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Application for beam time at ESRF – Experimental Method

The emergence of mammalian physiology: life histories inferred from dental parameters of the gomphodonts

The first mammals to appear in the Late Triassic underwent radical physiological changes such as the evolution of active lifestyles and rapid growth rates [1] laying the foundations for the features that characterise the group today. However, the origins of these defining traits remain unclear, due to an absence of data on life history and growth rates in extinct mammalian predecessors. To address this, we propose the collection of high-resolution synchrotron radiation-based X-ray computed tomography (SRCT) data of tooth cementum increments in some of the closest non-mammalian ancestors, the gomphodonts, allowing the first estimates of individual ages and growth rates in these important fossil species.

Scientific background :

We aim to study cementum growth in Triassic gomphodonts, a group that are among mammals' closest relatives and are abundant in the fossil record. Gomphodonts underwent a radiation in the Triassic that predates the origin of true mammals. They convergently evolved some of the same features linked to novel physiological changes in mammals, such as reduced tooth replacement frequencies and complex occluding teeth [1]. Together, these traits make gomphodonts an ideal group for studying the emergence of mammalian physiology. However there is currently no quantitative data on the cementum patterns or other indices of life history in this group - only a handful of thin-sections have been described [2].

Cementum is a unique mineralised dental tissue that grows throughout life and has typically been studied using destructive thin-section histological techniques. Under transmitted light, cementum comprises a series of alternating bands of differing optical density, forming thick 'light' increments and thin 'dark' increments. The dark increments represent lines of arrested growth (LAGS) that chart seasonal changes in growth rates [3]. Thus, LAGs act as dental age markers that provide a hard-tissue record of growth and development [4] that has been used for estimating age in a range of taxa—including humans—in archaeological, zoological and palaeontological contexts [5]. Dental age is important for inferring physiological evolution because the transition from continuous to once-in-a-lifetime tooth replacement indicates a switch between the slow, continuous growth strategies of mammalian precursors, and the rapid determinate growth that characterizes modern mammals.

Our previous experiments at the TOMCAT beamline imaged cementum increments in individual teeth of early mammals from the Jurassic to infer their chronological lifespan and understand the maximum age obtained by extinct mammals. These data provide proof-of-concept for the proposed work by piloting visualisation of fossil cementum increments (Fig. 1). Importantly, our proposal differs from those experiments because we aim to assess the age of multiple teeth along the tooth row from a single individual. By comparing the ages of multiple teeth, we can assess both relative and absolute patterns of tooth replacement. In modern mammals this replacement is a key innovation that occurs once in predictable patterns along the tooth row when the animal obtains adult size, and is coupled with a cessation of growth. This pattern appears to be present (with minor variations) from the earliest mammals. However, little is known about where on the tree of mammalian evolution this key innovation evolved. Combining these data with body mass estimates derived from the skeleton, and LAG data previously obtained for Jurassic and modern mammals, we will be able to reconstruct changing growth rates and life history patterns at key points in the mammal lineage.

Experimental technique(s), required set-up(s), measurement strategy, sample details (quantity...etc):

We have secured ten fossil specimens from four gomphodont genera representing several distinct growth stages, and therefore providing insights into growth rate and other aspects of life history. Each specimen comprises a partial dentary and/or maxilla containing 3-15 teeth, ranging in size from 3-10 mm length with a total of 145 teeth in the complete sample. We aim to characterise the cementum increments in every tooth in the tooth row. This will involve vertically mounting each specimen using florex dry foam and working systematically along the tooth row, imaging each preserved tooth. For each tooth, the region of interest is

the root from just below the crown, immediately below the alveolar margin (LAGs in this region show the highest correlation with actual age in living mammals [6] (Fig. 1).

