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Variation in enamel prism size in primate molars

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ABSTRACT

Objective: This study examines whether larger enamel prism diameters accommodate a greater enamel volume from the enamel-dentine junction (EDJ) to the outer-enamel surface (OES) in primate molars, and how prism size relates to enamel thickness and crown location.

Design: We assessed variation in enamel prism diameter in relation to crown location and enamel thickness in catarrhine lower molars ($n = 14$ species) and one platyrrhine. Prism diameter and enamel thickness were recorded in four locations (lingual lateral; lingual cuspal; buccal cuspal; buccal lateral), using a buccal-lingual section through the centre of the mesial cusps. Ten prism diameter readings were collected at inner (near the EDJ), outer (near the OES) and middle (between the two locations) enamel for each location.

Results: Mean prism diameter values for each species were similar (range: 4.06 μm to 5.81 μm). Prism diameter enlarged from inner to outer enamel, suggesting larger prisms help accommodate the increase in enamel volume from the EDJ to the OES. Average prism diameter does not associate with enamel thickness. Instead, cuspal positions had significantly smaller prism diameter at the EDJ than lateral positions, and larger prism sizes at the OES, leading to an overall similar prism diameter mean for all positions (Lingual lateral: 5.11 μm; Lingual cuspal: 5.04 μm; Buccal cuspal: 4.78 μm; Buccal lateral: 4.99 μm).

Conclusions: Our study revealed consistent average enamel prism diameters in various crown locations of lower primate molars, potentially contributing to the mechanical integrity and functional optimization of enamel in primates.

1. Introduction

Prisms (also known as rods) are a crucial component of enamel structure in many mammalian taxa, including primates (Boyde and Martin, 1984; Martin et al., 1988; Maas and Dumont, 1999). Studies on enamel prism size in primates have often focussed on single or few species, with the average diameter of prisms reported in the 3 μm − 7 μm range (Shellis and Poole, 1977; Boyde and Martin, 1987; Maas, 1994; Dean, 1989; Dumont, 1995; Wang et al., 2005; Cui and Ge, 2007; De Menezes Oliveira et al., 2010; Risnes and Li, 2019; Besnard et al., 2021). However, past studies are difficult to compare due to differences in methodology for prism diameter measurements and different crown positions considered.

Few studies have assessed prism diameter in different crown locations, and whether there is a significant change in prism diameter from inner to outer enamel is still subject of debate. Most past studies investigating variation in prism diameter throughout the enamel layer found prism size enlarges from the enamel-dentine junction (EDJ) to the outer enamel surface (OES) (e.g., Pickerill, 1913; Chase, 1924; Ockerse, 1963; Skobe and Stern, 1980; Osborn, 1981). Other studies have suggested either no variation in prism diameter, or a slight reduction in diameter from the EDJ to OES in lateral positions (Dean and Shellis, 1998; Busch et al., 2001; Radlanski et al., 2001). Some researchers have suggested the greater enamel volume from inner to outer enamel is accommodated by increasing the oblique orientation of prisms as they approach the OES, rather than an enlargement of prism diameter (Skobe and Stern, 1980; Radlanski et al., 1990; Busch et al., 2001). Greater enamel volume from the EDJ to the OES could also be achieved by a greater amount of interprismatic matrix or an increase in prism number, but neither of these hypotheses has garnered much support (Retzius, 1837; Mummery, 1916).

Whether variation in prism size is a major factor in accommodating enamel volume increase from the EDJ to the OES, it would be expected that variation in prism diameter would not be constant due to the complex crown morphology in primate molars. Dean (2004) reported that prism diameter in great apes was relatively uniform from inner to

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outer enamel in lateral positions, yet often larger in outer regions in cuspal enamel. Similarly, Radlanski et al. (2001) reported that prism diameter was relatively constant from the EDJ to the OES in human cervical enamel. Whether variation in prism diameter in the tooth crowns of primates may relate to functional adaptations such as stressors and forces in different crown areas has received little attention (e.g., Maas and Dumont, 1999; Macho et al., 2003).

In this study, we assessed prism diameter variation in four crown locations of lower molars in a wide range of primates, with a focus on Catarrhini. We hypothesize enamel prism size will be smaller at the EDJ than at the OES, and prism size will be associated to enamel thickness. To test this hypothesis, three predictions are proposed: 1) regions with thicker enamel (i.e., cuspal positions) will have larger prisms and show a larger difference in prism diameter between inner and outer enamel than lateral positions; 2) enamel thickness and prism diameter will be associated; 3) prism size will be smaller at the EDJ and larger at the OES in all crown locations. We also explored observable differences in prism diameter among species, and between buccal and lingual locations, since functional differences and variation in mechanical properties, mineral content and structure of enamel have been suggested between these molar sides (e.g., Towle et al., 2021; Yang et al., 2022; Towle et al., 2023a; b).

2. Materials and Methods

A range of Catarrhini (n = 14) and one Platyrrhini species were analysed. Most specimens were assigned to species level; however, some teeth were assigned to genus level (Table 1). Each species was represented by a single lower molar (n = 15 teeth). Most teeth studied were second molars (n = 10) and the rest first molars (n = 5). Unworn or moderately worn teeth were selected, with no visible signs of pathology, enamel defects or macroscopically visible post-mortem damage. Specimens belong to collections in New Zealand (Otago Museum; Dunedin Museum of Natural Mystery; Auckland Museum; Faculty of Dentistry, University of Otago), and the Primate Research Institute, Kyoto University, Japan. Information on each specimen is presented in Table 1, including species, tooth type, wild/captive status, and sex if known. Prior to testing, all teeth were stored dry, except for the human molar which was stored in formaldehyde. This human tooth was extracted as part of planned clinical treatment with patient consent.

Preparation protocols followed Loch et al. (2013a), and are summarised here. Before embedding in resin, tooth surfaces were cleaned

Table 1
Samples studied, including species, tooth type, sex, specimen number and collection.

