement yielding 12.0 µm/d using the method described in Dean and Cole (2013): see Methods section for explanation and SOM Table 2. Another stress line corresponding to the completion of the protoconid in the RM_2 allowed calculation of a DRER of 9.3 µm/d. Finally a DRER of 17.3 µm/d was calculated from the disto-buccal root of the RM_1.

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Another set of stress events, involving a double stress marking, was identified in the LP_4, the RM_2 and the LC_1 dentine. From the matching of the stress pattern between the dentine of the LP_4 and the LC_1 enamel, the latest accentuated line of this double stress event, i.e. the closest to death, was shown to have formed at 81 days before death (SOM Fig. 2). Measuring the distance between this stress event (i.e., the stress line of this pair closest to death which was easily recognizable in the dentine of LP_4 and RM_2) and death (i.e. the last deposited incremental growth layer, in each tooth) along the dentine tubules provided a local DDSR of 4.4 µm/d (measured from the distance at the blue double-arrow in SOM Fig. 2) and a local DRER of 10.8 µm/d for the LP_4 (green double-arrow in SOM Fig. 2), and a local DDSR=4.3 µm/d and a local DRER=10.0 µm/d in the RM_2. About two dozen Retzius lines were clearly visible at the cervix of the protoconid of the RM_2. In this region, a distance of 100 µm along the enamel prisms corresponds to 8 Retzius lines, or 24 days (see the description in the Methods section and in SOM Table 2). The corresponding EDJ length is 145 µm, yielding a cervical EDJ extension rate of 6.0 µm/d. Likewise, 100 µm measured along the dentine tubules just below the cervix corresponds to a root length of 323 µm but to a faster local DRER of 13.5 µm/d.

Figure 5 illustrates the cumulative increase in tooth height along the EDJ and CDJ (cemento-enamel junction) plotted against tooth formation time for the P_4 and M_2 in RUD 9. It demonstrates the earlier initiation time of the M_2 than the P_4 and also how closely these two teeth track each other in height gained over time. Early root growth rates in each tooth type are notably similar. Figure 6 shows the same data for M_2 in a comparative context. Few truly appropriate data are available for comparison, but the crowns and roots of *Gorilla* M_2s achieve greater heights than those of *Pan* by growing at faster rates. *Hispanopithecus* (Dean and Kelley, 2012) resembles *Pan* in growing a low squat crown more slowly while *Victoriapithecus* (Dean and Leakey, 2004),

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Oreopithecus (Zanolli et al., 2016) and *Anapithecus* show faster rates of crown and root

extension than *Pan* M_2s and more closely resemble those of *Gorilla*.

Crown (CFT) and root formation (RFT) times (Table 3 and SOM Table 2) Neither of the

deciduous premolars showed incremental growth lines or stress lines that could be reliably

identified, in either the enamel or in the dentine (SOM Fig. 3). Cuspal formation times were

calculated from measurements of cuspal thickness using the average CuDSR of 4.4 µm/d. Lateral

enamel formation times in the LC_1, LP_4 and RM_3 were calculated from counts of Retzius lines. In

the LC_1, the enamel and dentine growth lines meet the EDJ with a similar angle, suggesting

comparable secretion rates. Perikymata were visible on the 3D model of the LP_4 buccal aspect,

providing confirmation of the 2D count. Lateral enamel formation time was calculated from

perikymata counts for the RM_2 protoconid and the RM_1 hypoconid. In the case of the RM_1

protoconid, lateral enamel formation time was back-calculated from the age at death.

Despite the fact that no growth increments could be visualized in the LP_3 virtual 2D sections,

perikymata were visible on the 3D rendering of the OES except on the cuspal-most quarter of the

OES. Even though this may be speculative, an attempt was made to calculate CFT, RFT and

initiation time for this tooth. In addition to the 123 perikymata identified on the 3D model of the

LP_3 buccal aspect, the last cuspal-most portion of the OES was estimated to have 21 perikymata,

giving a total of 144 perikymata or 432 days. The cuspal formation time was calculated to be 95

days (418 µm-thick cusp), leading to a total CFT of 527 days. The DRER of the LP_4 was applied

to calculate the RFT: 734.75 µm / 11.4 µm/d = 64 days.

Initiation times Both the RM_1 and the LC_1 show two equally spaced stress lines in their cuspal

enamel (SOM Fig. 5). This temporal equidistance was confirmed by the identification of 13

Retzius lines between both stress lines, in each tooth. Although Retzius lines are difficult to

visualize reliably in the cuspal enamel, it seems very unlikely that there might be a large difference in the actual number of growth layers between both teeth. It follows that, as is commonly accepted for the permanent first molar, the stress line closest to the dentine horn in the canine is reasonably assumed to be the neonatal line (SOM Fig. 5). Since the existence of the NL was demonstrated in both LC_1 and RM_1, the time required to form the prenatal enamel was calculated by multiplying the distance between the dentine horn tip and the NL (along the enamel prisms path) by the average CuDSR of 4.4 μm/d. The canine was found to initiate 38 days before birth, while the RM_1 MB and the DB cusps started forming 49 and 33 days prenatally respectively (Table 4, SOM Table 2).

