Uneven distribution of enamel in the tooth crown of a Plains Zebra *Equus quagga*

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Unworn teeth of herbivorous mammals are not immediately functional. They have to be partially worn to expose enamel ridges which can then act as shear-cutting blades to break the food down. We use the Plains Zebra (*Equus quagga*) as a hypsodont, herbivorous model organism to investigate how initial wear of the tooth crown is controlled by underlying structures. We find that the enamel proportion is smaller at the apical half of the tooth crown in all upper tooth positions and suggest that lower enamel content here could promote early wear. Besides this uneven enamel distribution we note that the third molar has a higher overall enamel content than any other tooth position. The M3 is thus likely to have a slightly different functional trait in mastication, resisting highest bite forces along the tooth row and maintaining functionality when anterior teeth are already worn down.

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9   Introduction

10   Hypsodonty has been defined as the relative increase in the height of cheek teeth (Van Valen, 1960), or more
11   simply as larger tooth crown height compared to tooth crown length (White, 1959; Thenius, 1989; compare
12   Figure 1). It is a common evolutionary strategy of herbivorous mammals to counter high abrasive loads in the
13   ingested diet, which result in a high degree of dental wear. Following White (1959), cusp hypsodonty, tooth
14   base hypsodonty and root hypsodonty can be distinguished, depending upon which structure is elongated.
15   Hypsodonty can be easily achieved in all tooth positions by extending specific ontogenetic phases during tooth
16   development (von Koenigswald, 2011). Newly erupted hypsodont cheek teeth share a feature among all taxa:
17   they are not immediately functional. To comminute tough plant matter, the relatively rounded apex of the
18   (pre)molar tooth crowns has to wear down slightly, exposing the enamel ridges which may then act as shearing
19   blades during mastication. The rapid wear of the topmost tooth crown has been noted in selenodont molars
20   (Osborn and Lumsden, 1978), and several authors have hypothesised how this initial wear is facilitated. One
21   theory is that empty chewing movements (thegosis) sharpen teeth in adults and initiate wear in young animals
22   (Every, 1972; Teaford and Walker, 1983, Every et al. 1998). More often, however, such empty chewing is
23   considered a behavioural anomaly (termed bruxism or pathological thegosis) which appears in livestock, other
24   domestic or captive animals (e.g. Murray and Sanson, 1998; Troxler, 2007; 2012) and also in man (Manfredini
25   et al., 2013). We do not think that thegosis is needed to initiate functionality and in lack of empirical data see
26   not much support to this hypothesis.

27   We therefore propose that the apex of the tooth crown should be less resistant to all wear mechanisms.
28   Abrasion (tooth-to-food contacts including adhesive particles such as grit and dust) and attrition (tooth-to-
29   tooth contact during chewing movements) would hence be sufficient to promote early wear and expose
30   functional enamel ridges quickly. This could be accomplished by either building the apex of the tooth crown
31   from less and/or thinner enamel or by building a less resistant enamel microstructure. Both hypotheses
32   suggest that the apex of the tooth crown is structurally different from the rest of the tooth. Analysis of enamel
33   microstructure at different tooth crown heights is a destructive and time consuming method, therefore we
34   chose to study enamel distribution within the tooth crown of a subadult Plains Zebra (*Equus quagga* sp.) using
35   micro CT-scanning. The Plains Zebra is an ideal model organism for a large, hypsodont herbivore, because it is
36   adapted to grazing in both arid and savannah climates and therefore needs to have a high tolerance of
37   abrasional tooth wear. Its diet is composed of up to 90 percent grass but also includes some browse (Nowak,

38 1999). Grass lamina is generally narrower and more uniform in thickness than browse. Vascular bundles are
39 evenly distributed and generally very narrow. The fibre bundles in grasses are more evenly distributed and in
40 higher levels than in most dicotyledonous browse. This requires more energy to separate the grass into smaller
41 fragments and this is most economically achieved by shear-cutting, rather than crushing or grinding (Archer
42 and Sanson, 2002). Grasses have higher internal abrasive contents than browse (McNaughton et al., 1985;
43 Piperno et al., 2002) and often also bear more airborne grit and dust because they are common in arid,
44 savannah-like habitats. Specialised grazing species therefore tend to be the most hypsodont, as they
45 experience high abrasional wear. Amongst extant large herbivore species, the Equidae exhibit the highest
46 degree of hypsodonty, only equalled by a few Bovidae like *Bison bison* (compare hypsodonty indices in Janis,
47 1988).

