

Preliminary Report on the Deciduous Premolars of “Sasha”, the First Infant Woolly Rhino (*Coelodonta antiquitatis*) to be discovered

W. Dirks¹, O. Potapova², C. Witzel³, U. Kierdorf³, H. Kierdorf³, A. Protopopov⁴

¹ Department of Anthropology, Durham University, UK; ² The Mammoth Site of Hot Springs, USA; ³ Department of Biology, University of Hildesheim, Germany; ⁴ Department of Mammoth Fauna Studies, Yakutian Academy of Sciences, Russia

Introduction

The world's first frozen mummy of an infant woolly rhino was discovered in the Abyysky district of Yakutia, Russia in 2014, and given the nickname “Sasha” (Fig. 1). It has been dated to over 45.300 years cal BP, from the Karginsk interstadial of the Weichselian glacial. Our investigation aims to determine the age at death of the individual by microscopic analysis of incremental markings in the enamel and dentine of two deciduous premolars (right dP³ showing slight occlusal wear, Fig. 2, and unerupted, unworn right dP⁴, Fig. 3). In teeth whose formation extends from the prenatal to the postnatal period, a prominent incremental marking, the neonatal line (NNL), indicates the stage of tooth development at birth. This allows an age-at-death estimation based on increment counts in the postnatally formed tooth portions. In enamel this is possible only until the end of crown formation, while dentin formation continues after crown completion.

Materials and methods

Teeth were first assessed macroscopically and afterwards cut longitudinally as indicated (red lines) in Figs. 2 and 3. One of the resulting tooth halves was then embedded in epoxy resin. Polished block surfaces were inspected in the backscattered electron (BSE) mode in a Zeiss EVO MA 15 SEM. Individual grey scale figures were converted to pseudo-color images using Image J 1.46. Subsequently, 40-50 µm thick ground sections were prepared from the blocks and examined under transmitted plain light and transmitted light with phase-contrast in a Zeiss Axioskop2 light microscope. In the ground sections, incremental markings (laminations and prism cross-striations in the enamel and Andresen lines in the dentin) were recorded and their numbers counted.

Results and Discussion

In the dP³, a NNL was present in crown and root dentin (Fig. 4), but not in the enamel. The outcrop of the NNL at the root surface was marked by a prominent periradicular band (Fig. 5). In contrast, in the dP⁴ a NNL was present in both, the crown dentin and the enamel (figs. 6-8). These findings indicate that in the dP³ crown formation had been completed prior to birth, while crown formation in the dP⁴ was going on at birth. In both teeth, pre- and postnatally formed tooth portions could clearly be distinguished (Fig. 6).

Counting of daily incremental markings (laminations) in the postnatal enamel of the dP⁴ revealed a postnatal crown formation time (CFT) of about three months (independent lamination-counts varied between 87 and 92 days). In the postnatally formed dentin of the dP⁴, 40 Andresen lines were recorded. Of these, about 25 correspond to the postnatal period of enamel formation in this tooth. This indicates a periodicity of either three or four days for the long period lines in the dentin and a resulting postnatal lifespan between 120 and 160 days for the individual. This finding is in overall accordance with reports of gingival emergence of the dP⁴ in the white rhino (*Ceratotherium simum*) at an age of about 140 days (Hillman-Smith et al., 1986, *J. Zool., Lond.* 210A: 355-379).

In conclusion our study revealed that “Sasha” died at an age between 4 to slightly more than 5 months.



Fig. 1. The discovered remains of “Sasha”

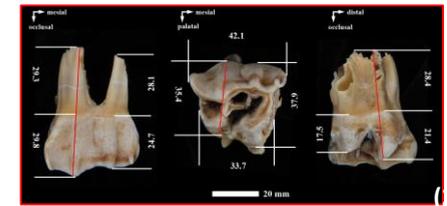


Fig. 2. Right dP³, viewed from buccal (left), occlusal (center), and palatal (right). Red lines marks the section plane.

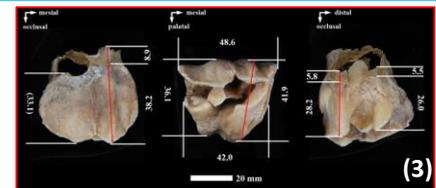


Fig. 3. Right dP⁴ viewed from buccal (left), occlusal (center), and palatal (right). Red lines mark the section plane.

Fig. 4. NNL in root dentin of the dP³ presents as a band of interglobular spaces. Prenatally formed dentine to the right of the NNL.

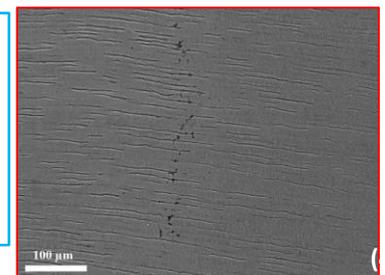


Fig. 5. Right dP³ viewed from distal. A prominent periradicular band (asterisks) marks the outcrop of the NNL at the root surface. Note further periradicular bands apically to the NNL. C: Coronal cementum.



Fig. 6. Prenatally formed dental hard tissues in the dP³ (right) and the dP⁴ (left) are shaded in red.