All the other initiation times were back-calculated by subtracting the CFT and potential RFT from the age at death (SOM Table 2). The LP_4 and the LM_2 were found to initiate 50 and 15 days after birth respectively. As highlighted above, the results for the LP_3 are less reliable than for the other teeth, but the LP_3 initiation occurred 108 days after birth, which is more than 50 days after the LP_4. Last, the RM_3 protoconid initiated 346 days postnatally (Table 4).

Developmental pattern

Age at death Because the cuspal enamel of the LC_1 contains evidence of the neonatal line (SOM Fig. 5), the postnatal crown formation time of this tooth could be used to calculate the age at death. Although the cervix is damaged on the mesial side, by matching several stress lines with the lateral enamel of the RM_3 is was possible to derive the information required to estimate age at death. The LC_1 postnatal cuspal enamel formed in 63 days (277 μm / 4.4 μm/d) and its lateral enamel contained 200 RL (i.e., 600 days) but some enamel was lost postmortem. Lateral enamel formation in the RM_3 could be matched with the LC_1 and continued on for an additional 12 RL.

These additional 12 RL from the RM_3 bring the lateral enamel formation time of the LC_1 to 3 days $\times$ 212 RL = 636 days. This gives an age at death of $63 + 636 = 699$ days or 1.9 years (Fig. 4, Table 4 and SOM Table 2).

The age at death together with the initiation times, the CFT and the RFT allowed a developmental chart to be constructed to visualize the sequence of initiation and crown completion of all teeth investigated up until the time of death (Fig. 4). The three stress markings identified in the RM_3 protoconid enamel and matched on the EDJ and root surfaces of the RM_2 and LP_4 (blue, green and pink lines; Figs. 2 and 4) appear on the chart as vertical lines and provided a means of checking for internal consistency of all the calculations made.

<u>Projection of dental development to completion</u> For comparative purposes it would be useful to attempt to predict the age by which RUD 9 would have completed its permanent dentition. However, since the M_3 had not quite reached crown completion, estimating its final root length and root formation time would be questionable. However, because the M_3 crown is so close to completion, we can at least estimate the time remaining to complete enamel formation based on morphological evidence from other molar teeth belonging to RUD9 and other *Anapithecus* specimens.

We observed that both M_1 and M_2 have complete crowns and that the buccal cingulum is located at ~50% of the total crown height (measured from the cusp tip to the most cervical point in the MB developmental plane – as described in the Methods section and in Smith et al. (2010a) – in the virtual section). This is also the case on the MB aspect of two mandibular third molars belonging to another *Anapithecus* individual, RUD 98, in which the buccal cingulum was located at 50.7% of crown height for the LM_3, and 53.5% for the RM_3. If we assume on this basis that RUD 9's RM3 would have followed the same pattern, then the additional near-vertical length of

EDJ (I would give the length here in brackets to one decimal place maybe?) corresponds to approximately 20 additional Retzius lines in the cervical enamel. This is equivalent to ~60 days to reach crown completion, giving a total CFT of 413 days.

Discussion

Reassessment of Anapithecus *dental development*

Overall, we found a pattern of rapid and precocious dental development in RUD 9, the *Anapithecus hernyaki* holotype, compared to other catarrhines, which was previously highlighted for the species by Nargolwalla et al. (2005). We estimated the age of death of RUD 9 to be 1.9 years. There were some key differences in approach between Nargolwalla et al. (2005)'s work and our study, which go some way towards explaining a number of divergent findings discussed in more detail below. First, it was the aim of Nargolwalla et al. (2005) to pool data from several individuals in order to describe and deduce the pattern and timing of dental development in *Anapithecus* generally, whereas in the present study our aim was to describe the timing of dental development in a single individual. Second, because Nargolwalla et al. (2005) relied on surface features revealed through microscopy and were not able to make histological sections, they made estimates of Retzius line periodicity, cuspal thickness and daily secretion rates, crown initiation times, and root extension rates. In this study, which was also non-destructive, synchrotron micro-tomography allowed us to reveal much of the internal tooth structure, and so enabled informed estimates of, for example, M_3 CFT.

Tooth formation times We found the average CuDSR of RUD 9 to be 4.4 μm/d, far lower than the estimate of 6.5 μm/d assumed for *Anapithecus* by Nargolwalla et al. (2005). Our direct

measurements of cuspal enamel thickness, combined with the calculated average daily secretion rate, considerably increase the cuspal formation time over previous estimates. It is possible that CuDSR might differ from one tooth type to another, but to resolve this would require additional high resolution cuspal scans for each tooth. We used direct counts of perikymata on the 3D models or of Retzius lines in standardized virtual 2D sections to calculate lateral enamel formation time. In cases where direct calculations were not possible, a back-calculation from age at death (by subtraction of any other available formation times) was performed. We thus present revised crown formation times, but based on one specimen of *Anapithecus,* compared to previous work, for example, an LC_1 formation time of 737 days rather than 769 days (Nargolwalla et al.,2005).