Species	Common name	Tooth	Collection	Specimen number	Sex
Cercocebus sp.	Mangabey	Lower right M1	Otago Museum	VT 2715	Not known
Chlorocebus pygerythrus	Vervet monkey	Lower left M2	Dunedin Museum of Natural Mystery	n/a	Male
Colobus sp.	Colobus	Lower left M2	Auckland Museum	LM 957	Not known
Macaca fascicularis	Crab-eating macaque	Lower right M1	Dunedin Museum of Natural Mystery	n/a	Female
Macaca fuscata	Japanese macaque	Lower left M2	Primate Research Institute	PRI 3383	Male
Macaca mulatta	Rhesus macaque	Lower left M2	Faculty of Dentistry	CU 138	Female
Macaca sinica	Toque macaque	Lower right M2	Otago Museum	VT 2707	Not known
Miopithecus sp.	Talapoin	Lower right M2	Otago Museum	VT 2702	Not known
Papio hamadryas	Hamadryas baboon	Lower right M2	Primate Research Institute	PRI 7148	Male
Papio ursinus	Chacma baboon	Lower right M2	Dunedin Museum of Natural Mystery	n/a	Female
Gorilla sp.	Gorilla	Lower right M1	Otago Museum	VT 2468	Male
Pan troglodytes	Chimpanzee	Lower right M1	Auckland Museum	LM 315	Not known
Pongo sp.	Orangutan	Lower right M1	Otago Museum	VT 3361	Not known
Homo sapiens	Human	Lower Left M2	Faculty of Dentistry	n/a	Not known
Cebus sp.	Capuchin	Lower left M2	Otago Museum	VT 2728	Not known

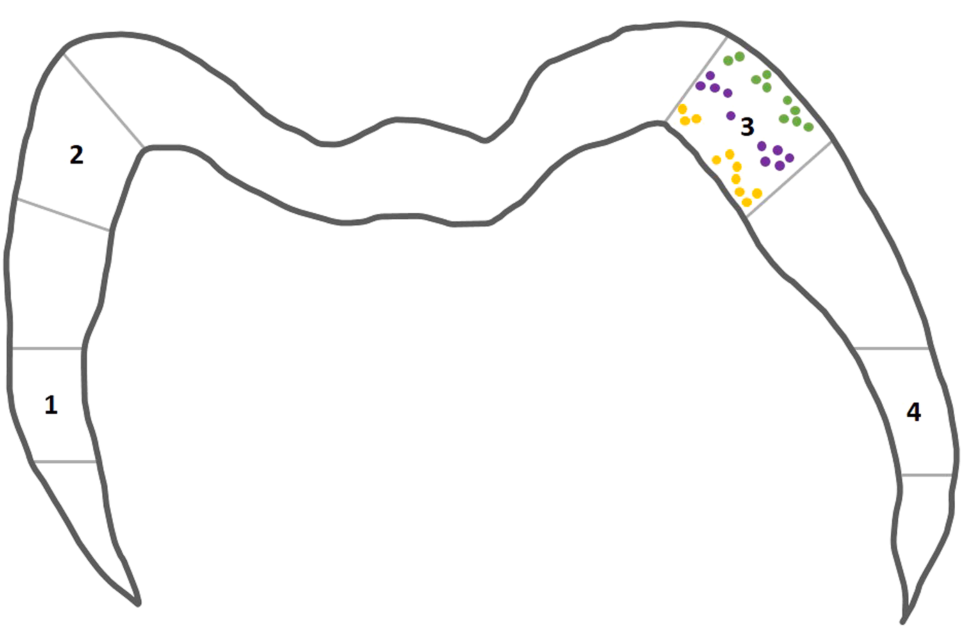

Fig. 1. A representation of enamel in a buccal-lingual slice through the mesial cusp tips. The four positions assessed are shown: 1) lingual lateral; 2) lingual cuspal; 3) buccal cuspal; 4) buccal lateral. 30 prism diameter readings were recorded in each position (as depicted in position 3), with 10 in outer enamel (green circles), 10 in middle (purple circles), and 10 in inner enamel (yellow circles).