48

49 Material and Methods

50 The selected individual (specimen no. 70335) is a loan from Museum für Naturkunde (Berlin). It shows very low
51 or no wear on the premolar and molar teeth and is therefore in the optimal stage to investigate enamel
52 distribution within all tooth positions of the same individual. The tooth eruption sequence for upper
53 permanent teeth in *Equus quagga* is M1, M2, I1, P2, P3, P4, I2, C, M3, I3 (Erz, 1964). Hence we see small
54 amounts of material loss in the earlier erupting teeth M1 and M2 compared to the unworn P2, P3, P4 and M3.
55 Before scanning the caps of the deciduous premolars must have been present, but were lost. The roots of all
56 premolars and molars seem to be open, however, some parts of the roots might have been damaged during
57 extraction from the skull. For this pilot study we did not use unworn premolars and molars of several
58 individuals, but one postcanine dentition of a single specimen. We focus on the upper permanent dentition,
59 because upper teeth are employed as the standard in studying dental characteristics (Archer and Sanson, 2002;
60 Fortelius and Solounias, 2000; Solounias and Semprebon, 2002) and functional traits should be more
61 pronounced as compared to lower teeth (Kaiser and Fortelius, 2003) due to the lack of gravity impact. High
62 resolution computed tomography (microCT) scans with a voxel size between 0.075 and 0.1 mm (see Appendix
63 1) were obtained at Steinmann-Institut für Geologie, Mineralogie und Paläontologie (Universität Bonn,
64 Germany) on the CT scanner v|tome|× s (GE phoenix|x-ray). All teeth were scanned individually after
65 extraction from the skull. The software VG StudioMax 2.1 (Volume Graphics, Heidelberg) was used for
66 reconstruction of virtual models and further processing. First, each tooth was reconstructed with all dental
67 tissues (enamel, dentin and cementum) as a voxel model using manual and automatic segmentation tools. All
68 models were cut at the base of the crown by fitting a clipping plane through the cementoenamel junction.
69 Then only the voxels above the clipping plane were selected to create models of the tooth crown. Next the
70 dental tissues enamel, dentin and cementum were individually segmented (Figure 2.a-c and Figure 3.a-b).

71

72 We created a pure enamel voxel model and two full tooth models, one with all dental tissues, one without
73 cementum, and cut them at approx. 75%, 50% and 25% of the initial crown height and created individual
74 models of four tooth sections: Section 1 from the apex at 100-75% crown height, Section 2 from 75-50% crown
75 height, Section 3 from 50-25% crown height and Section 4 from 25% down to the base of the crown (Figure

76   3c). Cutting planes had to be inserted manually and could therefore not exactly represent quarters of the tooth
77   crowns. Volumes of the enamel sections and full tooth sections were taken directly from the properties of
78   these models as displayed in VG StudioMax. We then calculated relative enamel content per section by
79   dividing the enamel volume of that section by total volume of the respective section. We further subtracted
80   the volume of cementum and repeated the same calculation without cementum contributing to the total
81   volume. Finally the quotient from enamel by dentin was calculated to express the ratio between those two
82   dental tissues.

