Palaeogeography, Palaeoclimatology, Palaeoecology 686 (2026) 113557

Contents lists available at ScienceDirect

Palaeogeography, Palaeoclimatology, Palaeoecology

journal homepage: www.elsevier.com/locate/palaeo

Quantitative histological analysis of dental variability in *Anchitherium*: insights into growth dynamics and dental development☆

Teresa Calderón [a,b,*], Andrea Cuccu [a], Jorge Morales [c], Beatriz Azanza [a], Daniel DeMiguel [a,d]

[a] Departamento de Ciencias de La Tierra, and Instituto Universitario de Investigación en Ciencias Ambientales de Aragón (IUCA), Universidad de Zaragoza, Zaragoza, Spain
[b] Naturalis Biodiversity Center, Leiden, the Netherlands
[c] Departamento de Paleobiología. Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain
[d] Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain

ARTICLE INFO

Editor: Howard Falcon-Lang

Keywords:
Enamel
Environmental change
Equidae
Hypsodonty
Pace of life
Palaeohistology

ABSTRACT

The correlation between enamel microstructure and life history has allowed researchers to gather important information on the dental growth and development of both extant and fossil taxa. The approach has been especially developed on primates but also applied to ungulates. Among them, equids have received particular attention, since the enamel microstructure has been examined in several species of hipparionines and *Equus*. However, this aspect has been completely unexplored in more basal equid taxa. In this work, we analyse the dental microstructure of upper and lower dental elements and calculate for the first time the dental parameters of *Anchitherium*, a three-toed equid that inhabited the Palaearctic region from the Early to Late Miocene. Results revealed significantly higher secretion rates in the first lower molar and similar extension rates in all the dental elements analysed. In addition, the study of both upper and lower elements in this work seems to indicate that the method of analysing only the lower dentition, as employed in many studies, may not provide the complete framework to make inferences (at least in *Anchitherium*) and hence, should be taken with caution. Comparing *Anchitherium* enamel parameters with more derived members of the family Equidae has allowed us to observe a clear correlation between enamel secretion rate and hypsodonty index, which increases over time, coinciding with the acquisition of hypsodont teeth. In parallel, a trend towards a faster pace of growth was identified over time, which could be related to the Miocene environmental changes. The present work deepens our understanding of equid dental development and the evolution of this iconic group of ungulates.

1. Introduction

Histological analysis of dental and bone microstructure is a valuable tool in vertebrate palaeontology, as it enables histologists to obtain life history data on extinct taxa (Padian and Lamm, 2013; de Buffrénil et al., 2021). The usefulness of this approach relies on two main aspects: the ability of hard tissues to record information and the durability of such tissues. Enamel is one of the best-preserved body tissues after death (Hillson, 2005). This tissue results from the maturation of the enamel matrix secreted by ameloblasts in accordance with metabolic rhythms, leading to microstructural marks of different periodicity (Dean, 2000). In addition to the periodic growth record, this tissue also records single or non-periodic marks, such as the neonatal line (Zanolli et al., 2011; Witzel, 2014) and other stressful events (Lemmers et al., 2021). As the matrix is secreted accretionally and remains unaltered, the tissue acts as a growth template that permanently retains a record of its development (Tafforeau et al., 2007). Thus, based on the periodic marks recorded in the template, dental parameters on the rate and duration of teeth growth can be calculated and examined to make inferences about the life history of the species (Hogg, 2018).

Histological studies aimed to analyse life history in mammals (e.g. Jordana et al., 2014, Nacarino-Meneses et al., 2017; Orlandi-Oliveras et al., 2019; Nacarino-Meneses and Chinsamy, 2022; Cuccu et al., 2025a) tend to use lower molars, specifically the m1 and m3, as their eruption correlates with important life history events, such as age at weaning and age at maturity, respectively (Hillson, 2005; Dean, 2006).

☆ This article is part of a Special issue entitled: 'Diamond Anniversary Paper' published in Palaeogeography, Palaeoclimatology, Palaeoecology.