For rooth growth, we were able to calculate local (apex) and overall DRERs that yielded mostly smaller values than previous estimates, except for the M_1 where only an overall measurement of DRER was possible (Table 1). In general, our new data support the fact that DRER may change according to tooth locus and also over the course of root growth (Macchiarelli et al., 2006; Dean and Vesey, 2008; Dean and Cole, 2013).

When considering tooth growth as a whole, figures 5 and 6 show that the CFTs of hominoid teeth vary greatly but those with absolutely taller crowns and longer roots appear to achieve this primarily by having faster extension rates in both enamel and root dentine (Zanolli et al., 2016).

Initiation, eruption and gingival emergence

RUD 9 preserves an unerupted permanent canine that we determined to have initiated more than 30 days before birth, *contra* previous estimates of 30 days *after* birth (Nargolwalla et al., 2005). Similarly, we found the M_1 to initiate slightly earlier, at -33 days for the hypoconid and -49 days

for the protoconid, both of which are close to the previous estimate of -30 days (Nargolwalla et al., 2005). The M_1 initiation times we observed for *Anapithecus* bear marked similarities to other catarrhines, such as monkeys (Swindler and Beynon, 1993) and apes (Beynon et al., 1991; Winkler, 1995; Kuykendall, 1996; Dirks, 1998; Kelley and Schwartz, 2010), in contrast to values reported for lemurs, for most of which all three molars are initiated prenatally (Schwartz et al., 2002, 2005 ; Godfrey et al., 2004, 2006 ; Catlett et al., 2010). In most of catarrhines, the M_1 is the only permanent tooth to initiate before birth (Oka and Kraus, 1969; Dean and Wood, 1981; Swindler, 1985). In *Anapithecus*, the canine also initiates before birth (Fig. 4). Because RUD 9 does not preserve any incisors, the overall pattern of eruption and initiation cannot be compared with previous research (Nargolwalla et al., 2005).

We calculated that the RUD 9 RM_2 initiated 15 days after birth, while Nargolwalla et al. (2005) estimated an initiation at 122 days based on its time of presumed gingival emergence corresponding in time to the canine crown completion (which was more securely determined). . Besides the impact of different values for cuspal formation time and periodicity, this difference also stems from the measured M_2 root length: Nargolwalla et al. (2005) reported 4 mm of root on the M_2. However, they likely measured root length in the middle of the mesial aspect of the tooth, while our measurement of RM_2 root length (2933 μm; Table 1) was taken on the developmental section of the protoconid on the buccal side, thus taking into account the corresponding calculated cusp formation time. The impact of this discrepancy is crucial in our calculation: root development does not start exactly at the same time in different points, i.e., at a given time there can be some amount of root already formed on one side of the tooth, while the crown is still developing on another side. We actually used our RFT (thus relying on our root length measurement) that we subtracted together with the M_2 CFT from the age at death to calculate the

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initiation of the M_2. Interestingly, the LP_4 buccal dentine horn is 1.3 times higher than the MB horn of RM_2 (5145 μm vs. 3869 μm; measurements on the developmental 2D sections), and takes 1.3 times longer to grow (502 days versus 367 days). In this context, it is worth recalling that the P_4 is still unerupted and lies deep within the corpus and below the LdP_3 roots, while the M_2 has almost reached gingival emergence. This pattern of eruption is the most common among Anthropoidea (Swindler and Meekins, 1991; Swindler, 2002).

The M_3 initiation is presently documented at 346 days in RUD 9, while Nargolwalla et al. (2005) estimated it to be 493 days. The initiation times for the different teeth reported in this study are even more precocious and closer in time to each other than was estimated by Nargolwalla and colleagues (2005), especially for the M_1 and M_2, which form very early and in rapid succession (Fig. 4). In catarrhines, the overall pattern of initiation reflects the fact that the M1 initiates shortly before birth, then the incisors follow shortly afterwards, and the canine starts mineralizing slightly later (see review in Nargolwalla et al., 2005). The absence of any significant delay between the initiation of the anterior teeth and the M_1 in *Anapithecus* is confirmed by the prenatal initiation of the canine (Fig. 4). Given the observation that in all catarrhines for which data are available, the canine initiates after the incisors (Swindler, 1985), it is reasonable to conclude that the incisors also initiated before birth in RUD 9, and this is the typical condition for strepsirrhines (Nargolwalla et al., 2005). This is supported by the observation that two other *Anapithecus* specimens—RUD 113 and RUD 156—are at the same dental developmental stage as RUD 9, and preserve their incisors crowns, which are fully formed. This tends to lend support to a prenatal initiation of the incisors.