83

84   Results

85   Data on enamel content in relation to the whole tooth volume and the whole volume without cementum are
86   given in Table 1. Though distribution of enamel content per section was variable between teeth, it was
87   consistently smallest in Section 1 (the most apical section) for all tooth positions when total volume was
88   considered. In relation to the tooth model without cementum, however, either Section 1 or Section 2 had the
89   lowest enamel proportions. This shifted ratio results from a higher cementum deposition at the apex of the
90   tooth. For the full tooth model, Section 4 had the highest enamel proportions within each tooth position.
91   When cementum was left out, either Section 3 or Section 4 had the highest relative enamel content (compare
92   Table 1). It is notable that M3 was composed of more enamel than all other teeth (>7% higher relative enamel
93   content than all other tooth positions). This is well expressed in the enamel/dentin-ratio (Figure 4.B). From P2-
94   M2, each section except P4 section 4 contained less enamel then dentin. For the M3, however, this ratio
95   reversed as each section contained more enamel than dentin.

96

97   Discussion

98   The results of this study support our hypothesis that the apex of the tooth crown is structurally different from
99   the rest of the tooth. We have shown that the overall enamel content is lowest at the crown apex and highest
100 in the lower half of the crown, regardless of the influence of cementum.

101 There are relatively more soft dental tissues (dentin and cementum) at the apex of the crown and therefore
102 this part of the tooth is prone to faster wear. We further note that the base of each tooth seems to be
103 structurally "enhanced", as the larger enamel content should strengthen it and help resist high pressure and
104 stress loads.

105 In *Equus quagga*, we find the third upper molar to be structurally different from all other upper teeth, as it has
106 the highest proportion of enamel along the tooth crown. We relate this phenomenon to adaptive pressures
107 related to two phenomena:

108   1.   Mechanical constraint: As the M3 is closest to the temporomandibular joint, the highest
109   masticatory forces can be generated here (Greaves, 2012). The high enamel content will then
110   prevent excessive wear and maintain evenly distributed chewing forces.

111   2. Biogenetic constraints: The M3 is the last tooth to erupt in most mammals, and also in the Zebra.
112   Therefore it is also the tooth position maintaining function when anterior teeth are already
113   wearing out.

114 In general, by being more resistant to wear, M3 can thus compensate for the functional loss of anterior teeth.
115 A similar observation was also made by Kaiser 2002 for the extinct equid *Cormohipparion occidentale*, in which
116 M3 shearing function is optimised in later ontogenetic stages. We observe that the apex of the M3 crown also
117 has the lowest enamel proportion compared to the remainder of tooth; however the enamel content is higher
118 than in the apex of any other tooth along the dentition. We hypothesise that it therefore takes longer for
119 enamel ridges on M3 to become exposed and that wear is hence delayed, which further adds to M3 serving as
120 a reserve once anterior teeth wear out.

121 Though *Equus quagga* is an appropriate model organism, these observations are still restricted to one single
122 specimen of this taxon. They can, however, help us to refine hypotheses on how mechanical and ontogenetic
123 constraints of wear and resistance may be solved in a biological system, by slight modification of a common
124 structure. The findings suggest that different functional requirements at different tooth positions and tooth
125 wear stages have shaped tooth morphology to a large extent and that even hypsodont forms are prone to high
126 levels of functional adaptation. As mechanical constraints triggering these adaptations must universally apply
127 to all mammals feeding on abrasive diets, we expect to find similar traits in other herbivorous species,
128 including bovids.