* Corresponding author at: Departamento de Ciencias de La Tierra, and Instituto Universitario de Investigación en Ciencias Ambientales de Aragón (IUCA), Universidad de Zaragoza, Zaragoza, Spain.
E-mail address: tcalderon@unizar.es (T. Calderón).

https://doi.org/10.1016/j.palaeo.2026.113557
Received 28 November 2025; Received in revised form 8 January 2026; Accepted 8 January 2026
Available online 12 January 2026

Only a few authors analysed other elements, such as the second lower molar (Emken et al., 2023) or upper elements (Tafforeau et al., 2007; Dirks et al., 2009). Even though different enamel secretion rates between upper and lower molars were documented (Dirks et al., 2009), there has been no attempt to examine the variability of growth parameters among the different dental elements of the same taxon. A matter of great importance, given that, especially with a limited paleontological sample, the possibility of including also the upper dentition could provide more material to widen the sample.

Initial histological analyses of mammalian teeth focused on primates (e.g. Dean, 1987, Bromage, 1991, Smith, 1991), but this approach was soon applied to other taxa, especially ungulates (Macho and Williamson, 2002; Iinuma et al., 2004, Tafforeau et al., 2007; Jordana et al., 2014; Kierdorf et al., 2013, 2019; Emken et al., 2021, 2023; Cuccu et al., 2025a, Nacarino-Meneses et al., 2025). Among them, the family Equidae has received special attention, with the enamel microstructure of several of its members having been explored. Nacarino-Meneses et al. (2017) compared the life histories of various extant and extinct *Equus* species, and Nacarino-Meneses and Chinsamy (2022) examined the enamel growth in a Pliocene hipparionine horse from Africa. On the other hand, Orlandi-Oliveras et al. (2019) analysed the dental histology of three species of Miocene hipparionine horse, comparing the enamel parameters with those of extant *Equus* spp. Considering the link between dental development and life history (Jordana et al., 2014), Orlandi-Oliveras et al. (2019) concluded that hipparionines experienced a slower pace of growth than extant *Equus* species. Despite these interesting results, the life history and dental patterns of the family Equidae remain unexplored in more basal taxa (a phylogeny of the group can be consulted in MacFadden (1988)).

One of these basal equid representatives was *Anchitherium*, a three-toed horse exhibiting low-crowned teeth (i.e. brachydont), with low cusps and lacking cement (MacFadden, 1994; Janis, 2007). This equid entered the Palaearctic region in the Early Miocene (Janis, 2007), had a moderately successful radiation (Forsten, 1991) until the late Miocene, when it coexisted briefly with *Hipparion* (Tleuberdina and Forsten, 2001) before going extinct. *Anchitherium* fossil remains have been found in several locations along the Palaearctic (e.g. Alberdi and Rodríguez, 2012; Klementiev and Sizov, 2015). In the Iberian Peninsula, the deposits of the Madrid Basin are particularly rich in fossil material (see Sánchez et al., 1998 for comprehensive information on the Madrid Basin sites), providing an excellent opportunity to study *Anchitherium* dental material. Specifically, the fossil record of *Anchitherium* from the Madrid Basin allowed us to study both upper and lower dentition, including different types of teeth (premolars and molars) and both complete and partially complete molar series (see Section 2).

This work holds, therefore, particular importance as it combines methodological and evolutionary perspectives. By analysing a great array of *Anchitherium* teeth, it establishes a detailed histological framework for the genus, which provides essential data on enamel growth and developmental rates at an early stage of equid evolution. In addition, the inferences on *Anchitherium* life history allow for comparison with other equids, shedding light on the variability of the life histories within this group of ungulates. Ultimately, the data herein provided may also serve as a reference for future studies on enamel growth and dental histology in other herbivorous mammalian groups, facilitating broader comparative and evolutionary analyses.

