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ORIGINAL ARTICLE

Life history data derived from the dental histological analysis of *Giraffa camelopardalis*: Implications for the palaeohistology of extinct giraffids

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Abstract

The analysis of incremental marks in the enamel, dentine and cementum of extant and extinct species provides important information about the rate and pattern of tooth growth, which permits inferences about key life history traits. Traditionally, such research has mainly focused on primates, while other mammalian groups have remained relatively unexplored. In some cases, this has led to the misidentification of incremental markings and the miscalculation of dental growth parameters in non-primate taxa, which has highlighted the importance of obtaining more reliable comparative frameworks. Here, we partially fill this gap by providing a detailed analysis of the dental microstructure in the extant giraffe *Giraffa camelopardalis*. We specifically studied the histology of the different cusps (i.e. protoconid, metaconid, hypoconid, entoconid and hypoconulid) of two first lower molars and two third lower molars with different degree of wear to identify the different incremental markings and to calculate dental growth parameters such as daily secretion rate and enamel formation front angle for each cusp and tooth. Our results show that incremental markings in enamel were more apparent as compared to those in dentine and/or cementum and have permitted a deeper analysis of the former tissue. Enamel laminations, which had a daily periodicity, were the most common incremental lines in all teeth. Supradaily Retzius lines and subdaily cross-striations and laminations were also recognised in dental enamel, revealing multiple secretory pulses of the ameloblasts in the giraffe. Generally, values of enamel growth parameters (i.e. daily secretion rate and enamel formation front angle) obtained for the first lower molar were comparable to those reported for closely related taxa, while those calculated for the third lower molar present a higher degree of variation that may be linked to differences in general somatic rates of growth. Nevertheless, enamel growth parameters were highly variable within each tooth, suggesting caution when making general (palaeo)biological inferences from dental histology. The giraffe dentine and cementum also register incremental lines. In the dentine, most of these features were classified as daily von Ebner's lines and their counting and measurement revealed values of secretion rates that agree with those previously reported in other

artiodactyls. The age calculated from the incremental lines in the dental cementum matches that deduced from dental wear, suggesting that the counting of yearly lines in this tissue is a reliable tool to estimate individual age in giraffids. This study further suggests ways to refine future analyses of dentine and cementum and sets the stage for dental palaeohistology of extinct giraffids and closely related ungulates for which life history information is still unknown.

KEYWORDS
daily secretion rate, enamel formation front angle, giraffids, incremental marks, tooth histology

1 | INTRODUCTION

Mammalian teeth are composed of three dental tissues: enamel, dentine and cementum (Carlson, 1990; Hillson, 2005). All these tissues are formed incrementally, leaving marks in their microstructure that reflect their secretion rhythm(s) (Carlson, 1990; Hillson, 2005; Klevezal, 1996). More specifically, cementum has a yearly periodicity (Klevezal, 1996; Lieberman, 1993; Ungar, 2010), while enamel and dentine register subdaily, daily and supradaily incremental markings (Bromage, 1991; Dean, 2000; Emken et al., 2021; Kierdorf et al., 2013; Smith, 2006). Analyses of these lines (odontochronology) permit inferences to key development and life history information about the extant and extinct mammals (e.g. Funston et al., 2022; Nacarino-Meneses & Chinsamy, 2022) and have shed light on diverse evolutionary questions (e.g. Newham et al., 2020; Newham et al., 2022). Incremental marks in dental cementum, for instance, have been used to reconstruct life history variables such as the age and/or the season of death of different taxa (Azorit et al., 2004; Burke & Castanet, 1995; Jordana et al., 2012; Klevezal, 1996; Lieberman, 1993; Newham et al., 2020; Veitschegger et al., 2019). Daily and supradaily lines in enamel and dentine, on the other hand, have been widely analysed to calculate timings and rates of tooth formation (Dean, 1987; Dirks et al., 2009; Emken et al., 2023, 2024; Jordana & Köhler, 2011; Köhler et al., 2021; Nacarino-Meneses et al., 2017; Nacarino-Meneses & Chinsamy, 2022; Orlandi-Oliveras et al., 2019; Tafforeau et al., 2007). These data are especially important for reconstructing the life history strategy of extant and extinct animals (Hogg, 2018), since some stages of mammalian dental development, such as the eruption of the first or the third permanent molar, might be tightly linked to key life history events (Dean, 2006). Precise information on the timing of some of these events, such as weaning, can indeed been obtained from the combined analysis of dental histology and tooth chemistry (Smith, 2013). Moreover, the study of incremental lines in dental enamel has also proven to be useful to shed light on other biological aspects apart from life history, such as illness or diet (Smith, 2013).

Traditionally, odontochronological research aimed at obtaining life history information had focussed on primates [see Smith (Smith 2008) for a review]. Over the last decades, however, this research has expanded considerably to other groups of mammals, including bovids (Jordana et al., 2014; Jordana & Köhler, 2011; Kahle et al., 2018; Kierdorf et al., 2013, 2012; Macho & Williamson, 2002), suids (Emken et al., 2021, 2023, 2024; Kierdorf et al., 2014, 2019), proboscideans (Dirks et al., 2012; Köhler et al., 2021; Metcalfe & Longstaffe, 2012), cervids (Iinuma, Suzuki, et al., 2004; Iinuma, Tanaka, et al., 2004; Jordana et al., 2014), rhinoceros (Tafforeau et al., 2007) and equids (Nacarino-Meneses et al., 2017; Nacarino-Meneses & Chinsamy, 2022; Orlandi-Oliveras et al., 2019), among others. There are, however, many mammalian taxa for which this information is still lacking. Moreover, previous research has shown that there exist important differences between the dental microstructure of primates and that of other mammalian orders (Hogg, 2018), which has been demonstrated to have caused the misidentification of dental incremental markings (e.g. Emken et al., 2021; Kierdorf et al., 2019) and the subsequent erroneous calculation of dental growth parameters in several ungulates (Kierdorf et al., 2014; Nacarino-Meneses et al., 2017). Hence, an increase in sampling and experimentation is urgently needed in mammalian groups other than primates (Hogg, 2018). This is particularly important for extant taxa, since they represent a solid framework that permits the comparison and analysis of fossil forms (de Ricqlès, 2011).

Here, we provide a detailed histological analysis of the growth and development of the lower molars of the extant giraffe *Giraffa camelopardalis*. In a pioneering study, Hall-Martin (1976) briefly described incremental lines in the dentine and cementum of giraffe teeth, but he did not provide any information about their growth parameters. This has remained the only analysis of giraffe dental histology to date. It is, thus, timely that a more thorough investigation of the histology of giraffe teeth is conducted using modern techniques, cutting-edge software and microscopes to obtain baseline information that can later be extrapolated to extinct giraffids.

2 | MATERIALS AND METHODS

We studied two first lower molars (m1) and two third lower molars (m3) of *G. camelopardalis* that present different stages of development and degrees of wear (Table 1, Supplementary Figure S1A–S4A). These teeth were selected because they are reliable to obtain life history data (e.g. Jordana et al., 2014; Nacarino-Meneses & Chinsamy, 2022), since their eruption is, respectively, correlated with the age at weaning and the age at skeletal maturity (Demisch & Wartmann, 1956; Orlandi-Oliveras et al., 2019; Smith, 2000).

TABLE 1 Sample studied.

Code	Teeth	Mandible	Collection	Estimated age
m1NW	m1	ZM39558	Iziko Museums	1–10 months
m3NW	m3	SAM36851	Iziko Museums	3.5 years
m1W	m1	UCTgm	Palaeobiology group	10 years
m3W	m3	UCTgm	Palaeobiology group	10 years

Dental notation in mammals generally follows two different systems (Smith & Dodson, 2003). On the one hand, veterinary sciences and (palaeo)primatologists commonly use capital and case letters to indicate permanent and deciduous teeth respectively (e.g. Hall-Martin, 1976). In this system, the position of the tooth within the mouth is indicated as a superscript if it is located within the maxilla or as a subscript if it is located within the mandible. Hence, the notation for a deciduous upper fourth premolar using this system would be as 'p^4'. On the other hand, palaeontologists usually use capital and case letters to identify maxillary and mandibular teeth, respectively, and the letter 'D' to indicate deciduous teeth (e.g. Smith & Dodson, 2003). Following this system, a deciduous upper fourth premolar would be abbreviated as 'DP4'. Both systems are universally accepted and the choice of one or the other is arbitrary. In the present work, we follow the second of these conventions.