129

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135 enhancement in the mammalian dentition—phylogenetic and ontogenetic impact on the masticatory
136 apparatus''.
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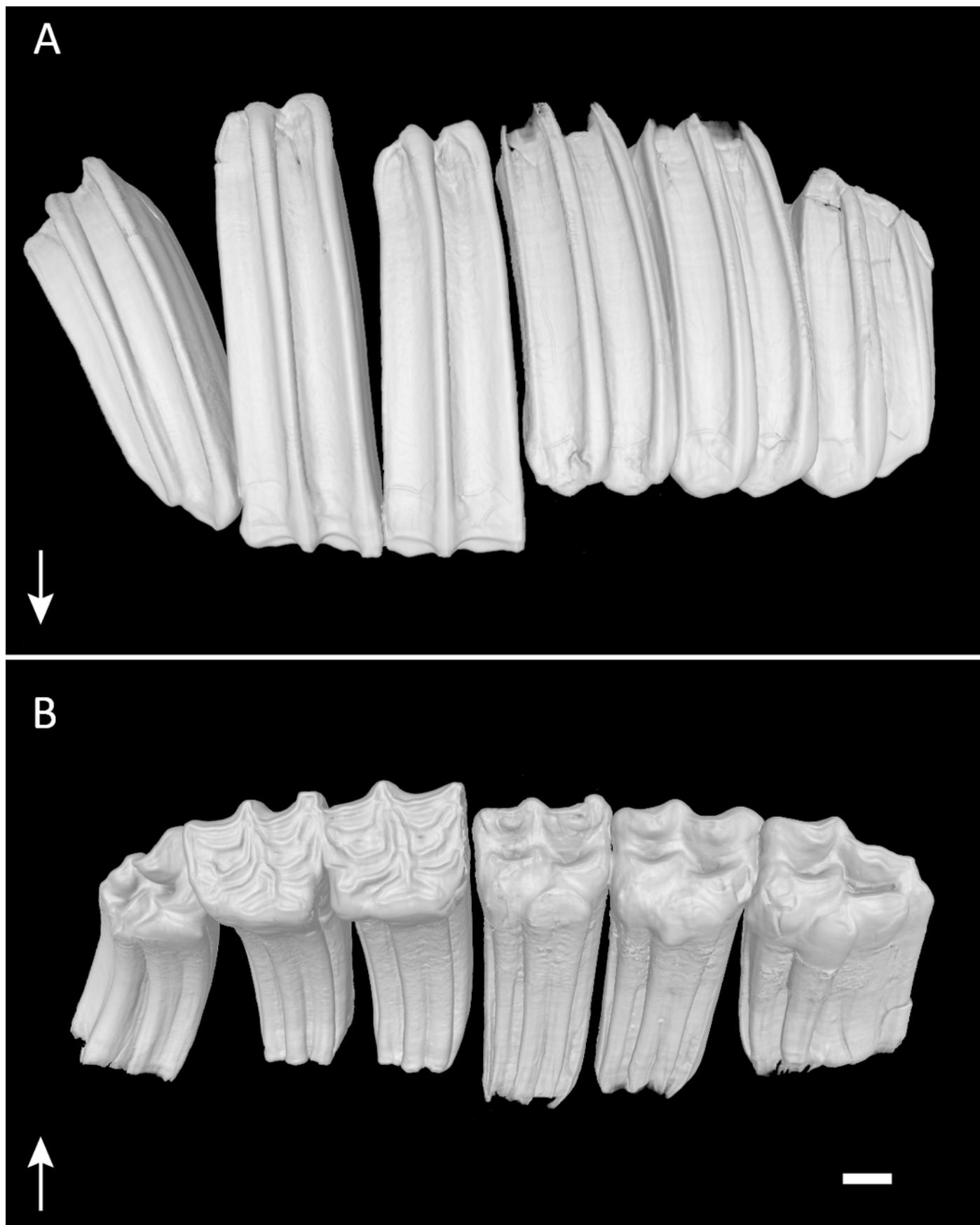
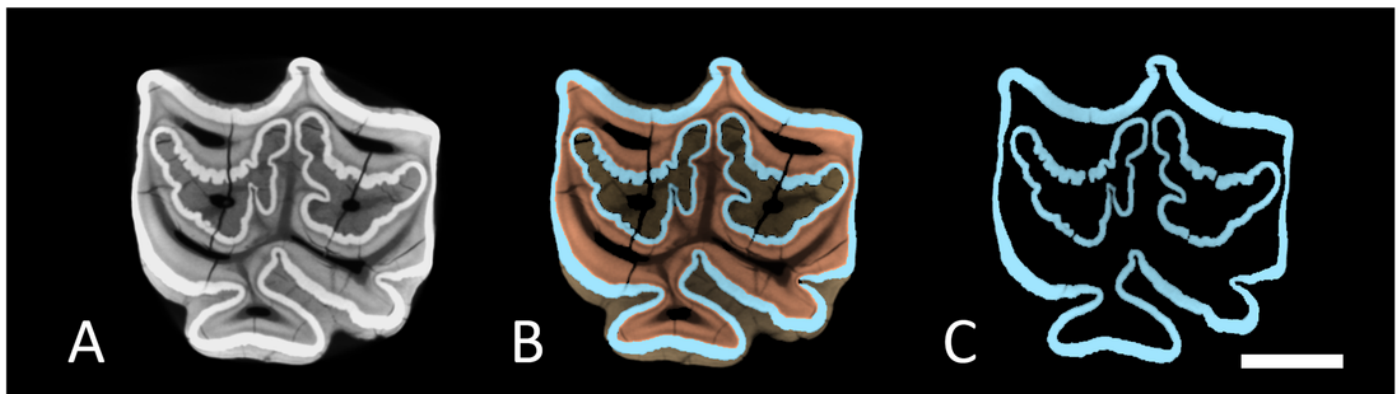
1