2. Material and methods

2.1. Dataset

The studied material consists of *Anchitherium* dentition from the site of Carpetana (Latitude: 40° 23′ 33.6582“, longitude: -3° 44’ 27.4698”; Madrid Basin, central Spain). This middle Miocene fossil site is located in the urban area of Madrid (Siliceo et al., 2020), near other classic Miocene sites (e.g. San Isidro, Hidroeléctrica). The macromammal fauna is dominated primarily by the equid *Anchitherium*, but there are also abundant artiodactyl remains from species such as the suid *Conohyus simorrensis*, the moschid *Hispanomeryx* sp., or the cervid *Heteroprox moralesi* (Pickford, 2013). In contrast, rhinos and proboscideans are quite rare. The rodent assemblage is characterised by the presence of two species of *Megacricetodon,* confirming the correlation of Carpetana (as well as other localities) to MN6, biozone F (Daams and Freudenthal, 1981; Hernández-Ballarín and Peláez-Campomanes, 2017).

The material considered for analysis is one second upper premolar (P2; 8/17/10348.HC), one upper first/second molar (M1/2; 8/17/10308.HC), one lower first molar (m1; 8/17/4013.HC), one lower third molar (m3; 8/17/2548.HC), and a series of lower m1-m2-m3 (8/17/0212.HC.1, 8/17/0212.HC.2, 8/17/0212.HC.3, respectively) and m1-m2 (8/17/10421.HC.1, 8/17/10421.HC.2, respectively) that belonged to two different mandibles (Fig. 1; Table S1). As the histological analysis implies the non-reversible alteration of the sample, the number of specimens was kept at minimum in order not to compromise the integrity of the fossil collection. Due to this, this work includes a limited sample size for some of the dental elements, having the subsequent implications for statistical power and results interpretation.

Prior to the histological procedures, we documented both qualitative and quantitative information. To this end, we photographed all the dental elements at occlusal, buccal, and lingual views, and made casts to preserve the external morphology of the teeth for future analyses.

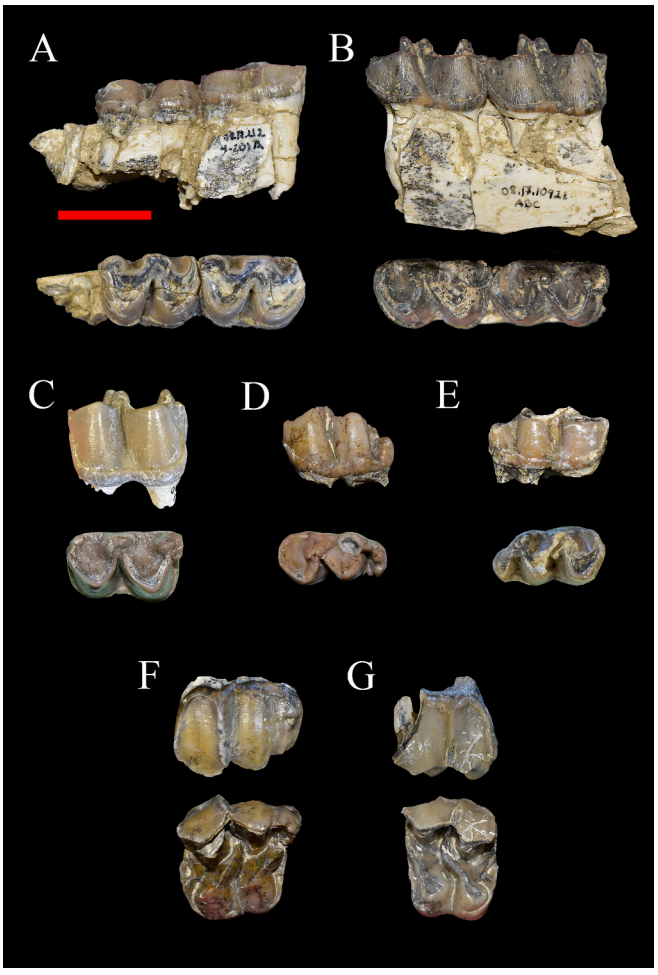

Fig. 1. Dental specimens selected for histological analysis in lateral and occlusal view: (a) m1-m2 8/17/0212.HC.1, 8/17/0212.HC.2; (b) m1-m2 8/17/10421.HC.1, 8/17/10421.HC.2 (c) m1 8/17/4013.HC; (d) m3 8/17/2548.HC; (e) m3 8/17/0212.HC.3; (f) P2 8/17/10348.HC; and (g) M1/2 8/17/10308.HC. Scalebar: 2 cm.