Teeth for this study were derived from three individuals: (i) ZM39558 from the Iziko Museum of South Africa (Cape Town), (ii) SAM36851 from the Iziko Museum of South Africa (Cape Town) and (iii) UCTgm, from the vertebrate comparative collections of the Department of Biological Sciences of the University of Cape Town. All teeth under study were located within their respective mandibles (Supplementary Figure S5). This allowed the estimation of the age at death of each specimen based on the observation and study of the eruption and wear patterns described by Hall-Martin (Hall-Martin, 1976). Mandible ZM39558, from which an unworn and unerupted m1 was extracted (i.e. m1NW), was aged 1 to 10 months (Supplementary Figure S5A,B). Mandible SAM36851, from which an unworn and unerupted m3 was extracted (i.e. m3NW), was aged 3.5 years (Supplementary Figure S5C,D). Mandible UCTgm, from which a worn and erupted m1 (i.e. m1W) and m3 (i.e. m3W) were extracted, was aged ca. 5–6 years and belonged to a female individual (Supplementary Figure S5E,F). All mandibles were photographed in different views, and teeth were extracted using a Dremel 3000 rotary tool. Mandibles ZM39558 and SAM36851 were also X-rayed before teeth extraction to study the degree of development of the unworn m1 and m3 respectively. Once extracted, these two teeth were further CT-scanned at X-Sight X-ray Services (Cape Town, South Africa) to obtain digital and 3D printed models.

A total of 10 thin sections were prepared at the level of the different tooth lobes in the bucco-lingual plane (Supplementary Figures S1–4) following standard procedures (Chinsamy & Raath, 1992; Nacarino-Meneses & Chinsamy, 2022). We took special care to obtain straight sections of the tooth crown, since oblique sections are known to highly influence the histological assessment of tooth growth (Smith et al., 2006). As a result of the latter, some of our slides did not preserve the true profile of the dentine horns, since in several cases the tooth roots are oblique to the tooth crown (e.g. Supplementary Figure S4C). Thin sections were studied under a polarising microscope Zeiss Ax10 Lab.A1 with an attached digital camera (Zeiss Axiocam 208 colour). All slides are curated at the Iziko Museums of South Africa (Cape Town, South Africa).

We analysed incremental marks in enamel, dentine and cementum. Although these features were recognised in all three dental tissues, they were better identified in enamel as compared to dentine and cementum (see Results section for further information). Therefore, detailed quantitative measurements were only performed in the enamel. However, it should be noted that the numerous Hunter-Schreger bands greatly hampered the identification of incremental marks in this tissue, especially in the cuspal enamel. For this reason, this area of the tooth usually has a limited number of observations as compared to other regions.

We measured and counted enamel incremental marks (Figure 1) of daily periodicity (i.e. enamel laminations) in the buccal and lingual band of each tooth cusp (protoconid, metaconid, hypoconid, entoconid and hypoconulid) using ZEN 3.0 and Image J software. From these measurements, we estimated the enamel growth parameters (i) daily secretion rate (DSR) and (ii) enamel formation front angle (EFFa) following previous methodologies (see (Nacarino-Meneses & Chinsamy, 2022) for a detailed description of these calculations). DSR was obtained by measuring the distance between enamel laminations following the enamel prims and not perpendicularly. We did not calculate enamel extension rate (EER) because of inconsistent observations of enamel laminations throughout the crown height. Since EFFa is directly related to the EER (Boyde, 1964), we preferred to rely on the values of EFFa. To describe the growth pattern of the giraffe teeth, we analysed the variation of DSR and EFFa among (i) different enamel areas (i.e. inner, middle and outer enamel; see Supplementary Figure S6 for further information), (ii) different crown areas (i.e. upper and lower crown; as divided by the horizontal plane that crosses the lowest part of the depression between cusps; see Supplementary Figure S6 for further information), (iii) different enamel bands of a tooth cusp (i.e. buccal and lingual band), (iv) different cusps within a tooth (i.e. protoconid, metaconid, hypoconid, entoconid and hypoconulid) and (v) different tooth type (i.e. first and third lower molar). When analysing the DSR of the giraffe lower molars, we identified differences between the different enamel areas (inner enamel vs. middle and outer enamel; see Results section). Hence, to simplify the results, statistical analysis to identify differences between enamel bands, tooth cusps and tooth

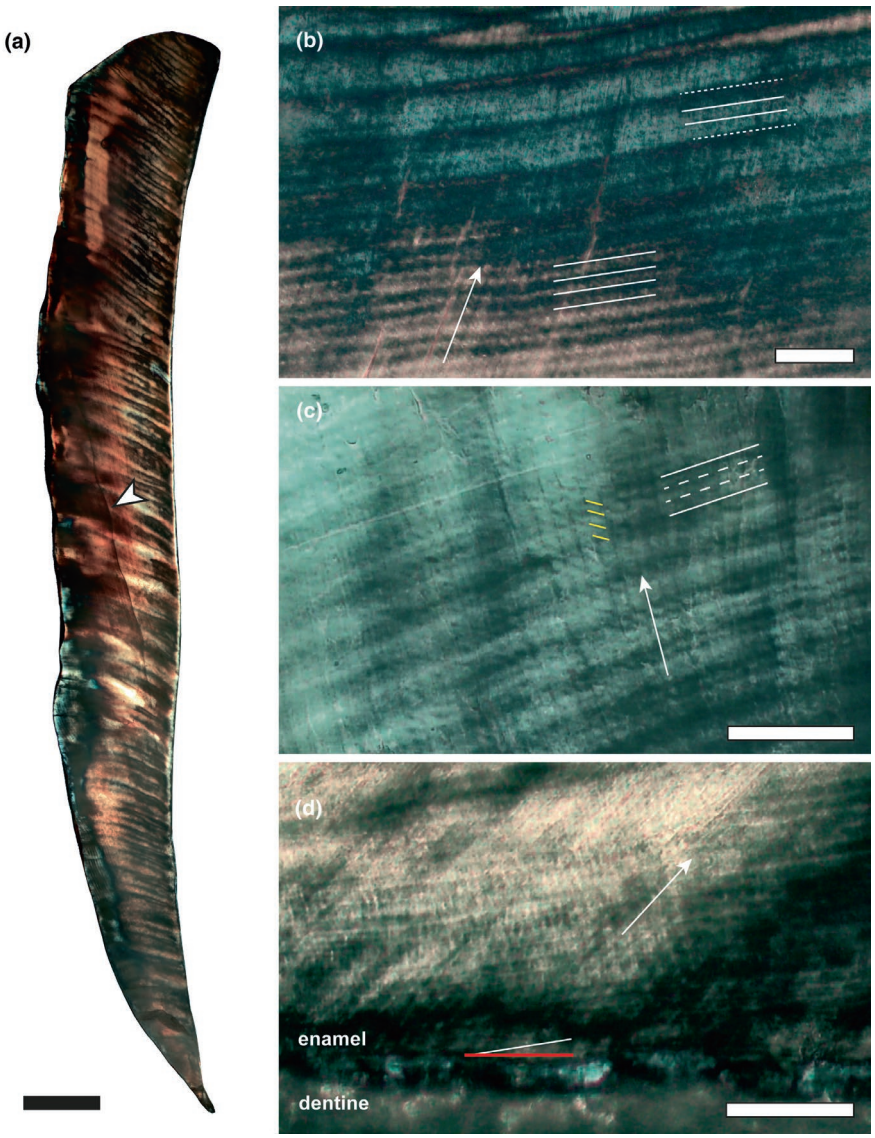

FIGURE 1 Enamel histology of giraffe lower molars. (a) Buccal enamel band of the hypoconid of m1W showing the neonatal line (white arrowhead). (b) Enamel laminations (white lines) and Retzius lines (white dotted lines) in the enamel of m3NW. (c) Enamel cross-striations (yellow lines), enamel laminations (white lines) and subdaily enamel lines that run parallel to enamel laminations (white dashed lines) in the enamel of m1W. (d) Inner enamel of m3W showing the enamel formation front angle, that is, the angle formed between enamel laminations (white line) and the enamel dentine junction (red line). The white arrow in panels b, c, and d indicates the path of the enamel prism. In these panels, cuspal direction is on the right of the image. Black scale bar: 1 mm, white scale bars: 500 microns.