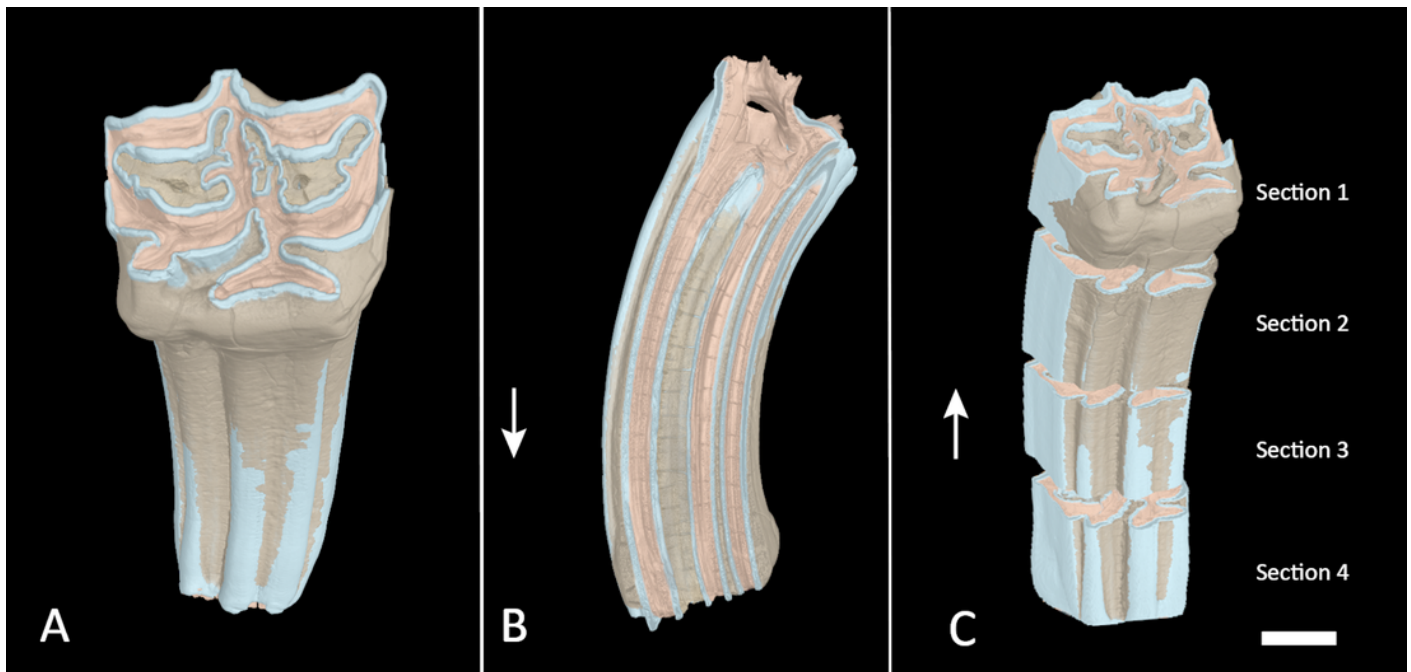
permanent dentition of *Equus quagga*

Figure 1.Virtual 3D-model of the permanent upper right dentition of *Equus quagga*, no. 70335. As typical in hypsodont species, the tooth crowns are much longer than wide. Arrows point towards apical. A. Anatomical position. Note that the caps of the deciduous premolars have been lost, but would have been present at this life stage B. Occlusal view. Scale bar 1cm.