Regarding the quantitative information, we took measurements of the total length, width and height of both the anterior and posterior cusps (see Table S1). Finally, we calculated the hypsodonty index (HI; tooth crown height) for the molars that showed minimal signs of occlusal wear (m1 8/17/4013.HC; m3 8/17/2548.HC; M1 8/17/10308.HC). We followed the method of Cantalapiedra et al. (2017) by dividing the maximal height of the crown by the tooth length of each molar.

2.2. Histological preparation

The specimens selected for the histological analysis (Fig. 1; Table S1) were embedded in epoxy resin. After 48–72 h of curing, blocks were cut transversally with a diamond saw through the protocone and paracone in the upper elements (Fig. 2a) and the protoconid and anterior valley in the lower molars. Then, the cut surfaces were polished with paper grinding discs and fixed to frosted glass using epoxy resin. The sample was cut cut a second time to obtain a sample of ca. 2000 μm thick attached to the glass slide. Finally, sections were ground and polished with paper grinding discs of successively finer grits (500p to 2500p) until reaching a final thickness of 100-120 μm. All these procedures were performed in the laboratory of the Servicio de preparación de rocas y materiales duros of the Servicio General de Apoyo a la Investigación – SAI (Universidad de Zaragoza). A full detailed description of the methodology employed in this work can be found in Cuccu et al. (2025b).

2.3. Enamel measurements

Thin sections were analysed through polarized light microscopy (Leica DM2700 P with digital camera Flexacam C3) located at the facilities of the University of Zaragoza. Enamel measurements were taken in the paracone of the upper teeth and protoconid of the lower ones (Fig. 2a) with LASX software (Lemmers et al., 2021) and Adobe Photoshop v.25.2 (Adobe Inc, 2019).

In the present study, the regular and most abundant enamel markings in our sample, laminations, were considered as representing daily increments, as was demonstrated for other equids (Nacarino-Meneses et al., 2017; Orlandi-Oliveras et al., 2019), in the same way that cross-striations are the equivalent daily increments in primates (Smith, 2006). Therefore, we relied on these growth marks for calculating the enamel parameters that define enamel growth, such as the Daily

