ESRF	Experiment title: **Life history variables at the dawn of mammals - using dental increments to make leaps in understanding**	**Experiment number**: ES152
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Shifts: 12	**Local contact(s)**: Vincent Fernandez	*Received at ESRF:*

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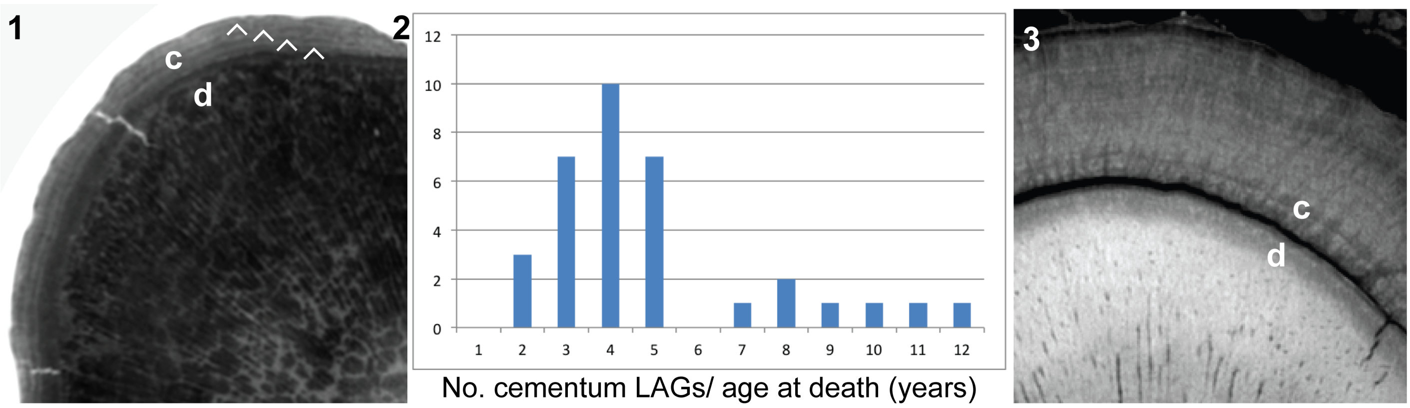
Report:

In this ID 19 experiment we used single distance phase contrast x-ray microtomography to study the molar teeth of the 200 million year old fossil early mammal *Morganucodon watsoni*. The purpose was non-destructive identification, mapping and quantification of growth increments (hereafter referred to as lines of arrested growth, LAGs) in order to reconstruct life history variables in the earliest mammals. The initial results are being analysed for presentation at an international conference in November, and, with suitable additional data, for publication in a high profile journal.

We used three different voxel resolutions, principally 0.35um but also 0.28um and 0.70um, and concentrated mainly on the cementum LAGs found in the tooth roots of *M. watsoni*. Cementum is a collagenous mineralised tissue that connects tooth roots to the periodontal ligament, supporting dentition within the alveolus against forces applied through mastication. Cementum LAGs have been shown to reflect seasonal changes, creating alternating bands within the tissue. As cementum grows continuously through life, these bands offer an absolute record of annual seasonal change, and hence an estimate of annular age and season of death. Cementum lines obtained through destructive thin sectioning have been used for ageing a range of taxa, including humans, and for archaeological, zoological, palaeontological and forensic purposes including conservation and identification of crime victims. However, limitations of the method have been identified, prinicipally due to difficulties in objectively quantifying LAG numbers from single histological thin sections of variable location and quality. We aim to improve the method through the use of tomographic acquisition of cementum LAGS, allowing more accurate quantification of LAG numbers due to the availability of whole root LAG data instead of single tooth root thin sections per specimen. Additionally, we are developing new methods for objective quantification of cementum LAG counts from tomographic data.

More than 160 measurements/scans were completed during the 12 shifts. Of these, over 130 were of *M. watsoni* tooth roots in order to observe cementum and dentine LAGs. A further 10 were of *M. watsoni* tooth crowns or lower jaw bones, in order to observe dentine and enamel LAGs, and bone LAGs, respectively. A small number of remaining scans were trials or to provide pilot data for future ESRF applications on this and closely related projects. Since each *M. watsoni* cheek tooth has two roots and the

region of interest volume size at these high resolutions does not encompass the length of an entire root, many isolated teeth required multiple measurements for complete coverage. Specimens were discarded if initial reconstructions revealed a lack of LAG resolution due to internal damage or chemical diagenetic alteration during fossilisation. This resulted in 85 individuals of *M. watsoni* being measured. Some were represented by single tooth roots and even single scan volumes, while others were jaws with teeth in situ, requiring multiple measurements to cover all teeth in the tooth row, all roots of a tooth, the entire volume of a tooth root, plus multiple regions of the bony dentary. In general, the number of cementum LAGs was extremely consistent between both roots of single teeth, between teeth along the jaw, and between tooth root cementum and dentary bone LAGs in specimens where both could be measured. However, LAG preservation varied substantially along individual roots in half of all specimens. This highlights a key advantage of using non-destructive tomographic imaging rather than conventional destructive histological thin sectioning, as we show here for the first time. Limited numbers of thin sections can be made from a single tooth root, and resulting LAG counts can vary depending on where these are taken. In contrast, tomographic data allows an overview of cementum LAGs across the whole root, and multiple areas with the clearest LAG preservation can be compared for consistency.



Figures 1-3. 1) Virtual cross section of *Morganucodon* tooth root**.** Cementum lags visible in outer cementum layer and highlighted with white arrows. **2)** Frequency distribution showing number of tooth root cementum LAGs and hence age in years at death of *Morganucodon* individuals, n=35. **3)** Virtual cross section of *Morganucodon* tooth root showing high number (~12) of cementum LAGs, corresponding with a high longevity/maximum lifespan of ~12 years for a small (ca. 30 grams) mammal. c = cementum, d = dentine.

Of the 85 individuals of *M. watsoni* for which measurements were made, preliminary analysis has shown that 35 individuals (41%) were of sufficient preservational quality to allow cementum LAGs to be resolved (eg Fig. 1). The remainder typically showed heavy diagenetic chemical alteration and/or damage to the cementum, either or both of which served to obscure the cementum LAGs. In addition some otherwise well preserved specimens entirely lacked the cementum layer, which could only be determined by examination of the synchrotron tomographic data. Preliminary counts of cementum LAGs for the 35 individuals allows a graph of the distribution of the annular age, and hence age at death, for a population sized sample of *M. watsoni* (Fig. 2). Results show a moderately flattened, heavily right skewed (kurtosis = 1.4, skew = 1.4) distribution with an age at death mean of 4.9 years, median and mode of 4 years, and maximum of ~12 years. This relatively high maximum cementum LAG number (Fig. 3) and hence maximum age/lifespan is towards the top of the range found for extant mammals of similar body mass, suggesting physiological differences between *M. watsoni* and living mammals..

We additionally analysed two specimens of the early mammal *Kuehneotherium* from similarly aged and nearby fissure deposits to the *M. watsoni* specimens, but with a different depositional and preparation history. LAGs can be clearly seen in the cementum of tooth roots in *Kuehneotherium*, which expands the potential for future comparative study of a larger sample of the earliest mammals. Trial measurements of the tooth root cementum of extant taxa of different sizes and morphology from *M. watsoni* required time consuming adjustments to experiment parameters, but we were able to obtain results sufficient to suggest that such specimens would also give good results in a future experiment. We therefore intend to apply for further beamtime on ID19 to allow these comparative analyses of new fossil and extant taxa to be conducted.