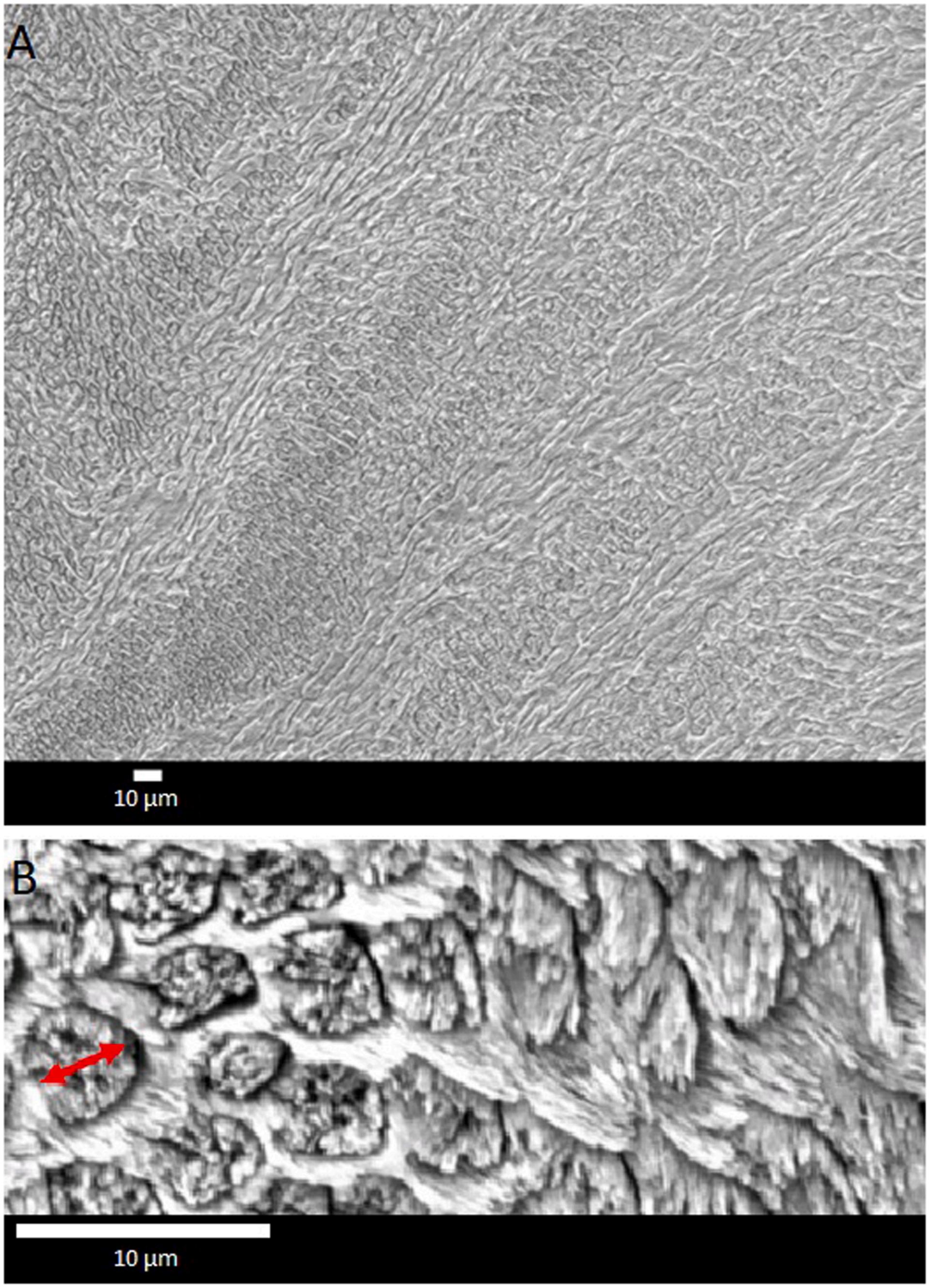

Fig. 2. Enamel decussation (i.e., Hunter-Schreger bands), in which the orientation of each prism varies between bands (A); this allows a location in which prisms are approximately perpendicular to the direction of sectioning to be located (B). Sample information: A) lingual cuspal position of the talapoin molar (magnification: 330x); B) lingual cuspal inner position of the capuchin molar (magnification: 2,000x).

with ethanol. Each tooth was placed in a silicone mould, and oriented to allow proper sectioning. Teeth were embedded in epoxy resin (Epofix™ Cold-Setting Embedding Resin, Struers, Copenhagen, Denmark). After setting at room temperature for 24 h, teeth were longitudinally sectioned through the buccal–lingual plane of the mesial cusp tips using a MOD13 diamond blade (Struers, Copenhagen, Denmark) under water irrigation in a high-speed saw (Accutom-50, Struers, Copenhagen, Denmark). Two tooth segments were produced (distal and mesial), and the distal portion used for SEM imaging. Sections were surface-cleaned with ethanol and polished on a TegraPol-21 polisher (Struers, Copenhagen, Denmark) with 1200, 2400 and 4000 grit silicon carbide paper (Struers, Copenhagen, Denmark) and ultrasonic cleaned in water for 3 min in between polishing sessions. After polishing, specimens were ultrasonically cleaned in ethanol for 3 min before etching with 2 M phosphoric acid for up to 10 s. Specimens were sonicated again in ethanol for 3 min. Samples were then coated with gold palladium prior to SEM analysis.

A JEOL JSM-6700 F Field Emission SEM (JEOL Ltd., Tokyo, Japan) operating at 5 kV and 10 µA was used for SEM imaging. Twelve locations were studied; these comprise the inner, middle and outer enamel in four positions: lingual lateral; lingual cuspal; buccal cuspal; buccal lateral (Fig. 1). The lateral/cuspal positions were standardised as follows: 1) lateral: within the lower half of the crown, but before enamel begins to taper towards the cementum-enamel junction; 2) cuspal: approximately in a straight line from the maximum height of the cuspal dentine to the outer enamel (if cusps were slightly worn, a cervical point was chosen).

Table 2
Average enamel thickness and prism diameter (PD) values for each sample studied (average calculated based on the four positions). All measurements in µm.

	Enamel thickness	PD Inner	PD Middle	PD Outer	PD Average
Mangabey	526.88	4.79	5.14	6.28	5.40
Vervet monkey	340.58	4.96	5.80	5.74	5.50
Colobus	478.88	4.63	5.06	6.33	5.34
Crab-eating macaque	592.71	4.22	4.89	5.26	4.80
Japanese macaque	680.82	3.34	4.66	5.35	4.45
Rhesus macaque	646.45	3.65	4.35	4.93	4.31
Toque macaque	576.71	3.14	4.29	4.76	4.06
Talapoin	390.06	4.06	5.27	5.98	5.11
Hamadryas baboon	1043.27	3.75	4.63	6.22	4.87
Chacma baboon	1151.33	3.87	5.57	5.82	5.09
Gorilla	789.76	5.20	5.70	6.52	5.81
Chimpanzee	837.52	4.96	5.90	6.21	5.69
Orangutan	882.06	4.04	5.39	6.24	5.22
Human	1168.79	3.86	4.43	4.69	4.33
Capuchin	483.67	4.00	4.80	5.38	4.73

Buccal and lingual refer to the two sides of the mesial cusps.

Prism diameter measurements were obtained using the JEOL software ('PC-SEM' version 3.04.00) via the 'point to point measurement' tool. Measurements were obtained in areas where prisms were approximately perpendicularly exposed to reduce effects of section angle on prism diameter. If the prism outline was not completely circular, the minimum diameter across the prism was recorded (Fig. 2). Ten prism diameter measurements were taken in inner, middle and outer enamel within the four positions (lingual lateral; lingual cuspal; buccal cuspal; buccal lateral; see Fig. 1, position 3; total of 120 readings per tooth). The SEM magnification was set at 2,000x for all prism diameter measurements.