type was restricted to the middle enamel, as this area usually presents intermediate values between the inner and the outer enamel (see Tables 2 and 3 and Nacarino-Meneses & Chinsamy (2022)). Statistical analysis to identify differences in DSR among different crown areas (i.e. upper and lower crown) did not account for enamel areas (i.e. inner, middle and outer enamel). Nevertheless, EFFa calculations revealed significant differences among the different crown areas (upper crown vs. lower crown; see Results section) and therefore we restricted statistical analysis regarding differences between enamel bands, tooth cusps and tooth type to measurements on the upper crown.

Measurements and counts of enamel laminations in m1W were also used to calculate postnatal crown formation time (CFT). Specifically, the CFT of this portion of the crown was calculated by measuring the distance on the EDJ between the neonatal line (Figure 1a) and the end of the crown following the course of the enamel prism. This value was later divided by the DSR of this area (Nacarino-Meneses et al., 2017; Nacarino-Meneses & Chinsamy, 2022). The postnatal CFT was later used to validate previous research on ungulates that report a daily periodicity of enamel laminations (Emken et al., 2021; Kierdorf et al., 2012; Nacarino-Meneses et al., 2017) (see Results section for further information).

Statistical analyses and graphs were performed using R Studio. Differences between groups were tested using non-parametric tests (Kruskal–Wallis test and Mann–Whitney U test), because not

TABLE 2 Results of daily secretion rate.

		Protoconid						Metaconid						Hypoconid						Entoconid					
		Buccal			Lingual			Buccal			Lingual			Buccal			Lingual			Buccal			Lingual		
m1		*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
Enamel area	Outer	26	13.46	1.39	9	13.73	1.97	13	14.28	1.37	12	12.5	1.62	19	14.22	1.37	16	13.72	1.32	17	15.11	1.03	10	12.6	0.94
	Middle	55	13.35	1.36	16	12.37	1.53	11	13.14	1.9	32	12.63	1.08	22	13.73	1.36	19	13.18	1.42	12	13.71	1.26	24	12.52	1.21
	Inner	9	11.61	1.24	16	10.13	1.4	5	9.97	1.24	10	11.18	0.88	29	11.35	1.15	7	9.76	0.92	6	9.65	0.46	16	10.24	1.61
Crown area	Upper	40	13.97	1.1	41	11.79	2.12	29	13.1	2.17	34	12.51	1.21	40	13.12	2.01	42	12.82	1.91	35	13.7	2.22	32	11.8	1.76
	Lower	50	12.6	1.41	NA	NA	NA	NA	NA	NA	20	12.05	1.4	30	12.54	1.49	NA	NA	NA	NA	NA	NA	18	11.81	1.56

		Protoconid						Metaconid						Hypoconid						Entoconid					
		Buccal			Lingual			Buccal			Lingual			Buccal			Lingual			Buccal			Lingual		
m3		*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
Enamel area	Outer	28	12.68	1.43	21	11.71	0.84	13	11.37	0.95	11	11.29	0.95	30	11.16	1.65	6	11.7	0.72	22	12.14	1.44	12	10.7	0.28
	Middle	53	12.55	1.24	22	11.42	1.16	8	11.19	0.7	32	11.38	1.01	35	11.19	1.64	32	11.38	0.94	24	11.94	1.06	17	10.96	0.62
	Inner	17	9.41	0.51	5	9.08	0.2	4	9.33	0.18	6	8.96	0.41	35	9	1.06	25	8.95	0.66	23	8.92	1.09	24	9.2	0.85
Crown area	Upper	37	13.07	0.91	48	11.3	1.23	25	10.99	1.07	23	11.41	1.13	43	10.9	2.02	63	10.44	1.47	69	11	1.9	31	10.01	1.04
	Lower	61	11.42	1.8	NA	NA	NA	NA	NA	NA	26	10.76	1.24	57	10.05	1.5	NA	NA	NA	NA	NA	NA	22	10.23	1.14

		Hypoconulid					
		Buccal			Lingual		
		N	Mean	SD	*N*	Mean	SD
Enamel area	Outer	16	11.02	1.14	13	11.19	1.26
	Middle	16	11.36	0.72	10	10.04	1.11
	Inner	9	9.36	0.39	7	8.93	0.91
Crown area	Upper	16	10.81	1.16	19	10.51	1.56
		25	10.78	1.17	11	9.89	1.12

TABLE 3 Results of enamel formation front angles.

		Protoconid										Metaconid									
		Buccal					Lingual					Buccal					Lingual				
m1		*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max
Crown area	Upper	3	2.25	0.53	1.66	2.68	68	3.21	0.82	1.45	4.65	6	1.98	0.41	1.43	2.46	22	3.09	0.49	2.19	4.01
	Lower	48	6.32	1.59	2.69	9.59	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	4.91	0.21	4.57	5.18
		Hypoconid										Entoconid									
		Buccal					Lingual					Buccal					Lingual				
		N	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max
Crown area	Upper	6	3.03	0.35	2.54	3.45	10	2.83	0.3	2.29	3.3	9	2.98	0.41	2.35	3.59	12	3.6	0.61	2.21	4.48
	Lower	39	4.44	0.74	2.88	6.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	18	4.72	1.38	3.17	7.48
		Protoconid										Metaconid									
		Buccal					Lingual					Buccal					Lingual				
m3		*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max
Crown area	Upper	3	4.47	0.42	4.05	4.89	12	3.73	0.81	2.32	4.57	3	3.304	0.38	2.89	3.64	6	3.08	0.79	2.14	4.3
	Lower	26	7.79	1.47	5.59	10.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	9	6.08	1.32	4.37	7.95
		Hypoconid										Entoconid									
		Buccal					Lingual					Buccal					Lingual				
		N	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max
Crown area	Upper	20	3.95	0.87	2.7	5.64	35	3.97	0.89	1.96	6.04	34	3.6	0.85	2.19	5.73	27	3.57	1.04	1.984	5.33
	Lower	37	7.13	1.23	4.98	9.32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	4.68	0.67	3.47	5.56
		Hypoconulid																			
		Buccal					Lingual														
		N	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max										
Crown area	Upper	4	5.93	0.48	5.34	6.51	6	3.87	1.28	2.45	5.89										
	Lower	8	9.1	1.57	7.44	11.69	6	6.25	0.95	4.95	7.19										

all variables adjusted to a normal distribution (Shapiro–Wilk test, *p*-value >0.05) or consisted of a large number of observations. Differences were deemed statistically significant at *p*-value <0.05.

3 | RESULTS

Giraffe tooth crowns were composed of enamel and dentine (Supplementary Figures S1–S4), while dental roots were composed of dentine (Supplementary Figures S1–S4). Roots were covered by a thin layer of dental cementum (e.g. m1W and m3W, Supplementary Figures S2 and S4). All dental tissues registered incremental lines of different periodicity, although these features were badly preserved in dentine and cementum as compared to dental enamel.