2

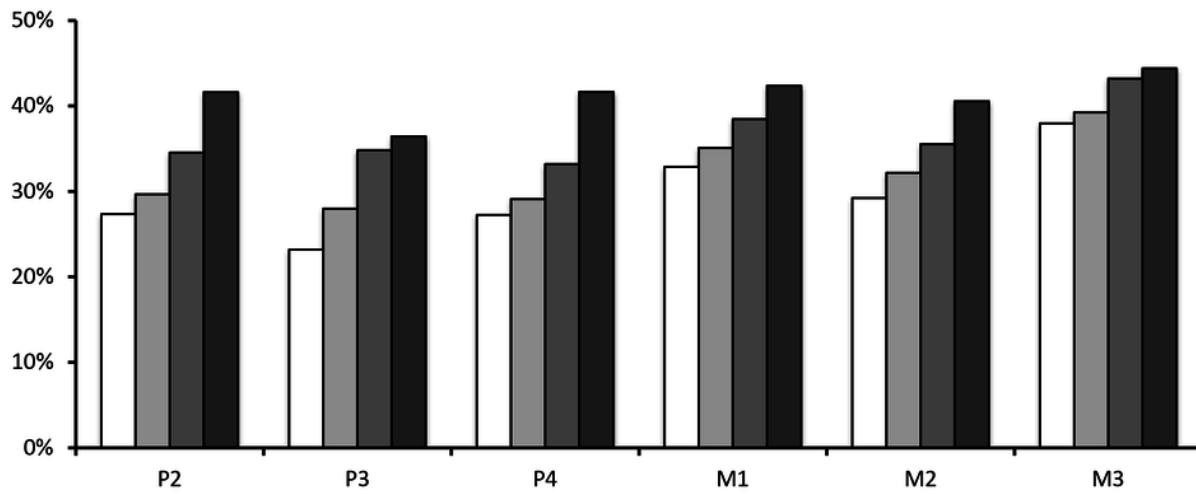
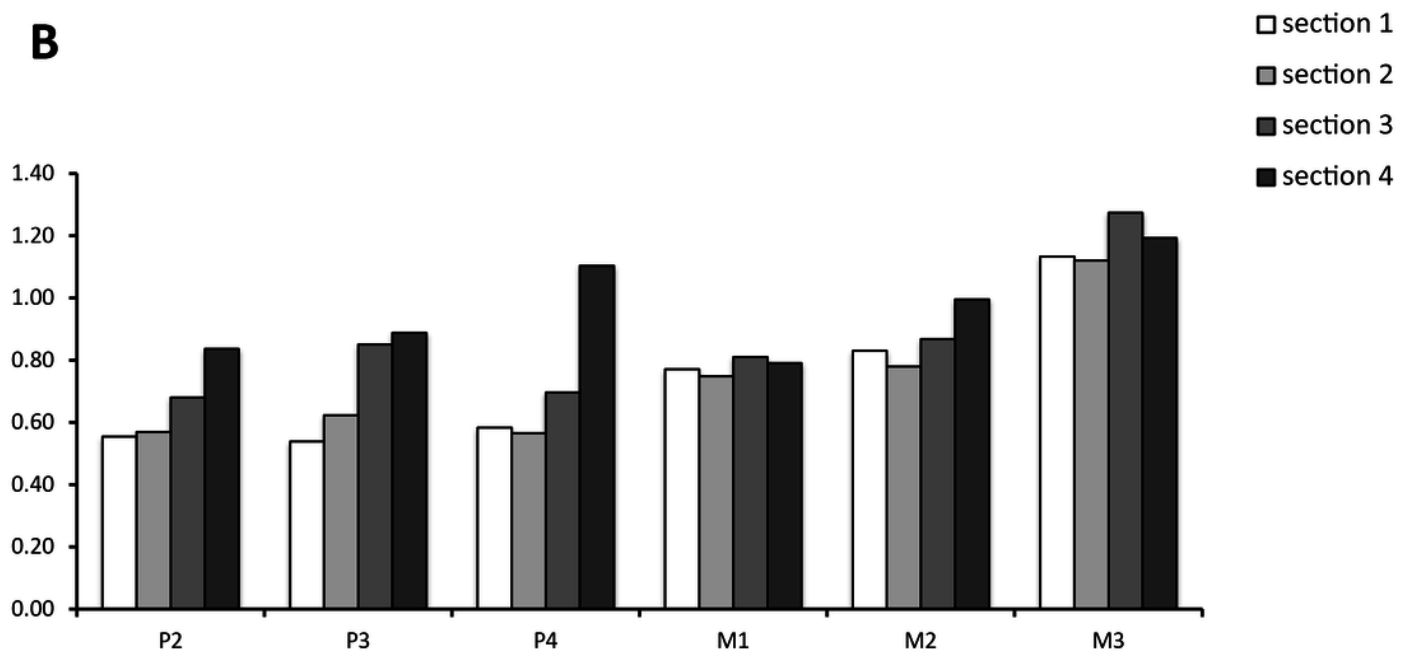
cross-sections through M1