Enamel thickness values (from the EDJ to the OES) were collected at the same locations. Ten thickness measurements were obtained per location and averaged. The association between prism size and enamel thickness, and differences in prism size among locations were assessed. Comparisons were made between cuspal and lateral, and between buccal and lingual positions. Both enamel thickness and prism diameter data were normally distributed (K-S test statistic on data from Table 2, enamel thickness: $D(15) = 0.14$, $p = 0.896$; prism diameter: $D(45) = .068$, $p = .977$). Comparisons of enamel thickness vs. prism diameter mean values were performed using a Pearson Correlation test with significance set at 0.05. A two-tailed T-test was used to compare prism diameter in different crown positions (e.g., buccal vs. lingual cusps). Average values for each site (inner, middle, outer) for the four positions

Table 4
Average prism diameter values for each position, with all samples combined.

Lower		Average Prism diameter (µm)
Buccal lateral	Inner	4.50
	Middle	4.91
	Outer	5.56
Buccal cuspal	Inner	3.76
	Middle	4.77
	Outer	5.82
Lingual cuspal	Inner	3.96
	Middle	5.28
	Outer	5.86
Lingual lateral	Inner	4.43
	Middle	5.27
	Outer	5.63

were used for statistical testing. The data that supports the findings of this study are available in the supplementary material of this article.

3. Results

Average prism diameter ranged from 4.06 µm in the Toque macaque to 5.81 µm in the Gorilla (Table 2). Average prism diameter was similar among the four crown positions (Table 3), close to 5 µm (Lingual lateral: 5.11 ± 0.62 µm; Lingual cuspal: 5.04 ± 0.59 µm; Buccal cuspal: 4.78 ± 0.62 µm; Buccal lateral: 4.99 ± 0.88 µm). Prism diameter enlarged from inner to outer enamel in all positions when all positions and samples were compiled in all but one species (Vervet monkey) (Tables 2 and 4; Fig. 3). Most prism diameter readings enlarge from inner to middle and middle to outer (79.1%; 95/120). Inner enamel within the cusp regions (buccal cuspal and lingual cuspal) is characterised by relatively small prisms, but larger prisms in outer enamel (Table 4). Lateral enamel had a lower range of prism size variation from inner to outer enamel.

There were no significant differences in average prism diameter between the four crown positions (e.g., cuspal lingual vs. cuspal buccal: $t(n = 15) = -1.62$, $p = 0.127$; lateral lingual vs lateral buccal: $t(n = 15) = -0.62$, $p = 0.543$), or when lateral and cuspal positions were combined to compare lingual and buccal differences ($t(n = 30) = -1.52$, $p = 0.14$). When inner, middle and outer averages for each specimen were compared, inner enamel prism diameter was significantly smaller than middle enamel ($t(60) = 7.66$, $p < 0.001$). Similarly, middle prism diameter was significantly smaller than outer enamel ($t(60) = 5.81$, $p < 0.001$). Inner enamel prism diameter was significantly larger in lateral than cuspal positions, with no significant differences observed for middle and outer enamel (Table 5).

Enamel thickness varied more substantially among samples than prism diameter, ranging from an average enamel thickness of 340.58 µm in the Vervet Monkey to 1168.79 µm in humans (Table 2). When all

Table 3
Average prism diameter (left value) and enamel thickness (right value) for each position, in each species. All measurements in µm.

Taxa	Lingual lateral	Lingual cuspal	Buccal cuspal	Buccal lateral
Mangabey	5.47; 409.13	5.75; 516.56	4.87; 695.36	5.53; 486.46
Vervet monkey	5.81; 301.10	4.60; 360.22	4.89; 469.60	6.71; 231.38
Colobus	5.81; 364.91	5.76; 446.99	4.68; 739.71	5.10; 363.92
Crab-eating macaque	4.67; 416.69	4.06; 586.99	4.90; 1003.14	5.56; 364.01
Japanese macaque	4.58; 700.51	4.79; 678.74	3.70; 614.34	4.73; 729.68
Rhesus macaque	4.59; 469.16	4.80; 560.12	4.52; 1349.80	3.33; 206.73
Toque macaque	4.22; 402.30	4.02; 618.97	3.82; 832.39	4.18; 453.19
Talapoin	4.46; 250.30	4.92; 440.68	5.52; 638.31	5.53; 230.95
Hamadryas baboon	5.13; 811.49	5.43; 1028.45	4.46; 1386.26	4.46; 946.88
Chacma baboon	5.41; 1192.80	4.94; 1122.11	4.92; 1305.05	5.08; 985.37
Gorilla	6.29; 596.45	5.82; 945.78	5.88; 1066.25	5.24; 550.55
Chimpanzee	5.54; 751.27	5.79; 789.60	5.36; 954.67	6.08; 854.52
Orangutan	4.92; 889.00	5.29; 694.38	5.52; 1132.15	5.16; 812.71
Human	4.41; 972.07	4.73; 1214.02	4.40; 1740.61	3.77; 748.45
Capuchin	5.34; 345.65	4.84; 508.44	4.34; 664.67	4.39; 415.92