Gingival emergence is an important measure of maturation in primates and some further observations about this in *Anapithecus* are now possible. The M_1 must have been the first

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permanent tooth to erupt, and is the widely established eruption pattern in higher primates (e.g.,

Schultz, 1935). Some attempt can be made to estimate the times of gingival emergence and

attainment of functional occlusion, with the most reliable estimates being for M_2. If one assumes

that the RM_2 is actually very close in time to reaching gingival emergence with just 2933 μm of

root formed on the mesio-buccal side formed over 317 days, then summed with the M_2

protoconid initiation time (15 days postnatally) and its CFT (367 days), this roughly corresponds

to functional occlusion at ~2 years. This is a little more than RUD 9's age at death of 1.9 years.

The time taken to erupt from gingival emergence to the level of the occlusal plane is presumed to

be a short, but is clearly unknowable. Regarding the order in which the permanent canine and

third molar would have erupted, our observations would favor an eruption of the canine before

the M_3 as suggested in Kordos and Begun (2001), rather than a simultaneous eruption as

proposed in Nargolwalla et al. (2005). In spite of the fact that the fossil does not offer any

physical evidence of this, the most parsimonious hypothesis would imply the same order of tooth

eruption as seen in most catarrhines (Table 6). The observation of another specimen of

Anapithecus hernyaki, the female RUD 98, which preserves both the lower canines and an M_3 at

a more advanced developmental stage than RUD 9 adds support to this conclusion. Its M_3s lack

any wear facets but these teeth are probably close to gingival emergence (Nargolwalla et al.,

2005; confirmed on SOM Fig. 7). RUD 98 M_3s have however, little root formed (despite the

breaks, the 2D slice in SOM Fig. 7 confirms it was little expanded — less than 2 mm of root

formed on either side of the 2D section). 3D models of the canines, which have over three

quarters of their root formed (~7 mm), show no sign of any wear (mesial, distal, honing facets)

on this tooth either. The RUD 98 M_3 is only slightly more developed than that of RUD 9 as it is

crown complete with only less than 2 mm of root. In contrast, the RUD 9 canine only has 1.2 mm

of root formed on the lingual aspect, while in RUD 98 the root is ~7 mm long. Thus, by the time of death of RUD 98, three quarters of the canine root were formed when the M_3 developed a ~ 4 mm- high crown (see on SOM Fig. 7) and a ~2 mm-long root. The crucial observation here in RUD 98 lies in the fact that the canine is well ahead of the M_3 in term of development, and is thus more likely to have been also more advanced in terms of eruption or even achievement of the occlusal plane as well. This very short M_3 root would not have been developed enough to ensure sufficient anchorage of the tooth in its socket to function in occlusion during mastication. Based on the amount of root formed, it is likely that the canine had at least erupted through the gingival tissues, by the time of RUD 98's death. In known-age chimpanzees, Kuykendall (1996) has documented emergence of both the lower canine (~8.5 years) and M_3 (~10.5 years) at developmental stage 6; i.e., when the root length is equal to or greater than the crown height. Kelley et al. (2009) have shown, however, that the amount of root formed by the time of emergence on molar teeth is very variable in great apes and it is even more so in modern humans (Liversidge, 2016). For all three great ape taxa (*Gorilla*, *Pan* and *Pongo*), and for all root measurements pooled (mesial, distal and mesio-buccal), Kelley et al. (2009) report a root length at the time of emergence ranging overall between 2.1 and 7.9 mm for M_1, and between 3.2 and 8.5 mm for M_2. Dean and Cole (2013) have further shown that M1 in *Pan* emerges with a shorter length of root formed than in *Gorilla* and *Pongo*, but their M2s emerge with a similar root length formed in all three taxa. Data for M3 in *Pan* is limited but suggests a longer root length may be formed at gingival emergence than in the two other great ape taxa. These authors also suggest that the high furcation level of the *Gorilla* molar roots (i.e., close to the cervix) may represent an alternative to growing a long root — *Gorilla* has longer roots than *Pongo* and *Pan*— by increasing the surface area of attachment of the periodontal ligament to withstand the

considerable chewing forces exerted at the time teeth come into functional occlusion (Dean and Cole, 2013). RUD 98 does not preserve teeth within the alveolar bone, so their relative position to the alveolar margins in situ remains unknown. Nonetheless, the tooth formation stages tend to suggest that the canine would have erupted first in *Anapithecus*, as it has over half of its root formed, and the M_3 only ~ 2 mm formed (SOM Fig. 1).