Cementum is composed of inorganic hydroxyapatite (45%), an organic component of mostly collagen fibers (33% by volume), and water (22% by volume) [7]. From our previous experience and published work [9], fossilised cementum is denser to synchrotron X-rays than 'fresh' cementum. LAGs range from ~1-15 µm in transverse thickness. We thus require a combination of high X-ray energies and <1 µm isotropic voxel sizes to accurately image these fine details in our fossil samples. Single propagation distance phase contrast reconstruction techniques are also essential for our data, providing an exponential increase in contrast sensitivity for localised tomographic imaging such as ours.SRCT data will be processed by EP and EN using Fiji/ImageJ [8]. Quantitative morphometry for LAGs will include measures such as number, thickness, separation and their respective variations. These data will be obtained by both manual measurements and algorithmic computer vision approaches developed by EN.

Beamline(s) and beam time requested with justification :

The sub-micron isotropic voxel sizes offered at the BM05 imaging beamline are crucial to this study due to the μm size of cementum LAGs. Data on cementum increments have been obtained from similarly sized human teeth using the BM05 beamline [9], achieving voxel sizes of 0.615 μm. Our team has developed a robust methodology and optimization procedure through previous experiments imaging cementum at SLS (TOMCAT) and ESRF (ID19). Our samples comprise 145 teeth from 10 partial skulls and skull fragments. Based on our previous experience, we anticipate an estimated 6-8 hours for experimental setup and optimisation of settings. This will be followed by individual scans of the same cementum region of each tooth, ranging in time from 12-15 minutes. The number of teeth varies between specimens, but we estimate 37 hours to scan 145 teeth. We estimate additional time for mounting and transferring specimens, and region selection of approximately 30 minutes per jaw (5 hours total). We therefore require approximately 50 hours total scan time.

Results expected and their significance in the respective field of research :

Our experiment will constitute a novel use of SRCT to understand growth and longevity in the closest relatives of mammals, with implications for evolutionary biology, palaeontology, anatomy and zoology. This experiment will allow us to answer two fundamental questions in palaeontology: 1) what is the macroevolutionary patterns of the emergence of the mammal lineage; and 2) what is the physiology of stem mammals. It will also provide the first-ever quantitative data on cementum increments in gomphodonts, which can be used to understand more specialist questions in the group such as the age at death of individuals.

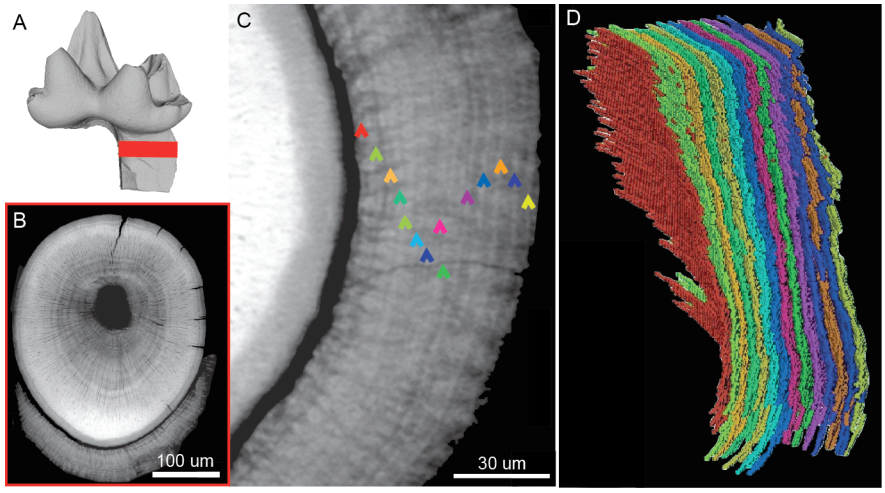

Figure 1: (A) Three-dimensional model of molar tooth of Jurassic mammal, showing optimal area for cementum retrieval (red); (B) SR CT slice of a Jurassic mammal with high increment count; (C) detail of cementum increments (arrows); (D) 3D model of increments imaged in (C).

References

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