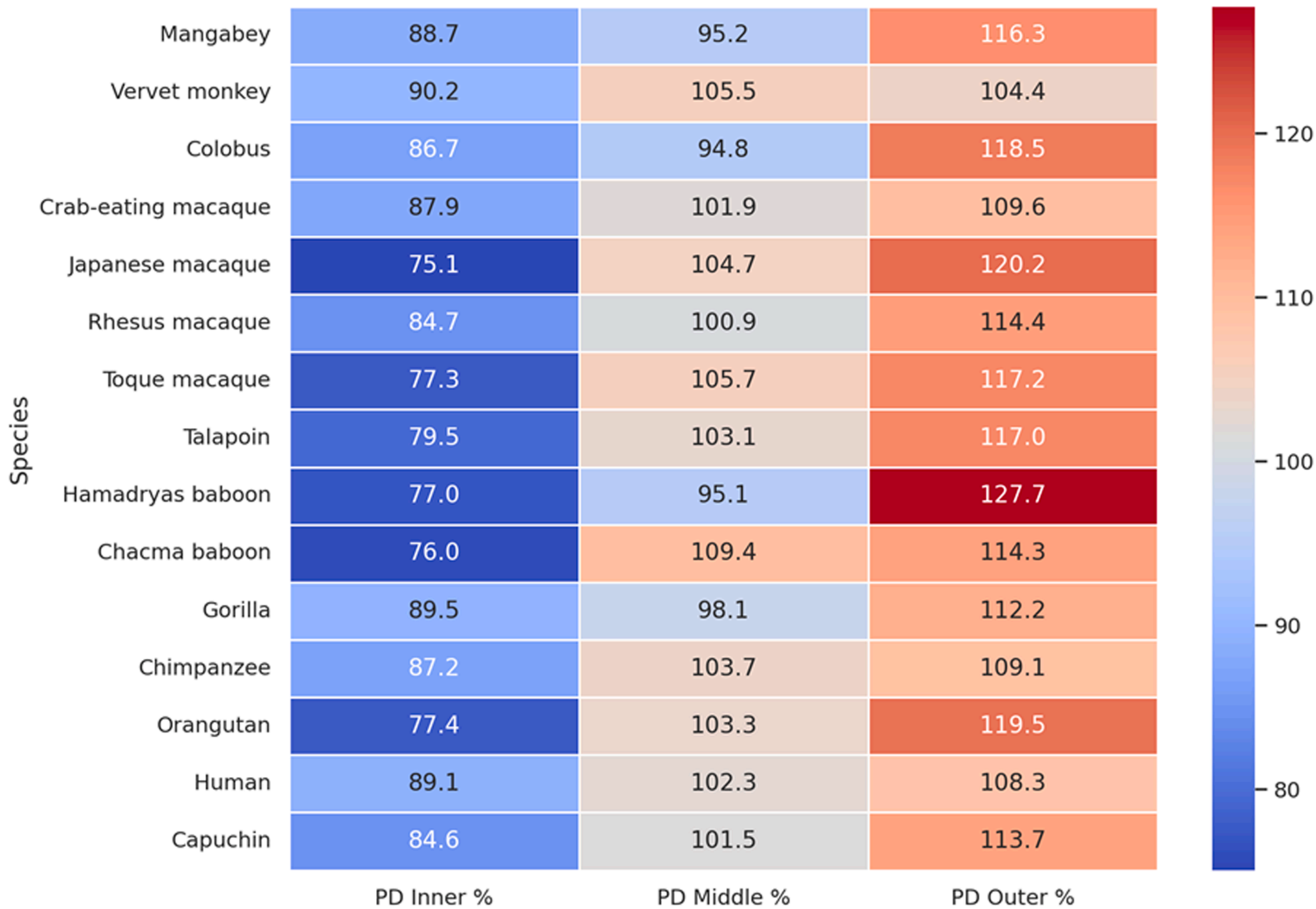

Fig. 3. Heatmap of enamel prism diameter (PD), with inner, middle and outer mean values displayed as a percentage of the overall tooth average.

samples were combined, the two lateral positions had similar enamel thickness (lingual lateral: 591.52 µm; buccal lateral: 558.71 µm), enlarging at lingual cuspal enamel (700.80 µm), while buccal cuspal enamel had almost twice the enamel thickness observed in lateral enamel (972.82 µm). The thickest enamel was observed in buccal cuspal positions in all samples studied, with the exception of the Japanese macaque in which all four positions had similar values (Table 3). When the four positions were compared in different species, there was no significant relationship between enamel thickness and prism diameter ($r(n = 60) = -0.07$, $p = 0.595$; Fig. 4).

4. Discussion

The hypothesis tested in this study can only be partially accepted. The results show prism diameter enlarges from inner to outer enamel in all four crown positions studied; however, no significant association between prism diameter and enamel thickness was observed. Similarly, no significant differences in average prism diameter were observed among the four positions. A larger range in prism diameter values from inner to outer enamel was seen in areas of thicker enamel (e.g., cuspal positions mean difference = 2 µm, lateral positions mean difference = 1 µm). These differences arise due to the starting diameter of prisms in cuspal positions being smaller near the EDJ but larger near the OES than in lateral positions, leading to similar overall mean values (Fig. 5).

Further research is needed to fully understand how ameloblasts function in different crown locations, but current evidence suggests that the average size of enamel prisms remains fairly consistent across different parts of a tooth crown, despite variations in the thickness of the enamel. Previous studies have noted differences in enamel formation, including the size of prisms, between cuspal and lateral locations (Osborn, 1968; Skobe and Stern, 1980; Paulson et al., 1984). This includes observations that the initial size of enamel prisms near the EDJ varies over short distances, especially around cusp tips (Paulson et al., 1984). Additionally, the trajectory of these prisms from the EDJ to the outer enamel surface also varies greatly depending on their location on the crown (Skobe and Stern, 1980; de Sousa and Hara, 2023). Consequently, to explain the findings of this study, a range of processes involved in the formation of enamel must be considered.

The thicker enamelled functional cusps (buccal) and thinner non-functional cusps (lingual) had no significant differences in average prism diameter, despite substantial differences in enamel thickness. Based on the results of this study, it appears prism diameter does not vary between functional and non-functional cusps, despite varying enamel properties between these locations (e.g., Cuy et al., 2002; Yang et al., 2022; Towle et al., 2023a; b). Whether enamel properties are associated with observed variation in wear and chipping across primate molars (Towle et al., 2021), other enamel structural and compositional properties such as prism orientation, enamel composition, and crystal arrangement/orientation are likely playing a more important role than prism size (e.g., Angker et al., 2004; Shimizu and Macho, 2008; Xie et al., 2009; Jeng et al., 2011; Stifler et al., 2021).