3.1 | Enamel

Dental enamel (Figure 1) was recognised in all teeth under study (Supplementary Figures S1–4). Nonetheless, enamel bands of m1NW were still under formation and not fully mineralised (Supplementary Figure S1). Cuspal enamel was not present in m1W and m3W (Supplementary Figures S2 and 4) due to dental wear.

We identified multiple types of incremental marks in the giraffe dental enamel (Figure 1). The most common incremental features were enamel laminations (Figure 1b), which were identified throughout the tooth crown. In the outer enamel of the lower crown of both molar types, we further recognised Retzius lines (Figure 1b). Usually, we counted two laminations between consecutive Retzius lines, which indicates a repeat interval of 3 days for this mammal. Enamel cross-striations, on the other hand, were found in a few small areas of the outer enamel (Figure 1c). In these regions, we further observed several lines that run parallel to enamel laminations and that presented the same spacing as enamel cross-striations (Figure 1c). Frequently, two cross-striations and two of these closely spaced lines appeared between consecutive laminations (Figure 1c).

To verify if enamel laminations followed a daily periodicity in the giraffe, as they do in other artiodactyla (Emken et al., 2021; Kierdorf et al., 2012), we estimated the postnatal CFT of the buccal band of the hypoconid of m1W (Figure 1a3) by considering these features as daily markings. The calculations performed revealed a CFT of around 3 months, an acceptable result considering that this tooth erupts at the 11 month of life (Hall-Martin, 1976) and it has to develop the roots before that. Instead, if we consider that the closely spaced lines that appeared parallel to laminations are the daily markings, we would obtain a CFT of around 11 months, leaving no time for root formation before eruption. Our results therefore confirm the daily periodicity of enamel laminations in the giraffe, as well as the subdaily periodicity of both cross-striations and the closely spaced lines found in the enamel of this taxon.

The enamel daily secretion rate (DSR) of the giraffe lower molars ranged between 9 μm/day and 15 μm/day (Table 2, Figures 2 and 3). In both molars, the DSR of the inner enamel (m1: 10.5 μm/day; m3: 9.1 μm/day) was significantly lower as compared to that of the middle (m1: 13.1 μm/day; m3: 11.3 μm/day) and the outer (m1: 13.7 μm/day; m3: 11.5 μm/day) enamel (Figure 2, Table 2, Supplementary Information). The only exceptions to this general finding occurred on the lingual side of the m1 metaconid and the m3 hypoconulid, where the DSR of the inner enamel only differed significantly from that of the middle or outer enamel respectively (Figure 2, Supplementary Information). Conversely, the DSR did not vary widely along the tooth height (i.e. from cervix to cusp, Figure 3, Table 2, Supplementary Information). Significant differences between the upper and lower crown were only detected in the protoconid of both molars and the hypoconid of the m3 (Figure 3, Supplementary Information). Within a tooth cusp, the buccal enamel band usually presented somewhat higher values of DSR as compared to the lingual enamel band (Figure 2, Table 2, Supplementary Information). Significant differences, however, were only noted within the protoconid and entoconid of both molars and the hypoconulid of the m3 (Supplementary Information). Interestingly, we could not identify variations on DSR among the different cusps of the m1 (Figure 2, Table 2, Supplementary Information). In the m3, however, the DSR of the buccal side of the protoconid was significantly higher as compared to that of the metaconid, hypoconid and hypoconulid (Figure 2, Table 2, Supplementary Information), while the DSR of the lingual side of the hypoconulid was significantly lower as compared to that of the protoconid and the hypoconid (Figure 2, Table 2, Supplementary Information). In all cases, the DSR of the m1 was significantly higher as compared to the DSR of the m3 (Figure 2, Table 2, Supplementary Information).

The enamel formation front angle (EFFa) varied from 2° to 6° in the m1 and from 3° to 9° and the m3 (Figure 4, Table 3). In both teeth, the lower crown consistently showed significantly wider angles as compared to the upper crown (Figure 4, Table 3, Supplementary Information). We further detected significant differences between the EFFa of the lingual and buccal enamel in the m1 metaconid and entoconid (Figure 4, Supplementary Information). In the m3, however, EFFa of the buccal band only differed significantly from that of the lingual band in the hypoconulid (Figure 4, Supplementary Information). In both teeth, the buccal enamel band showed narrower angles as compared to the lingual one. When comparing tooth cusps, we identified significant differences in EFFa between the m1 buccal side of the metaconid and that of the entoconid and hypoconid (Figure 4, Supplementary Information), which presented wider angles (Table 3). In this tooth, the EFFas found at the lingual side of the hypoconid were significantly narrower as compared to that of the entoconid (Figure 4, Supplementary Information). Differences among cusps in the m3 were only found in the buccal band of the enamel, where we detected significantly narrower EFFas in the hypoconulid than in the entoconid and hypoconid (Figure 4, Supplementary Information). Generally, the m3 usually presented wider EFFas as compared to the m1 (Figure 4, Table 3), although these differences only were statistically significant for the lingual side of the protoconid and hypoconid, and the buccal side of the metaconid, hypoconid and entoconid (Supplementary Information).

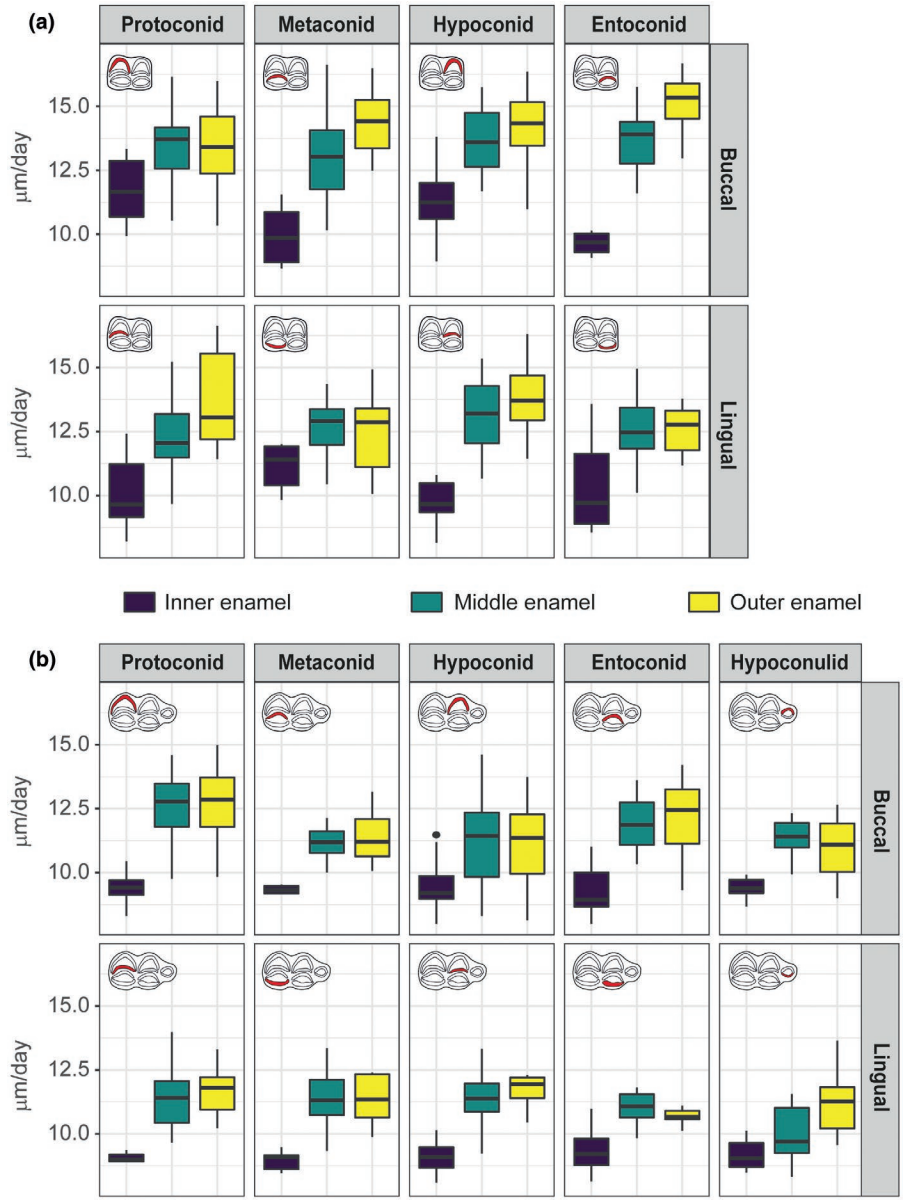

FIGURE 2 Enamel daily secretion rate in the different enamel areas of the first (a) and third (b) lower molar of the giraffe. On each graph, there is a schematic drawing of the occlusal view of the tooth with the enamel band under analysis indicated in red.