Fig. 7. Pseudo-color BSE-SEM image of polished cut surface of the longitudinally sectioned dP⁴. Each color represents a grey-level band of 16 grey levels covering the range from black (grey level 0) to peak white (255), thereby indicating the varying degrees of tissue mineralization. Only the cuspalmost enamel and dentin are (almost) fully mineralized. Arrowheads mark the course of the NNL in the dentin.

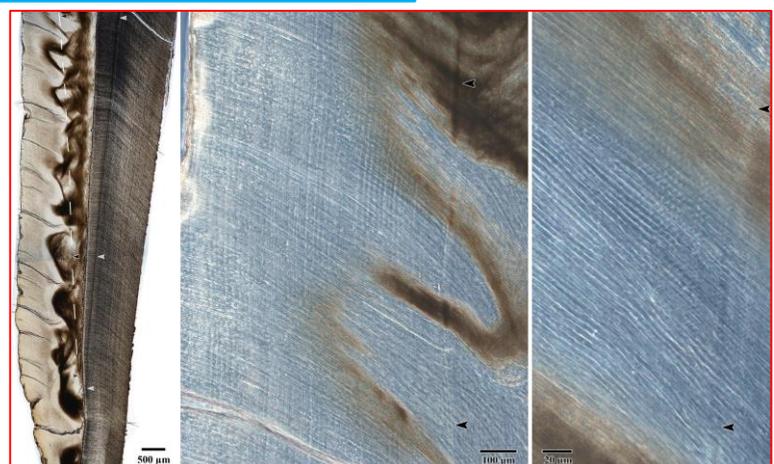
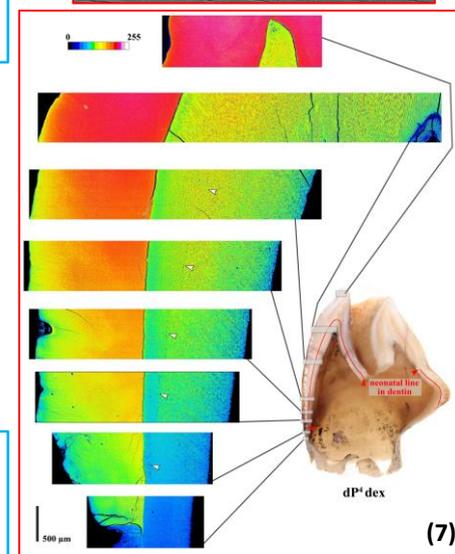


Fig. 8. Longitudinal ground section through dP⁴ showing the course of the NNL (stippled line in enamel and arrowheads in enamel and dentin) in cervical enamel and dentin (left image). Note prominent incremental markings in the postnatal dentin. Higher magnifications (phase-contrast) of mid-coronal enamel demonstrate daily incremental markings (laminations, central image) and sub-daily prism cross-striations (right image). Occlusal to top of images.

Friday plenary poster R9

**PRELIMINARY REPORT ON THE DECIDUOUS PREMOLARS OF “SASHA,”
THE FIRST INFANT WOOLLY RHINO (*COELODONTA ANTIQUITATIS*) TO BE
DISCOVERED**

**W. Dirks^{1*}, O. Potapova², C. Witzel³, U. Kierdorf³, H. Kierdorf³, and A.
Protopopov⁴**

¹*Durham University, Dawson Building, Department of Anthropology South Road, Durham,
DH1 3LE, UK*

²*The Mammoth Site of Hot Springs, SD, Hot Springs, SD 57747, USA*

³*University of Hildesheim, Department of Biology, Hildesheim, 31141, Germany*

⁴*Yakutian Academy of Sciences, Department of Mammoth Fauna Studies, Yakutsk 677007,
Russia*

*wendy.dirks@durham.ac.uk

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The world's first frozen mummy of an infant woolly rhino was discovered in the Abyysky district of Yakutia, Russia in 2014, and given the nickname “Sasha.” The partial mummy is remarkably well preserved, with soft tissue, including skin and fur, as well as most of the skull and teeth. It has been dated to over 45,300 calBP, from the Karginsk interstadial of the Weichselian glacial. Our team is undertaking the analysis of the DP3 (slight occlusal wear) and DP4 (unerupted) These analyses will include determination of the age at death from histological sections, trace elemental analyses to determine the process of weaning, and isotopic analyses to determine season of death. Eruption order of the deciduous premolars in both black and white rhinos is DP3, DP2, DP4, DP1. The first deciduous premolar erupts at ~ 12-14 months. In Sasha, the order of deciduous premolar eruption appears to differ from that in the extant African rhinos. The DP1 is already in place but the dp1 has not erupted through the gingiva. Our preliminary investigations suggest that Sasha is much younger than a year. The position of the neonatal line, visible in the dentine of the DP4, is very close to the end of crown formation, indicating that most of the crown was formed prenatally. The root stock has partially formed but not yet divided, also indicating that the calf was quite young at death, probably only a few months old, which will be determined with histological analysis. Neither the enamel nor the dentine is fully mineralized. Hypoplastic enamel defects are visible in both analyzed deciduous premolars. Trace element analyses combining dental histology and LA-ICP-MS will determine the age at which the calf began to eat solid food, which can be as young as a week in extant rhinos. SEM observation revealed that the enamel is highly decussated, as was reported in previous studies of rhino enamel. Our analyses will add significantly to the understanding of the biology and ontogeny of woolly rhinos.



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