Interestingly, at the time of death, RUD 9 still preserved deciduous premolars with long unresorbed roots, the dP_4 being only relatively slightly worn (SOM Fig. 1). Besides, the underlying premolar germs are crown complete, while the M_1 is in full occlusion with some moderate wear. The M_2 had erupted to a height about the equivalent of gingival emergence and the P_4 is still unerupted deep in its crypt. Although this configuration seems characteristic of RUD 9, one cannot definitively say whether the M_2 eruption was advanced over the P_4 eruption or vice versa in all specimens of *Anapithecus*. The retention of the deciduous dentition while the molars are reaching gingival emergence close to each other is confirmed by the observations by Kordos and Begun (2001) who also stressed that there was only a subtle difference in stage of wear between the three permanent molars once they are in functional occlusion. All in all, the developmental overlap (initiation and crown formation) between M_1 and M_2 is greater than previously thought, whereas M_3 initiates towards the end of the M_2 crown formation. The early initiation of the first two molars close to birth and their following substantial developmental overlap combined with the relatively early eruption of the M_2 can be interpreted as evidence of rapid dental development and probably life history.

Relatively late premolar eruption may alternatively represent a strategy to maintain a considerable amount of efficient posterior chewing surface throughout ontogeny with deciduous molars retained as long as they are functionally effective given their smaller size and thinner

enamel. The thick layer of cementum on the roots of the deciduous premolars – as well as the already considerable amount of cementum on the incompletely formed M_1 roots – could attest for a demanding loading regime. This could mean that the young had to process its solid food by itself rather early. This juvenile maintained a large chewing efficiency (two deciduous premolars and two permanent molars) before shedding its deciduous premolars, possibly for assuring an efficient foraging strategy when the individual needed to gain its feeding independency towards its mother (weaning) or to cope with a seasonal availability in preferred food items. Several scholars (Eaglen, 1985; Godfrey et al., 2004) have related the occurrence of two waves of eruption for permanent teeth in lemurs with an adaptation to environmental constraints. The eruption of the anterior molars would coincide with a dietary shift which would require relying more on a folivorous diet (Eaglen, 1985). In the case of *Anapithecus*, we do see a first wave of dental eruption with the M_1 and M_2, and later the second wave involving the P_3, P_4 and M_3 (not necessarily in that order). This strategy of adapting dental eruption to the environmental constraints would match the findings by Deane et al. (2013) suggesting that *Anapithecus* was primarily frugivorous and would have relied on leaf-consumption as a seasonal fall-back. The increased seasonality in the Late Miocene was shown to induce a diversification in dietary adaptations among Miocene hominoids with the combination of feeding on soft and hard fruits (Eastham et al., 2016). Paleoclimatic and isotopic data from R. II at Rudabánya have highlighted a clear seasonality, comparable to a present-day subtropical or at least humid warm-temperate climate, with ripe-fruit shortage on a short-term that would compel *Anapithecus* and the sympatric hominoid *Rudapithecus* to rely on harder fruits (Eastham et al., 2016), and *Anapithecus* on foliage, perhaps so as to avoid competition between young *Anapithecus* individuals and *Rudapithecus* (Deane et al., 2013). It has been further argued that a seasonal environment could

exert a strong selective pressure on species to develop fast when the preferred, food resources are available and therefore the timing in dental development would be crucial (Harvey and Clutton-Brock, 1985).

Finally, it has been noted that *Anapithecus* and *Rudapithecus*, with overlapping body masses and possibly overlapping feeding strategies, may have experienced competitive dietary selection pressure resulting in specialized adaptations in each taxon (Deane et al., 2013). Concerning *Anapithecus*, rapid dental development and rapid growth, may indicate that *Anapithecus* was able to achieve shorter generation times than *Rudapithecus*, for which there is evidence of a hominid-like extended life history (Smith et al., 2010b). More work on the paleobiology of *Anapithecus* is needed to work out the adaptive consequences of its pattern of growth and development.

Periodicity and body mass

We have observed a long-period line periodicity of 3 days in RUD 9 (Fig. 3). It was on the grounds of a body mass to periodicity correlation that Nargolwalla et al. (2005) favored a periodicity of 4 days (at most 5 days) in *Anapithecus* but rejected a 3-day periodicity. This decision is based on Dean and Leakey (2004) who noted the body mass estimate of *Victoriapithecus macinnesi,* one of the oldest known cercopithecoids (15 Ma), to be 3-5 kg and that two molars both had a periodicity of 3 days. Body mass and long-period line periodicity are assumed to covary in mammals as a result of a common systemic rhythm, although the reasons for intra-group variation remain unclear (Dean, 1995; Dean and Scandrett, 1996). Whether this correlation is positive or negative remains subject to on-going debates (Smith , 2008; Bromage et al., 2009, and Bromage et al., 2016, respectively). A factor that may influence the robustness of the correlation between periodicity and body mass is the still poorly documented intraspecific