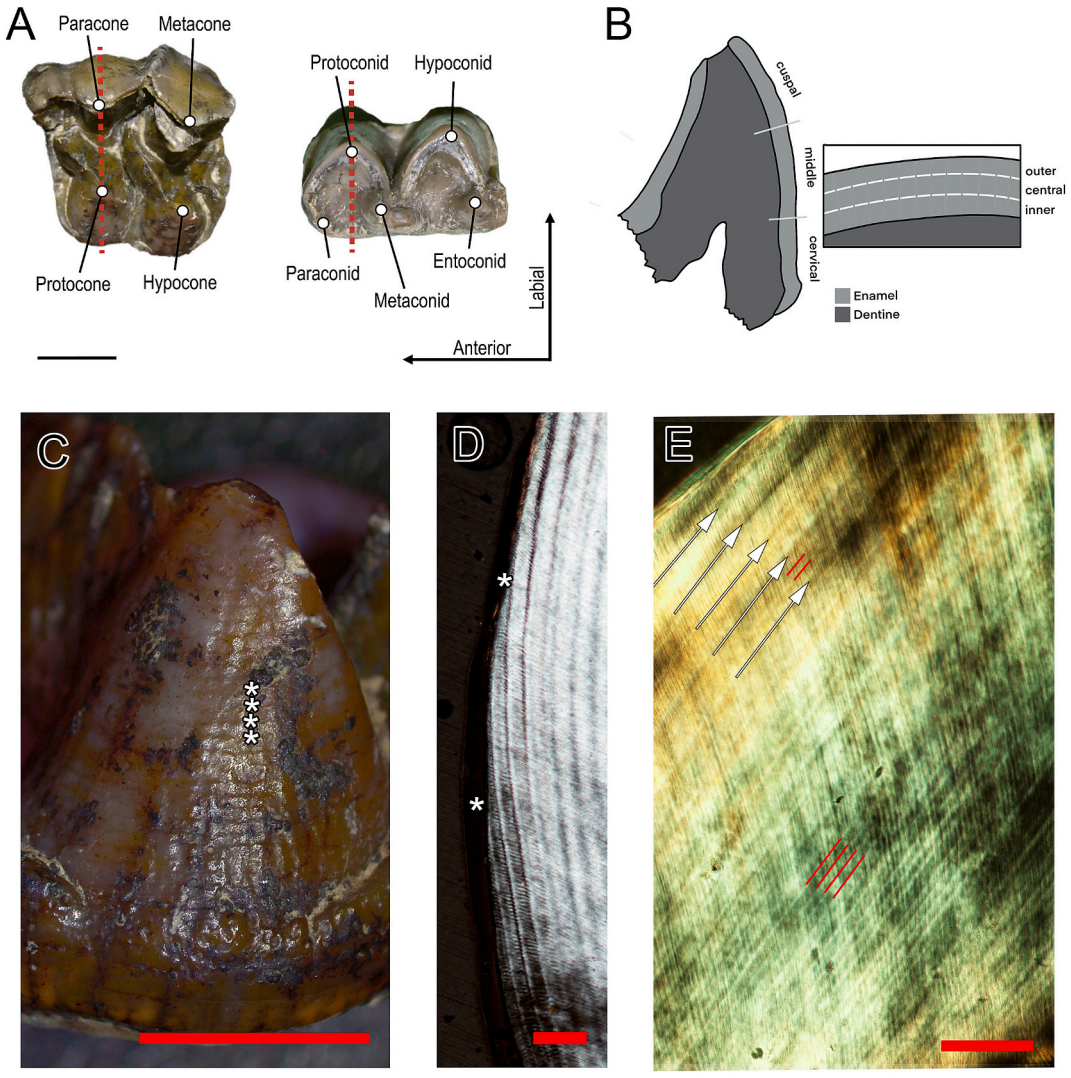

Fig. 2. Macro and micro-photographs of *Anchitherium* dental material (a) Information on the dental features and cutting plane (red line) of specimens 8/17/10348. HC (P2) and 8/17/4013.HC (m1) (flipped horizontally) (scalebar: 1 cm); (b) schematic representation of the nine virtual enamel regions; (c) external surface of specimen 8/17/10421.HC.1 showing the perikymata grooves (white asterisks) (scalebar: 500 μm); (d) Retzius lines on the outer enamel surface coinciding with the perikymata grooves (scalebar: 100 μm); and (e) histological details of incremental marks of different periodicity (red lines are laminations and white arrows are Retzius lines) (scalebar: 100 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Secretion Rate (DSR), Enamel Extension Rate (EER), and the Crown Formation Time (CFT). The DSR illustrates the amount of enamel secreted by the ameloblasts per day. To calculate this parameter, the distance among several incremental lines was measured and divided by the number of increments. These measurements were taken in three different enamel regions along the crown height: cuspal, middle, and cervical parts, as previous studies documented differences between these regions (Orlandi-Oliveras et al., 2019; Cuccu et al., 2025a; Nacarino-Meneses et al., 2025).

The EER indicates the rate of ameloblast differentiation along the EDJ at the time of molar formation. In the methodology here employed, based on works from Jordana and Köhler (2011) and Orlandi-Oliveras et al. (2019), the time required to form a portion of enamel between two incremental lines (t1) was calculated by dividing the distance between the lines (x1), along the prism course, by the DSR. Then we calculated the EER by dividing the distance between the two incremental lines at the EDJ level (y1) by the time required for their formation (Fig. 2c).

$$\frac{x1}{DSR} = t1; EER = \frac{y1}{t1}; CFT = t1 + t2 + t3 + \ldots + tn$$

We repeated this procedure along the entire EDJ to cover the full crown surface. All the molars in our sample showed a fold-like structure in the cervical region (Fig. 2b,c), challenging the EER calculation in that region as it causes a deformity of the EDJ. To overcome the problem, we considered this region separately (Fig. 4).

The CFT (i.e. the time required for the formation of the entire crown) was calculated by adding the formation times previously obtained (t1 + t2 + t3 + … + tn).