Pickerill (1913) reported human prism diameter values of 2.5 - 3.1 µm close to the EDJ, rising to 5.7 - 6.5 µm near the OES. More recent studies suggest similar findings (e.g., Skobe and Stern, 1980; Osborn, 1981; De Menezes Oliveira et al., 2010), which are also in line with human values reported in the present study. Studies on other primates

Table 5
Statistical comparisons for all samples combined, using a paired T-test. Bold indicates significant relationship.

Position	Buccal lateral	Buccal cuspal	Lingual cuspal	Lingual lateral
Inner				
Buccal lateral		**t(n = 15) = 3.1, p = .008**	**t(15) = 2.3, p = .036**	t(15) = 0.3, p = .789
Buccal cuspal	**t(n = 15) = 3.1, p = .008**		t(15) = 1.2, p = .247	**t(15) = 3.5, p = .004**
Lingual cuspal	**t(15) = 2.3, p = .036**	t(15) = 1.2, p = .247		**t(15) = 2.9, p = .012**
Lingual lateral	t(15) = 0.3, p = .789	**t(15) = 3.5, p = .004**	**t(15) = 2.9, p = .012**	
Middle				
Buccal lateral		t(15) = 0.4, p = .685	t(15) = 0.9, p = .361	t(15) = 1.2, p = .259
Buccal cuspal	t(15) = 0.4, p = .685		t(15) = 1.9, p = .082	t(15) = 1.5, p = .159
Lingual cuspal	t(15) = 0.9, p = .361	t(15) = 1.9, p = .082		t(15) = 0.05, p = .962
Lingual lateral	t(15) = 1.2, p = .259	t(15) = 1.5, p = .159	t(15) = 0.05, p = .962	
Outer				
Buccal lateral		t(15) = 1.1, p = .307	t(15) = 1.1, p = .298	t(15) = 0.3, p = .760
Buccal cuspal	t(15) = 1.1, p = .307		t(15) = 0.3, p = .794	t(15) = 0.8, p = .411
Lingual cuspal	t(15) = 1.1, p = .298	t(15) = 0.3, p = .794		t(15) = 1.2, p = .246
Lingual lateral	t(15) = 0.3, p = .760	t(15) = 0.8, p = .411	t(15) = 1.2, p = .246	

are also consistent with the present results, including overall prism diameter average values and prism diameter enlarging from inner to outer enamel (Dumont, 1995; Ockerse, 1963; Campbell, Cuozzo, Sauther, Sponheimer & Ferguson, 2012). Larger prism diameters in inner compared to outer enamel, and prism size variation in different regions of the crown, has also been suggested for other mammals (Kallistová, Horáček, Šlouf, Skála & Fridrichová, 2017), which adds further support to our findings.

Earlier studies suggested that 'supplemental prisms' were present in middle and outer enamel, accommodating the increase in enamel volume in these areas (Retzius, 1837). Radlanski et al. (1986), (1988) suggested that prisms approached the OES at an angle (increasing cross-sectional area) with the actual prism diameter remaining constant from EDJ to the OES. A similar hypothesis has been suggested by other researchers, although with the caveat that prism diameter variation should still be considered (Skobe and Stern, 1980). Neither of these two hypotheses seem supported by the present study, since only prisms that were perpendicular to the histological section were recorded, and a consistent enlargement in prism diameter was observed.

The mechanisms behind prism diameter being larger at the OES compared to the EDJ are not well understood; however, they seem consistent with ameloblasts increasing enamel matrix production as