3.2 | Dentine

Dentine was observed in all teeth under analysis. In the still-developing m1NW and m3NW, we only recognised primary dentine (Supplementary Figures S1 and 3). This type of dentine also conformed to most of the tooth crown and roots in m1W and m3W (Supplementary Figures S2 and 4). In these teeth, however, we also recognised a thin layer of secondary dentine covering the pulp chamber.

Dentine incremental marks were only visible in some areas, and they were usually better recognised in the tooth crown than in the root (Supplementary Figures S1–4). Due to the blurred appearance of the lines, measurements were only taken in crown areas close to the EDJ. Here, most of the incremental lines were 15–20 µm apart, and likely corresponded to daily von Ebner's lines (Figure 5). Supradaily Andersen lines were also discernible in some areas, but preliminary measurements delivered inconsistent results to provide a reliable repeat interval.

3.3 | Cementum

Dental cementum was only identified in m1W and m3W, where a thin layer of 200–400 mm covered the root (Supplementary Figures S2 and S4). The tissue mainly consisted of cellular cementum and, in some areas, it presented the characteristic layered structure with incremental lines. These features appeared quite blurred throughout the cementum band, and they were only easier to recognised in

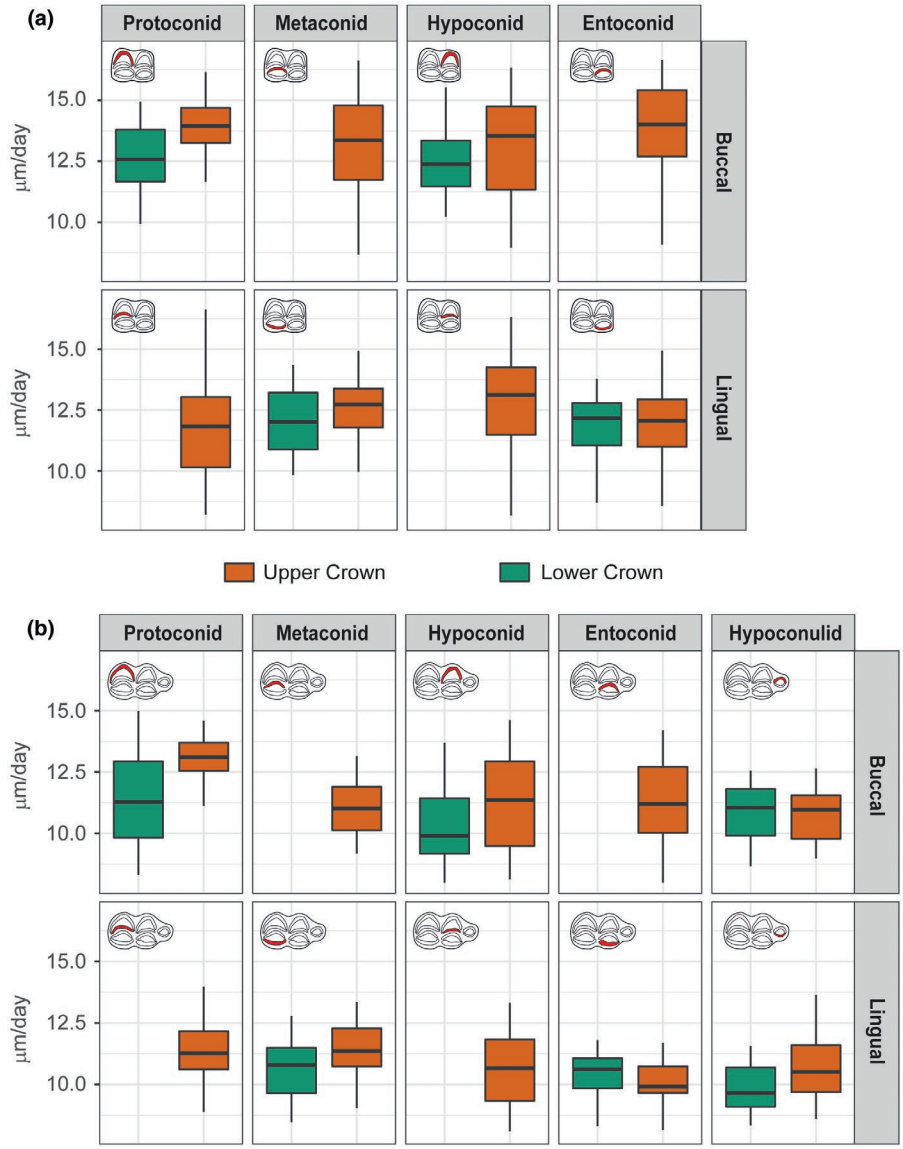

FIGURE 3 Enamel daily secretion rate in the different crown areas of the first (a) and third (b) lower molar of the giraffe. On each graph, there is a schematic drawing of the occlusal view of the tooth with the enamel band under analysis indicated in red.

acellular cementum at the beginning of the tooth root (Figure 6). In this area, we were able to count four and three incremental lines in the m1W and the m3W respectively (Figure 6).

4 | DISCUSSION

Our research provides a novel comprehensive description of the dental histology of the lower molars of *G. camelopardalis*, the extant giraffe. The only other study of the dental microstructure of this animal was by Hall-Martin (1976), who briefly reported incremental lines in the dentine and cementum of the upper first molars. In the current study, we identify incremental features in the dentine, enamel and cementum of the first and third lower molars of *G. camelopardalis* (Figures 1, 5 and 6), although incremental lines are more extensively documented in dental enamel (Figure 1). In the dentine and the cementum, these features appear patchily (Figures 5 and 6), which are likely an artefact of the thin sectioning (i.e. thin section thickness and obliquity) (Hillson, 2005).

4.1 | Periodicity of enamel incremental lines

In giraffe dental enamel, incremental lines appear registering circadian, ultradian and supradaily rhythms. As described in other ungulates (Emken et al., 2021; Iinuma, Tanaka, et al., 2004; Jordana et al., 2014; Jordana & Köhler, 2011; Kierdorf et al., 2014, 2019, 2013, 2012; Nacarino-Meneses et al., 2017; Nacarino-Meneses & Chinsamy, 2022; Orlandi-Oliveras et al., 2019; Tafforeau et al., 2007), enamel laminations are the most prominent incremental lines in the lower molars of *G. camelopardalis* (Figure 1b – d), where they also have a daily nature. This result further agrees