Figure 2. Cross section trough a virtual 3D-model of M1. A. Full tooth model with all dental tissues. B. Segmented tooth model with enamel in blue, dentin in orange and cementum in brown. C. The same enamel only. Scale bar 1cm.

3

segmented M1 and sections

Figure 3.A. Segmented tooth model of M1. B. Vertical cross section trough M1 in anatomical orientation with open roots. C. The same as in A but with each section separated. The lower boundary of section 4 was cut approximately at the cementoenamel junction. Arrows point towards apical. Colours as in Figure 2. Scale bar 1cm.

4

enamel content per section and tooth position

Figure 4.A. Relative enamel content per section and tooth position. Proportions are calculated using the whole tooth volume with all dental tissues (enamel, cementum, dentin). B. The value of enamel versus dentin expressed as the quotient from enamel/dentin per section and tooth position.

Enamel proportions with and without cementum

Tab.1. Enamel proportions calculated with the whole tooth volume (enamel, cementum, dentin) and for the whole tooth without cementum.

2   Tab.1 Enamel proportions calculated with the whole tooth volume (enamel, cementum, dentin) and for the whole tooth without cementum.

stage	P2		P3		P4		M1		M2		M3	
	Enamel proportion whole tooth	Enamel proportion without cementum	Enamel proportion whole tooth	Enamel proportion without cementum	Enamel proportion whole tooth	Enamel proportion without cementum	Enamel proportion whole tooth	Enamel proportion without cementum	Enamel proportion whole tooth	Enamel proportion without cementum	Enamel proportion whole tooth	Enamel proportion without cementum
1	27.34%	37.58%	23.17%	35.23%	27.23%	39.98%	32.86%	48.28%	29.22%	46.50%	37.96%	53.61%
2	29.64%	37.63%	27.97%	38.99%	29.08%	38.47%	35.09%	45.62%	32.15%	44.62%	39.22%	52.72%
3	34.50%	41.89%	34.80%	46.69%	33.17%	42.19%	38.45%	48.45%	35.52%	47.31%	43.20%	57.01%
4	41.61%	47.11%	36.43%	46.99%	41.62%	54.64%	42.35%	47.31%	40.55%	50.56%	44.37%	54.72%
total	31.69%	40.17%	28.75%	40.39%	31.33%	42.64%	36.91%	47.63%	34.00%	47.14%	41.34%	54.62%