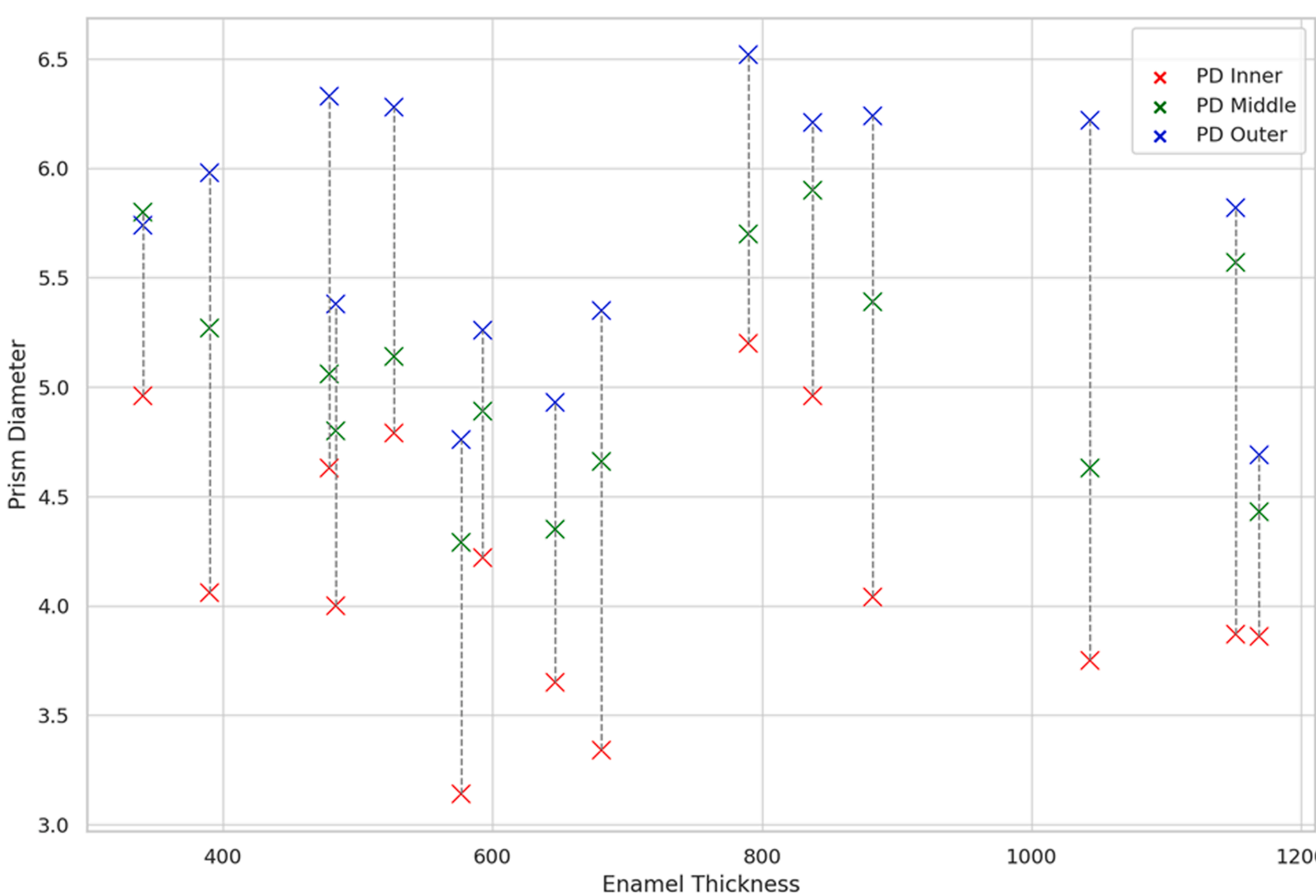

Fig. 4. Combined scatter plot of mean prism diameters for inner, middle and outer enamel for each species, plotted against the mean enamel thickness across all four crown positions. Both measurements in μm.

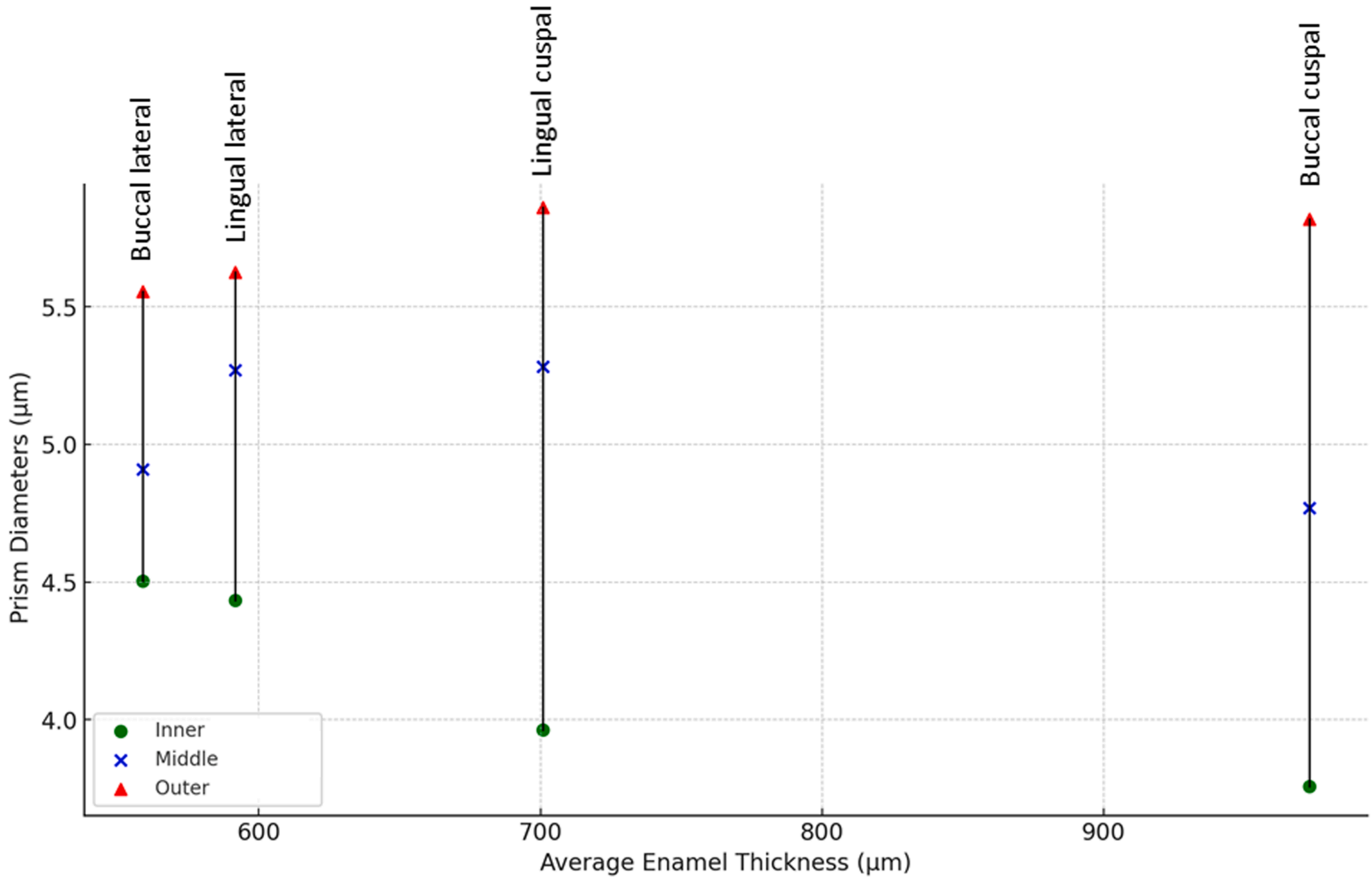

Fig. 5. Mean enamel thickness and prism diameter for inner, middle and outer for all species combined, split into the four tooth positions.

they move towards the OES (Smith, 2004). Changes in hydroxyapatite crystals size could also contribute, although there is disagreement in the literature regarding size and shape variation in hydroxyapatite crystals (e.g., Rönnholm, 1962; Boyde, 1964; Sakae et al., 1997; Palamara, Phakey, Rachinger & Orams, 1980). Some papers suggest crystallite width increases from inner to outer enamel (Sakae, 1988; Palamara et al., 1980). Rönnholm (1962) proposed larger prisms at the OES to be associated with larger crystallite diameters, and Sakae (1988) suggested less ionic substitutions near the OES may accommodate the increase in crystallite and prism size at this location.