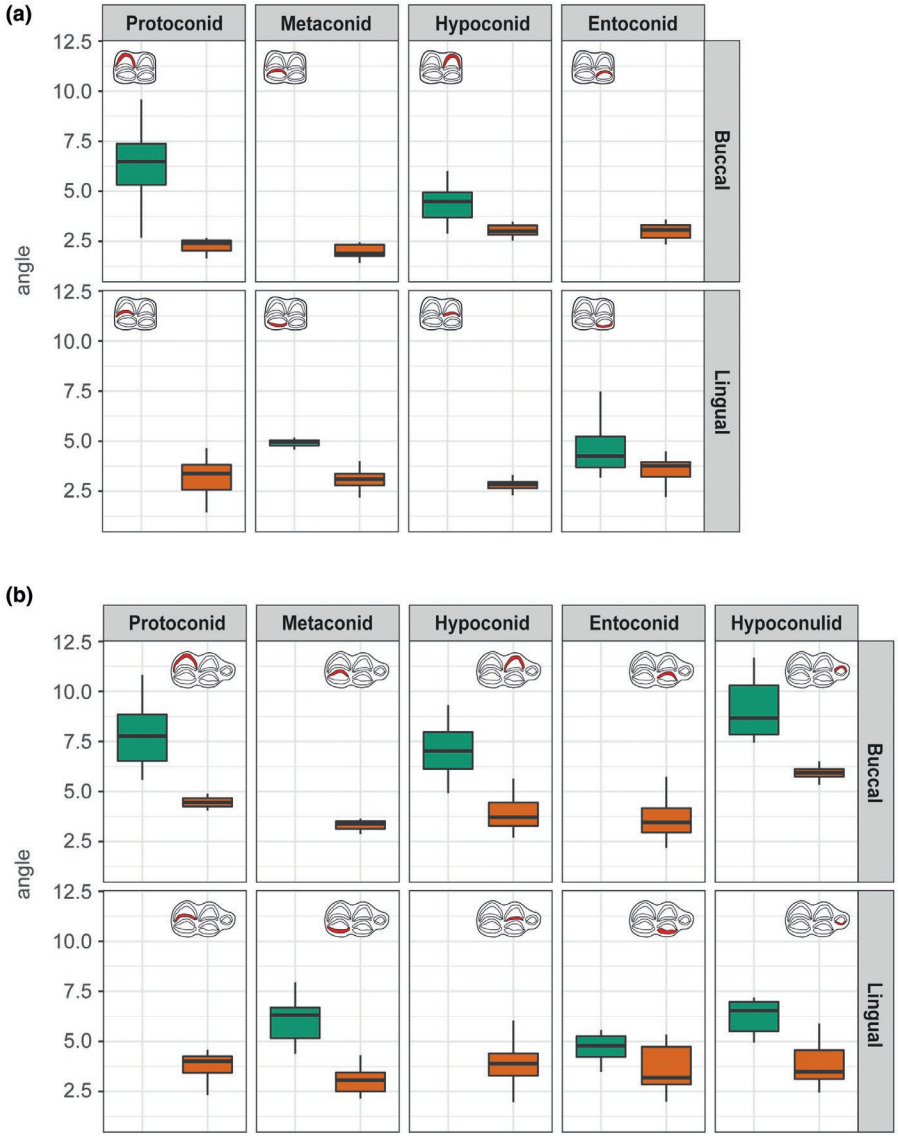

FIGURE 4 Enamel formation front angle in the different crown areas of the first (a) and third (b) lower molar of the giraffe. On each graph, there is a schematic drawing of the occlusal view of the tooth with the enamel band under analysis indicated in red.

with previous findings on equids (Nacarino-Meneses et al., 2017), bovids (Kierdorf et al., 2013, 2012), cervids (Iinuma, Tanaka, et al., 2004), suids (Emken et al., 2021; Kierdorf et al., 2019) and primates (Smith, 2006), which also reported a daily periodicity of enamel laminations.

Along with daily enamel laminations, we also identified long-period Retzius lines in the outermost part of the giraffe cervical enamel (Figure 1b). Considering the number of daily laminations found between these lines, we estimated a repeat interval of 3 days. This observation is comparable to the results provided by Kierdorf et al., (2019) and Emken et al., (2021) for the wild boar (3 days) and the domestic pig (2 days). Previous research had found a positive correlation between the repeat interval and the adult body mass in primates and proboscideans (Bromage et al., 2012, 2009), but our results do not support such a relationship in Artiodactyla: the adult body mass of *G. camelopardalis* (800 kg, (Tacutu et al., 2018)) is more than six times that of *Sus scrofa* (130 kg, (Tacutu et al., 2018)), yet they have the same repeat interval. Kierdorf et al., (2019) and Hogg et al., (2018) also reported that the association between adult body mass and infradian growth cycles in the enamel of pigs and dogs does not apply at the intraspecific level. Thus, it is apparent that further research is needed to better understand the biological significance of the repeat interval.

In small patches of the outer enamel, we identified some closely spaced markings that run parallel to daily laminations (Figure 1c), as well as enamel cross-striations (Figure 1c), that record ultradian growth rhythms within this tissue. Similar subdaily incremental markings have already been described in primates (Smith, 2006), bovids (Kierdorf et al., 2013) and suids (Emken et al., 2021; Kierdorf et al., 2014, 2019). In these artiodactyls, five subdaily growth increments have been reported between consecutive laminations (Emken et al., 2021; Kierdorf et al., 2014, 2019, 2013), while two or three

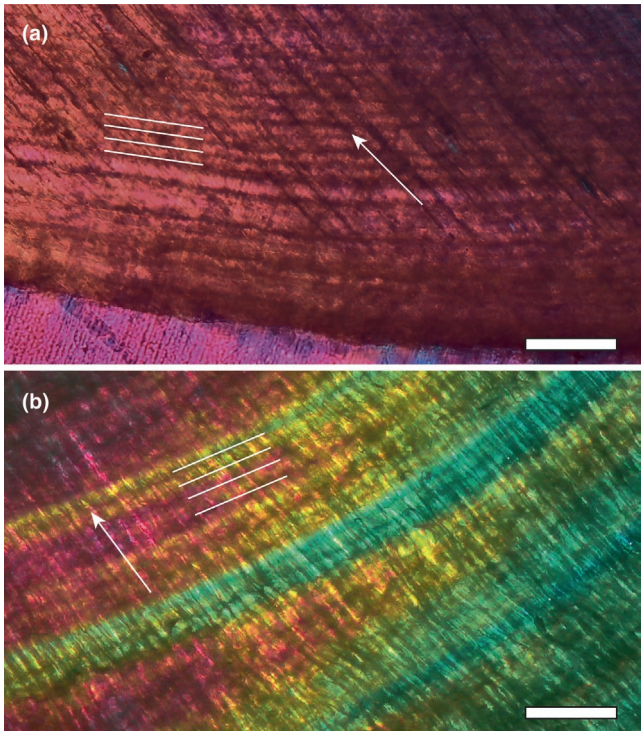

FIGURE 5 Dentine incremental lines (white lines) in the teeth of the giraffe. (a) m1NW. (b) m3W. The white arrow indicates the path of the dentine tubules. Scale bars: 100 microns.

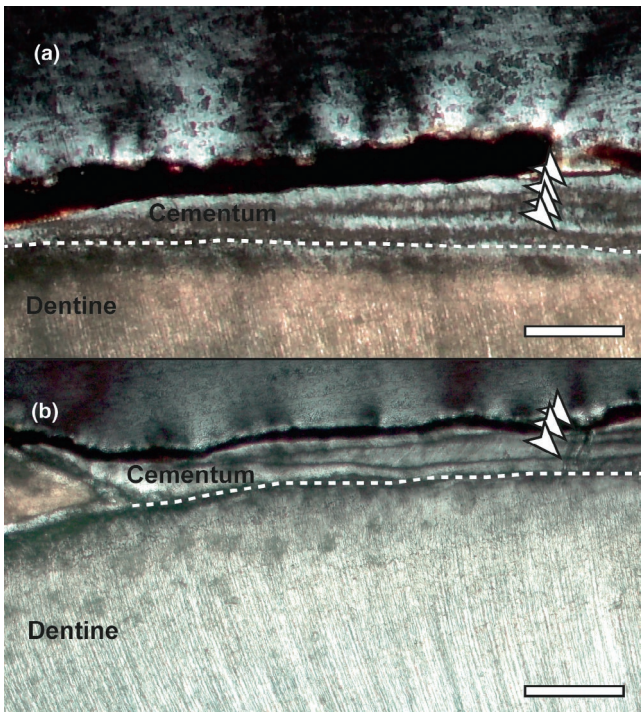

FIGURE 6 Cementum incremental lines (white arrows) in the teeth of the giraffe. (a) m1W. (b) m3W. White dashed line: Cementum dentine junction. Scale bars: 250 microns.

subdaily increments between consecutive daily lines have been described in the enamel of macaques (Smith, 2006). Surprisingly, subdaily cross-striations and laminations in the giraffe enamel also record three growth increments between daily lines (Figure 1c), suggesting that there is a high variability in the ultradian rhythms of ameloblasts' secretion within Artiodactyla.