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variability in periodicity in some fossil taxa. This has been demonstrated by T.M. Smith et al. (2015: Fig. 2), who have reported ranges from 6 to 12 days in *Paranthropus robustus*, from 6 to 11 days in *Australopithecus africanus* and 5 to 12 in recent modern humans. Among fossil species, *Megaladapis edwardsi* has a low periodicity (3 days) for a large body mass (~88 kg), early initiation of its permanent molars, high CuDSR (~7 µm/d) and overall rapid dental development (Schwartz et al., 2005). Similarly, in *Palaeopropithecus ingens*, a periodicity of 2 days was found for an animal approaching 45 kg, in combination with accelerated dental development and high CuDSR (Schwartz et al., 2002; Schwartz and Godfrey, 2003). These two examples illustrate that it is possible for large-sized individuals to have a fast pace of development. In addition, in strepsirrhines: low variability in enamel periodicity has been documented in both extant and extinct species in this suborder together with high variation in related metabolic variables (extant species) and body mass (from a few kg to >80 kg; Jungers et al., 2008; Bromage et al., 2009). Biomolecular investigations have recently shown that the protein sequence of amelogenin is different in strepsirrhines than in other primates, which Lacruz et al. (2011) have argued might potentially impact on enamel formation rates in this group. This reveals how difficult and potentially error-prone it can be in general to estimate a periodicity (by as little as 1 or 2 days) from an estimated body size. Our findings allow us to revisit the reasoning of Nargolwalla et al. (2005) and provide new evidence of a 3-day periodicity in the female holotype RUD 9 which body mass was estimated to be close to 15 kg, based on orbital area (Kordos and Begun, 2001; Begun, 2002). However, the modal periodicity of *Anapithecus hernyaki* still remains unknown until a larger sample of individuals has been investigated and it remains possible that *Anapithecus* individuals with 4- or 5-day periodicities (as proposed by Nargolwalla et al., 2005) would be found in sufficiently large samples. Clearly, however,

periodicity estimates within a range of 3-4 days cannot be rejected on the basis body size estimates without data from large samples of individuals. Interestingly, when one plots RUD 9 on Fig. 3a in Hogg et al. (2015; ln[periodicity] vs. ln[body mass]), RUD 9 falls right on the strepsirrhine regression line (although this regression does not seem to be significant at $r^2 = 0.05$). In sum, caution is warranted regarding any of the arguments that have been used previously for estimating enamel periodicities in primates other than by direct observation.

Anapithecus dental development in a comparative context

In comparison with M_2 in *Victoriapithecus* (Dean and Leakey, 2004), for which initiation and gingival emergence have been reported to be 1.95 years apart, this time period is reduced to less than one year in the *Anapithecus* M_2. Addressing the variability of overlap in CFT in sequential molars on a large radiographic sample of modern humans, Liversidge (2015), has however shown that the stage of crypt formation is much more variable than that of cusp initiation. She also showed that M_3 initiation is delayed relative to M_2 more than M_2 is to M_1, as is also observed here in *Anapithecus*.

The crown completion times reported in Beynon et al. (1998) showed that *Ekembo heseloni*[1]— two individuals from the Kaswanga primate site on Rusinga Island: one juvenile (Individual IV, ground section HT3/91G RM_2) and one adult (Individual III, ground sections HT2/91C RM_1 and HT2/91D RM_2) — develops faster than *Ekembo nyanzae* — two adults: RU 1721 RM^1 and RU 1695 RM_2 —. In comparison, RUD 9 achieves the formation of its C_1, P_3, P_4, M_1, M_2, and M_3 (for the last tooth locus, this involves using our estimation of crown completion) crowns faster respectively by 20%, 22%, 18%, 33%, 29% and 35% than *E. heseloni* and by 33 to

[1] Species of *Ekembo* were formerly included in *Proconsul*, see McNulty et al. (2015).

57% (C_1), 60% (M_1) and 57% (M_2) than *E. nyanzae*. While *E. heseloni* completes the formation of all its permanent crowns before the age of 3 years (Beynon et al., 1998, Fig. 13), *Anapithecus* appears to complete all its crowns in just a little more than 2 years of age (Fig. 4). Specific comparisons of molar CFTs are difficult but Zanolli et al. (2016) report a formation time for two permanent second molars of *Oreopithecus bambolii* of 1.96 years. With respect to *Oreopithecus*'s M_2s, the molar crowns of *Anapithecus* appear to form in a considerably shorter amount of time than this (59% faster for M_1, 49% for M_2 and 44% for M_3).

Unlike *Ekembo*, for which an increasing gradient in CuDSR has been observed (Table 6), little variation was observed between the inner and outer CuDSR of *Anapithecus* (Tables 2 and 6). Similar to *Victoriapithecus* (Zanolli et al., 2016), *Anapithecus* shows only a 15%-increase from inner to outer enamel CuDSR. The inner CuDSR of RUD 9 is closest to that of *Afropithecus turkanensis*(17-17.5 Ma; Smith et al., 2003), while the middle CuDSR is most similar to that of *Pongo pygmaeus*(Dean, 1998), and the outer CuDSR approaches the *Pan troglodytes* rate (Smith et al., 2007), all reported in a comparative context in Mahoney et al. (2007). The average CuDSR observed in RUD 9 is very close to those observed in a *Ouranopithecus macedoniensis* RM_3(Smith et al., 2004), and to the average rates of *Afropithecus turkanensis* and of *Pongo pygmaeus* (Mahoney et al., 2007).