5. Limitations and future research

In light of the recent findings by Beniash et al. (2019), which revealed a complex arrangement of nanocrystals within enamel prisms, our study acknowledges the importance of considering enamel at varying scales, including macro (e.g., variations in enamel structure relative to thickness and occlusal morphology), micro (e.g., the relationship between increasing prism size and the decussation patterns of enamel prisms), and nanoscale (e.g., structural variation of individual crystals within prisms at different crown locations). Our findings suggest that the average prism diameter enlarges from inner to outer enamel, indicative of a gradient in prism size across the enamel thickness. While our study primarily focused on the micro scale, a nanoscale perspective could further enrich our understanding of the structural complexity of enamel. It may also reveal potential scaling laws or length-scale dependency that govern the mechanical behavior and biology of enamel.

The integration of artificial intelligence (AI) tools, particularly deep learning and machine learning methods as demonstrated by Lew et al. (2023), can significantly contribute to this analysis. AI methods can analyse complex patterns and relationships within large datasets, enabling a nuanced understanding of enamel structure. For example, enamel indenter maps created using AI could be applied to analyse variation in prism size and structure at different enamel depths and locations across multiple primate species. Extending this research to include a broader range of primates presents both challenges and opportunities. One of the primary challenges lies in the variability of enamel characteristics across species, which may require methodological adaptation to account for these differences. Additionally, obtaining a sufficient and robust sample size for each species could be challenging. However, the analysis of a broader range of primates could yield valuable insights into the evolutionary adaptations of enamel in response to dietary and environmental pressures in primate lineages. This comprehensive approach could elucidate any species-specific variation in enamel prism size and structure, revealing how these differences contribute to the mechanical and functional optimization of primate molars.

The categorization of prism ‘types’ in primate enamel has been based on prism/crystallite shape, structure, and surrounding interprismatic matrix. Although anthropoids tend to have similar prism structure/shape, there has been debate as to which ‘types’ are presented in different species, or even different regions of the crown (Boyde, 1964; Shellis and Poole, 1977; Shellis, 1984; Martin et al., 1988). As suggested by Carlson and Krause (1985), the classification of prism pattern types is often difficult. All primates in the present study were described following the simplified methods of Carlson and Krause (1985), as having small, uniform, circular prisms. In the samples studied, prism diameter was an appropriate measurement to be assessed, due to the approximate circular shape of the prism outline in the different positions. This may not be the case for all primates and other mammalian groups. Olek et al. (2020) studied human, cattle and porcupine teeth, and noted that although prism diameter was an appropriate dimension for human samples given their shape, it was not for cattle. In this case, they recommend considering two dimensions (length and width), since bovine prisms may be three times longer than wide.

The assessment of enamel prism diameter in primate tooth sections is subject to potential errors and requires careful methodological considerations. A notable challenge emerges when prisms are oriented obliquely to the cutting plane. In these instances, the perceived prism diameter could be overestimated. Our study focused on species characterized by significant enamel decussation, as evidenced by distinct Hunter-Schreger bands. Such decussation aids in pinpointing regions where prisms appear approximately perpendicular to the cutting plane (when prisms are approximately circular in shape). However, prisms also exhibit variability in cross-sectional shape. This complicates the determination of prism angles, thus introducing a degree of error into our measurements. In species with enamel showing minimal or no decussation, the assessment of prism orientation is even more difficult. The orientation of ameloblasts and the consequent direction of enamel formation during development needs consideration. When teeth are sectioned in a horizontal plane, which aligns with the direction of ameloblast movement during secretion, more uniform prism diameter results could be achieved. This could reduce uncertainties with prism angulation relative to the cutting plane, although the examination of multiple sections would be required, which was not feasible in this study.

This study used a small number of teeth, and hence a phylogenetic analysis was not feasible. However, the study does suggest some potential areas for further exploration. Although prism diameter does not seem associated with enamel thickness, there may be other allometry factors to consider. In the present study, the six non-human primates with mean prism diameter of under 5 μm have average body mass of under 20 kg, whereas those with average prism diameter over 5 μm, half ($n = 4/8$) have body mass of over 20 kg (body mass data from Galán-Acedo, Arroyo-Rodríguez, Andresen & Arasa-Gisbert, 2019). Future research with a larger sample size could test the relationship between prism size and body mass within a phylogenetic context. In this study, average prism diameter was similar between species, but variation in average prism size is present among mammals, suggesting phylogenetic differences in prism size among distantly related groups (Olek et al., 2020). In primates, prism diameter seems to be conserved, at least compared to other enamel characters such as thickness (this study; Thiery et al., 2017).

In the current study, the effect of tooth wear on prism size assessments could have been exacerbated by sample size. Minimally worn teeth were selected, and locations away from the cusp tips were chosen to minimise such effects (Fig. 1). However, cuspal positions could have had more wear than lateral positions. This would not affect the main findings of this study, suggesting that prism diameter has varying widths in different crown positions (since enamel near the EDJ will likely be unaffected by wear). Mean prism diameter was similar among crown positions, despite substantial variation in enamel thickness, and this seems to be achieved by varying the prism size at the EDJ. A study with a larger number of species and specimens, including multiple teeth for each species, is also required to further explore the relationship between enamel thickness and variation in prism diameter, especially in relation to overall changes in tooth morphology.

CRediT authorship contribution statement

Towle Ian: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Loch Carolina:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors have no competing interest to disclose.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.archoralbio.2024.105895.

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