4.2 | Enamel daily secretion rate

Measurements of the daily enamel laminations allowed for the estimation of the DSR of this tissue. As indicated in Figures 2 and 3, and Table 2, we estimated a mean value of DSR of 10–15 μm/day for the m1 and of 9–13 μm/day for the m3 of the giraffe. The results obtained for the m1 match those reported in other artiodactyls (9–19 μm/day, (Iinuma, Tanaka, et al., 2004; Jordana et al., 2014; Jordana & Köhler, 2011; Kierdorf et al., 2012; Kierdorf et al., 2013; Skinner & Byra, 2019)), but the DSRs found on the m3 are lower than those described in suids (11–24 μm/day, (Kierdorf et al., 2014, 2019)). Moreover, the values obtained for both teeth are lower than those described in equids (15–19 μm/day; (Nacarino-Meneses et al., 2017; Nacarino-Meneses & Chinsamy, 2022; Orlandi-Oliveras et al., 2019)) and higher than those found in archaic ungulates (2.5–5 μm/day; (Dirks et al., 2009)). The very low rates of enamel secretion reported in the latter should however be considered with caution since they were calculated by measuring enamel cross-striations instead of enamel laminations (Dirks et al., 2009) and, according to our research and that of Kierdorf et al. (Kierdorf et al., 2014, 2019) and Emken et al., (2021), they likely present a subdaily periodicity in Artiodactyla. Nevertheless, variation in DSR has generally been attributed to several factors, including tooth morphology (Jordana et al., 2014), tooth size (Dirks et al., 2012) and general somatic growth (Emken et al., 2023; Orlandi-Oliveras et al., 2019). From our data, it is difficult to ascertain precisely which of these factors had a higher impact on the DSR differences found between the giraffe lower molars and that of other mammals, although some preliminary inferences can be drawn. Conversely to (Jordana et al. 2014), our observations suggest that tooth size and morphology might play a small role in these DSR differences. On the one hand, the giraffe has larger teeth as compared to equids and suids, but lower values of DSR. On the other hand, the different kinds of cuspal morphology of the giraffe teeth (i.e. selenodont) as compared to that of suids (i.e. bunodont) and equids (i.e. lophodont) might explain the differences found on the m3 but not the similarities observed for the m1, since both molars present the same morphology. Instead, the lower values of enamel DSR found in the giraffe m3 could correlate with a lower general somatic growth rate expected from its slower pace of life and larger body size (Tacutu et al., 2018). Differences related to general somatic growth should also be expected for the m1, but these are not recorded in our results. Perhaps, they are masked by the generally high growth rates that mammals present at these earlier ontogenetic stages.

Along with interspecific differences in DSR, we have further analysed how this enamel growth parameter varies within a tooth. In agreement with previous research performed in other mammals

(e.g. Emken et al., 2021; Kierdorf et al., 2014; Kierdorf et al., 2019; Nacarino-Meneses & Chinsamy, 2022), our results show that the inner enamel of the giraffe lower molars is formed at lower secretion rates as compared to the middle and outer enamel (Figure 2, Table 2). However, we did not find statistical differences in DSR along the height of the giraffe crown (Figure 3, Table 2), except for the protocone of both molars under study and the hypocone of the m3, where the DSR decreases from the cusp to the root. This observation generally agrees with previous investigations in extant and extinct equids (Nacarino-Meneses & Chinsamy, 2022; Orlandi-Oliveras et al., 2019), pigs (Emken et al., 2023; Kierdorf et al., 2014, 2019) and sheep (Kierdorf et al., 2013). Within a tooth cusp, the buccal enamel consistently shows higher DSR values as compared to the lingual enamel (Figures 2 and 3, Table 2), although statistical support for this observation has only been found in some cusps (Supplementary Information). (Kierdorf et al., 2013) also noticed differences in DSR between the buccal and lingual enamel on the lower m1 of sheep, even though their analyses are not restricted to the same tooth cusp (i.e. they analysed the buccal and lingual lateral enamel, which would correspond to the buccal band of the protoconid and the lingual band of the metaconid, respectively, for the mesial lobe). Finally, we have observed that the DSR varies widely among the m3 cusps in the giraffe, but it remains the same in the different cusps of the m1 (Figures 2 and 3, Table 2). Previous research on humans and chimpanzees reported no differences on DSR among molar cusps (Mahoney, 2008; Smith, 2004; Smith et al., 2007), suggesting that the m3 of artiodactyls might show a higher variability in DSR as compared to that of primates.

Variations in DSR among molar types were also detected in the giraffe, with the m1 secreting enamel at higher rates than the m3 (Figures 2 and 3, Table 2). Differences among molar types have also been reported in extinct hipparionins (Nacarino-Meneses & Chinsamy, 2022; Orlandi-Oliveras et al., 2019), but were not found in other taxa, such as hominoids (Smith, 2016; Smith et al., 2007). Following previous suggestions (Orlandi-Oliveras et al., 2019), we attributed the differences between tooth types to the general somatic growth of the animal during the formation of the specific molar.

4.3 | Enamel formation front angle

The study of enamel incremental lines permitted the calculation of the enamel formation front angle, an enamel growth parameter that reflects the number of ameloblasts that are active at the same time (Hogg, 2018). The EFFa is also closely linked to the rate of enamel extension (Boyde, 1964) (i.e. the rate at which the tooth crown grows in height), with larger angles indicating slower rates (Boyde, 1964) provided that the DSR remains constant along the EDJ (Smith, 2006). Our analysis revealed that the EFFa in the giraffe ranges from 2° to 9° (Figure 4, Table 3). The m1 presents slightly lower values (2°–6°) as compared to the m3 (3°–8°) (Figure 4, Table 3), although these differences are statistically significant only for some cusps and enamel bands (Supplementary Information). Generally, the results obtained for the giraffe m1 fairly match those reported in other ungulates, including some bovids, cervids and hipparionines (2°–6°, (Jordana et al., 2014; Nacarino-Meneses & Chinsamy, 2022)). They differ, however, with the lower EFFas observed in the more hypsodont *Equus* (1°–12°, (Nacarino-Meneses et al., 2017)). The EFFas found in the m3 of the giraffe also present similarities with the ones described in hipparionines (3°–7°; (Nacarino-Meneses & Chinsamy, 2022)), but they are much more acute than those observed in pigs (6°–24°; (Kierdorf et al., 2019)). Results for both molars also differ from the much wider angles described in primates (9°–48°, (Guatelli-Steinberg et al., 2018; Hogg & Walker, 2011)). Previous studies have proposed that molar EFFa positively correlates with some life history characteristics, including the age at first reproduction in ruminants (Jordana et al., 2014), and the body mass, the brain mass and the encephalisation quotient in primates (Guatelli-Steinberg et al., 2018; Hogg & Walker, 2011). However, caution should be taken when using the EFFa to classify a species along the slow–fast life history continuum (Jordana et al., 2014), since this enamel growth parameter has an important phylogenetic effect (Hogg & Walker, 2011). Our results confirm that the relationship between EFFa and life history traits is complex, even in phylogenetically related taxa, and that it requires further study. For instance, the positive correlation proposed by (Jordana et al., 2014) between the age at first reproduction in ruminants and the EFFa of their first lower molar does not seem to apply for the third lower molar at a higher taxonomic level (i.e. Artiodactyla); if it had been the case, we should have found the opposite results for the EFFa in the giraffe as compared to pigs.