Root extension rates are known to vary during root growth in hominoids but little information exists for other primates. Within a single root, in humans and living great apes, root extension rates reach a peak and then fall off towards root completion. Between teeth, DRERs have also been shown to decrease from M1 to M2 to M3 in recent modern humans and *Pan* (Dean and Vesey, 2008; Dean, 2010; Dean and Cole, 2013). How roots grow in other primates has remained largely unknown with the exception of a few fossil primates (Zanolli et al., 2016

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[*Oreopithecus bambolii*]; Dean and Leakey, 2004 [*Victoriapithecus macinnesi*]; Dean and Kelley, 2012 [*Hispanopithecus laietanus*]). The present study is among the first to provide concrete evidence that root extension is not linear in pliopithecoids, as shown with the DRER of the MB RM_2 root, with a cervical rate of 13.5 µm/d, an apical rate of 10.0 µm/d and an overall rate of 9.3 µm/d. In addition, rates seem to differ following the same pattern among tooth loci, with higher rates at the cervix (LC_1 = 12.7 µm/d, RM_2 MB = 13.5 µm/d) than at the developing root apex (LP_4 = 10.8 mm/d, RM_2 MB = 10.0 µm/d).

Conclusions

The present study explores dental development in the dentition of the *Anapithecus* holotype RUD 9, using non-destructive synchrotron virtual histology techniques. This suite of techniques empowers dental anthropologists to retrieve a maximal amount of information from isolated or in situ teeth belonging to a single individual non-destructivelyThis analysis of RUD 9 illustrates the benefits of being able to cross-check information between the 3D and the 2D data. As such it is the first to reach this fine level of detail from a single individual non-destructively.

The fast and precocious nature of *Anapithecus* dental development has been confirmed in this investigation in a single individual dentition, that of the holotype RUD 9. The permanent first molar and canine initiated over a month before birth. The pattern of eruption of RUD 9, as far as this can be determined, seems distinctive with respect to M_2 and P_4, which have the same amount of root formed, while the P_4 still lies deep in its crypt below the unexfoliated deciduous premolars, and the M_2 is close to, as far as can be judged in a fossil specimen, gingival emergence. We conclude that the P_4 erupted much later than the M_2 relative to other catarrhines, possibly to increase the functional lifespan of the chewing surfaces in newly weaned individuals.

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Anapithecus appears to retain a very primitive pattern, closer to strepsirrhines than anthropoids, with early and fast development and persistence of deciduous molars in a mixed dentition that includes a nearly complete M_3 crown. Our analysis suggests that the pliopithecoids, represented by *Anapithecus*, preserve many aspects of the ancestral morphology of the catarrhines in terms of dental development (e.g., fast dental development, early initiation especially of the canine and probably the incisors, M_1 and M_2). Although they are stem catarrhines, they are also representatives of a long lineage dating back to at least 30 Ma, but one that only appears in the fossil record at about 15 Ma in Europe, possibly earlier in Asia (Harrison et al., 1991; Begun, 2002). It is possible therefore if *Anapithecus* had derived characters not found in older stem catarrhines. For example, it is likely that certain aspects of dental development in *Anapithecus* represent adaptations specific to diet, competition, predation or other selection pressures unique to this portion of the pliopithecoid radiation.

Given that dental development in *Anapithecus* more closely resembles that of lemurs than does the dental development of any other catarrhine, we suggest that the condition we have identified in *Anapithecus* may be primitive for catarrhines. . Drawing further conclusions at this point is speculative but the suggestion must be that growth in *Anapithecus* was in general fast and quite contrasting to that of the sympatric hominoid *Rudapithecus* known from the same localities.

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Figure and Table captions

Figure 1. Three-dimensional overview of the RUD 9 dental material. Due to a black coloration of the original specimen caused by taphonomic alterations, the occlusal relief is difficult to see on the original or on photographs. Therefore, enamel and dentine were separately segmented and rendered in 3D. (See SOM Fig. 1 for more details).

Figure 2. Stress matching between the EDJ, root surface and 2D internal structure of LP$_4$, RM$_2$ and RM$_3$. The matching of stress markers on the EDJ and on the 2D internal structure (A for RM$_3$), on the root surface and the 2D section (B for RM$_2$), and between EDJ and root surface (C for RM$_3$, LP$_4$ and RM$_2$) allows cross-checking of the calculations underlying the developmental chart. This allows also visualization of how much tooth was formed across the dentition, and how fast the different tooth types may have grown. This is based on three stress events which are visible as stress lines in the RM$_3$ enamel, in the RM$_2$ dentine and onto the 3D models of the RM$_3$ EDJ, the LP$_4$ and LM$_2$ root surface and the LP$_4$ enamel cap. "H1" is the earlier stress occurring in the RM$_3$ enamel at ~105 RL from death, i.e. at the ~384th day and thus at 1.1 years of the RUD 9 life. "H1" corresponds to the completion of the RM$_2$ protoconid. "H2" is a stress corresponding to the end of the formation of the RM$_3$ cuspal enamel, and occurring at 63 Retzius lines from death (~510th day), i.e. at 1.4 years of life of RUD 9. "H3" corresponds to the completion of the buccal cusp of the LP$_4$, at 49 pk from death, i.e., ~552nd day or at 1.5 years of life of RUD9. The red asterisk on the EDJ of the RM$_2$ (C) figures another stress (no precise time determined) mentioned in the morphological description of the teeth.