We also detected differences in EFFa among the different areas of the tooth crown, the enamel bands and the tooth cusps. As reported in other taxa (e.g. Kierdorf et al., 2019; Nacarino-Meneses et al., 2017), the upper crown of in the giraffe tooth crown present narrower EFFas as compared to the lower crown (Figure 4, Table 3). This indicates a decrease in the number of active ameloblasts during the formation of the tooth (Hogg, 2018). Given that DSR largely remains constant along the molar height (see before), it also suggests a decline in the rate of enamel extension during crown formation (Boyde, 1964). The lingual and buccal bands of each tooth cusp also differ in EFFa. Generally, the buccal band presents wider angles as compared to the lingual band in the m3, while the opposite pattern can be observed in the m1 (Figure 4, Table 3). Statistical significance for these trends, however, was only detected in some cusps (Supplementary Information). Interestingly, (Kierdorf et al., 2019) also reported generally wider EFFa in the buccal than in the lingual enamel band of the porcine third lower molars. Why the relationship between EFFas and enamel band is the opposite in the m1 remains unresolved, and further research is needed to address this issue. In this sense, the findings for the m1 presented here should also be considered with caution, since the number of observations varies widely among the two enamel bans in some cusps, and therefore they can be

statistically skewed (Figure 4, Table 3). Nevertheless, in both molars, we have further detected differences in EFFa among different cusps (Supplementary Information), with the m1 showing lower variability than the m3 (Figure 4, Table 3). Comparative data are unfortunately very limited, but a previous study by (Smith et al., 2004) also found differences in EFFa among the different cusps of the third lower molar in a late Miocene hominoid from Greece. Differences in EFFa observed in the m1 might be reflecting differences in EER among tooth cusps, since DSR does not vary among the different cusps of this tooth (Figures 2 and 3, Table 2, see previous section). (Smith et al., 2006) also observed different EER in the different cusps of *Macaca nemestrina*. Differences in EER in the giraffe m3, however, cannot be directly inferred from EFFas, since we have identified significant differences in DSR among the different cusps for this tooth (Figures 2 and 3, Table 2, see previous section).

4.4 | Incremental lines in dentine and cementum

As previously indicated, incremental lines in dentine and cementum appeared more patchily and blurred as compared to those in dental enamel. (Hall-Martin 1976) reported some difficulties to identify these features in undecalcified thin sections, and he was only able to clearly recognise them in stained decalcified dental slides. An extensive study of these features in the dentine and cementum of the giraffe is beyond the scope of the present research, but we do provide some preliminary results that could inform future investigations.

In agreement with (Hall-Martin, 1976), we recognised incremental lines in the dentine of the giraffe lower molars (Figure 5). In our study, these features seem to correspond to both daily von Ebner's lines and supradaily Andersen lines (Dean et al., 1993). Generally, both kind of incremental marks appeared more clearly in the giraffe tooth crown as compared to the root, and especially in areas close to the EDJ or the cementum-dentine junction (CDJ) than to the pulp cavity (Supplementary Figures S1–S4). In these regions, we estimate a daily secretion rate of 15–20 μm/day for the dentine of the giraffe, a value that fairly matches those reported in the cuspal area of the crown of other ungulates, including pigs (Emken et al., 2024) and sheep (Kahle et al., 2018). The dentine secretion rate estimated for the lower molars of the giraffe is however much higher as compared to that observed in deer (6–8 μm/day, (Iinuma, Tanaka, et al., 2004)). Differences between our results and those reported in deer can be explained by the fact that measurements in the latter taxon were restricted to the cervical area of the crown (Iinuma, Tanaka, et al., 2004), which is known to present lower values as compared to the cuspal region (Emken et al., 2024; Kahle et al., 2018).

Regarding dental cementum, we identified four incremental lines in the first lower molar and three incremental lines in the third lower molar (Figure 6). Both teeth were extracted from the same mandible (i.e. UCTgm, Table 1), but differences in the number of incremental lines were expected since these teeth differ in their time of eruption (Hall-Martin, 1976). Previous research has shown that cementum lines are generally laid down annually (e.g. Lieberman, 1993), and therefore they are widely used to estimate age at death in extant and fossil samples (e.g. Azorit et al., 2004). For the giraffe, (Hall-Martin, 1976) specifically suggests that individual age should be calculated as the number of broad dark lines in the first molar plus one, since this tooth erupts at the age of 1 year and no cementum lines are formed prior to eruption. Considering this, the mandible under study should be aged 5 years. This matches our estimation of 5–6 years based on tooth wear and confirms the previous observations by (Hall-Martin, 1976) suggesting that yearly lines in dental cementum constitute a reliable methodology to estimate individual age.

5 | CONCLUSIONS

Our histological analysis of the lower molars of *G. camelopardalis* permitted the identification and calculation of dental growth parameters that will serve as a baseline for future studies on extinct giraffids and closely related ungulates. Generally, enamel growth marks were better preserved as compared to those in the dentine and cementum, and therefore our analyses focussed on those in the enamel. Results obtained in this tissue are especially important to obtain reliable estimations about the dental growth in extinct giraffids, and therefore to make life history deductions in these taxa. Establishing the exact periodicity of enamel incremental lines, on the one hand, is crucial to calculate crown formation times, and therefore to obtain important life history data. Other enamel growth parameters calculated here, such as the enamel secretion rate or the enamel formation front angle, however, present a more complex relationship with life history traits.

Concurring with previous investigations on other ungulates, daily enamel laminations are the most common incremental features in the giraffe. Supradaily Retzius lines and their repeat interval (3 days) suggest, contrary to primates, that there does not appear to be a correlation between this parameter and the adult body mass in Artiodactyla. We found that in *G. camelopardalis* subdaily enamel cross-striations and closely spaced enamel laminations indicated an 8-h ultradian rhythm of ameloblasts' secretion, as well as a high variability in the periodicity of these features within Artiodactyla. Values of DSR obtained for the giraffe m1 agree with those reported in closely related artiodactyls, while those of the m3 are slightly lower, probably reflecting differences in general somatic growth between taxa. Similarly, values of EFFa observed in the giraffe m1 match those previously reported in other ungulates, but those of the m3 differ from the EFFa described in pigs. Our results caution against a direct relationship between EFFa and life history traits, even in phylogenetically related taxa. Our findings also showed that both enamel growth parameters, that is, the DSR and the EFFa, show a high variation in the enamel of different enamel areas (i.e. inner, middle and outer enamel), crown areas (i.e. upper and lower crown), enamel bands of a tooth cusp (i.e. buccal and lingual band), cusps within a tooth (protoconid, metaconid, hypoconid, entoconid and hypoconulid) and tooth types (i.e. first and third lower molar).

These findings hinder the comparison among species and highlight that comparisons should only be made in comparable regions. Thus, it is important that researchers precisely indicate where their analyses are performed so that reliable comparative information can be utilised when extrapolating to the fossil record.

Regarding dentine, we also document some preliminary findings about the presence and periodicity of incremental lines in this tissue, which could be expanded and assessed more comprehensively in future studies comprising larger sample sizes. Finally, our investigation revealed that incremental marks in dental cementum concur with age estimation based on wear, corroborating the use of cementum lines to estimate individual age in giraffids.

Overall, this study has demonstrated the usefulness of dental histology to deduce life history information and sets the stage for future palaeobiological research on the histology of dental tissues of extinct giraffids.

AUTHORS CONTRIBUTION

CNM contributed to the concept/design, acquisition of data, data analysis/interpretation, drafting of the manuscript, critical revision of the manuscript and approval of the article. JMJ was responsible for acquisition of material, critical revision of the manuscript and approval of the article. ACT contributed to the concept/design, acquisition of material, critical revision of the manuscript and approval of the article.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they do not have any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in this article or in its supplementary material.

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