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Figure 3. Long-period line periodicity in the metaconid of the RUD 9 RM₃. Sets of three laminations (delineated by green lines) are visible in between Retzius lines (red arrows) on the cuspal 0.638 μm scan, yielding an enamel long-period line periodicity of 3 days.

Figure 4. Developmental chart of the RUD 9 permanent dentition. Initiation, cuspal and lateral enamel formation times as well as root formation times are reported for each tooth investigated. The calculation of each temporal spacing between noticeable stress markings such as birth, three hypoplastic lines and death lock each tooth into the chart chronologically.

Figure 5. Plot of tooth height along the EDJ and continuing on along the cemento-dentine junction against tooth formation time for the LP_4 (B cusp) and RM_2 (protoconid) of RUD 9.

Figure 6. Plots of cumulative growth in M_2 crown and root height along the EDJ and CDJ against tooth formation time (Years). Data for the *Anapithecus* (RUD 9) M_2 and P_4 are compared with published data for five *Gorilla* M_2s (green diamonds) and 10 *Pan* (blue triangles) M_2s and with *Hispanopithecus* (an M^1 with root: IPS-1781, and an M^2 crown: IPS-1794; Dean and Kelley, 2012), *Oreopithecus* (an M_2: FS#BC-nn; Zanolli et al., 2016) and *Victoriapithecus* (an M_2: KNM MB 19841; Dean and Leakey, 2004). The *Pan* and *Gorilla* samples are largely derived from the Elliot Smith Collection at University College London, and are described and referenced in Dean (2010) and Dean and Cole (2013).

SOM Figure 1. Three-dimensional rendering of all of the RUD 9 teeth under the six anatomical angles.

SOM Figure 2. DRER and DDSR in LP_4 and RM_2, by stress matching in the dentine. Several stress lines were identified in the LC_1 (which gives their spacing in time thanks to the corresponding Retzius lines count), in the LP_4 and RM_2 dentine. DRER of the developing roots were thus calculated (since the roots are broken, the final root length is estimated in green from the angle formed by the external root surface and the developing front of dentine internally), as well as DDSR (N.B.: that of the RM_2 MB has been measured on another image following the same principle) over a period of 81 days, corresponding to the time between death and the line closest to death in the double stress (pink arrows).

SOM Figure 3. Developmental stages of the RUD 9 mixed dentition. Developmentally optimized virtual sections have been taken for best representing crown and root development.

SOM Figure 4. The neonatal line was found in the LC_1 and the two M_1 (only the protoconid of the RM_1 is shown here). The neonatal line (pink arrow) was identified as the closest stress to the M_1 dentine horn. A second stress (turquoise arrow) is present in both teeth, roughly corresponding to the first perikymata, and distant of about 13 Retzius lines from the neonatal line.

SOM Figure 7. RUD 98 (*Anapithecus hernyaki*) permanent mandibular teeth. Isosurfaces rendered in Avizo from conventional micro-CT scans acquired at MPI-EVA (Leipzig, Germany) on a Skyscan 1172, at 100 kV and 0.1 mA, with 0.5 mm of Al and 0.04 mm of Cu, and voxel sizes of 10.1 μm (RP_4, LM_2 and LM_3), 13.7 μm (LM_1), 15.8 μm (RC_1) and 17.4 μm (RI_1, RI_2 and RP_3). The labels of the teeth that have reached functional occlusion and thus have wear facets (red arrows) are underlined. Note the amount of root formed on the buccal and apical views of the LM_3, as well as on its mesial virtual 2D section (plane of section in turquoise on the apical view), in comparison to the amount of root formed on the lingual view of the canine.

Table 1. Daily root extension rates (DRER) and dentine secretion rates (DDSR) in several RUD 9 permanent teeth.

Table 2. Cuspal daily secretion rate (CuDSR) in the metaconid of the RUD 9 RM_3.

Table 3. Crown formation times in RUD 9 permanent teeth.

Table 4. Developmental summary of the RUD 9 dentition.

Table 6. Pattern of eruption of the permanent canine and M₃ in Primates. Literature review and estimations from this study.

SOM Table 1. Scanning parameters employed for the synchrotron acquisitions of the RUD 9 dentition.

SOM Table 2. Detailed calculations regarding age at death and the developmental pattern.