In addition to the calculation of enamel parameters, we obtained previously published data on DSR and EER from more recent equids—hipparionines and *Equus* (Nacarino-Meneses et al., 2017; Orlandi-Oliveras et al., 2019; Nacarino-Meneses and Chinsamy, 2022)—for comparison (Table 1).

2.4. Statistical analyses

We compared enamel parameters (both within the different enamel regions and from different tooth positions) using non-parametric tests. In that way, we employed the Kruskal-Wallis to compare data between groups. If significant comparisons were obtained with the Kruskal-Wallis test, we applied the Dunn test (with Bonferroni correction) as a post-hoc analysis. In addition, we performed a linear regression analysis to study the relationship between the DSR and the HI for different equid genera. All the analyses were conducted using RStudio, 2024.12.0 (Posit Software, PBC).

3. Results

The microscopic examination of the dental material of *Anchitherium* from Carpetana revealed the presence of incremental lines in all the specimens except for the m1 8/17/10421.HC.1. In order to standardize the analysis of incremental lines and, hence, dental parameters, enamel was divided into three different regions relatively to the position along the crown (cervical, middle, cuspal) and into three different locations within the linear enamel thickness from the EDJ to the outer enamel surface (inner, central, outer) (Fig. 2b). The most common incremental lines were laminations (i.e. daily incremental marks). Supra-daily incremental lines containing 3 to 4 daily increments were quite common on the outer portion of the enamel (Fig. 2d,e), ending at the outer enamel surface in perikymata grooves (Fig. 2c). The latter were visible at sight before the embedding process (Fig. 2c). It was also possible to distinguish closely-packed lines of sub-daily nature corresponding to 1/2 or 1/3 of the distance between laminations in the cervical part of specimens 8/17/0212.HC.1, 8/17/0212.HC.2, 8/17/0212.HC.3, 8/17/10421.HC.1 and 8/17/10348.HC. In addition to the incremental lines described above, it was possible to identify non-periodic, well-marked lines running from the inner cervical part to the outer cuspal part in the m1 and M1/2 specimens.

Table 1
Enamel parameters (in μm/day) calculated for the different teeth of *Anchitherium* compared with data obtained by Orlandi-Oliveras et al. (2019) for *Hipparion* species (*H. concudense*, *H. gromovae* and *H. periafricanum*) and *Equus quagga*, and Nacarino-Meneses and Chinsamy (2022) for *Eurygnathohippus hooijeri*. Abbreviations: m1 = first lower molar; m3 = third lower molar; M1/2 = first or second upper molar; P2 = second upper premolar; DSR = daily secretion rate; EER = enamel extension rate; mean ± standard deviation; n = number of occurrences; cp = cuspal; m = middle; cv = cervical.

Element	Taxon	DSR	EER_cp	EER_m	EER_cv
m1	*Anchitherium*	13.19 ± 1.23 (*n* = 75)	147.0 ± 45.9 (n = 2)	73.0 ± 16.8 (*n* = 5)	24.6 ± 6.90 (*n* = 17)
	H. concudense	15.76 ± 0.96	82.52 ± 38.96	177.08 ± 24.71	211.50 ± 27.25
	H. gromovae	16.17 ± 1.15	55.75 ± 35.85	131.32 ± 29.63	144.73 ± 28.05
	H. periafricanum	15.96 ± 1.37	37.27 ± 19.84	121.14 ± 8.11	166.62 ± 33.08
	E. quagga	16.98 ± 1.63	78.37 ± 73.25	316.18 ± 146.18	441.69 ± 152.37
	Eu. hooijeri	15.5	220	160	45
m3	*Anchitherium*	11.68 ± 0.59 (*n* = 32)	105.0 ± NA (n = 1)	51.5 ± 16.5 (n = 5)	14.7 ± 4.53 (*n* = 16)
	H. concudense	14.08 ± 1.73	41.30 ± 22.43	95.54 ± 18.87	150.39 ± 45.50
	H. gromovae	15.37 ± 1.78	43.06 ± 20.59	95.36 ± 14.25	119.09 ± 10.51
	H. periafricanum	13.72 ± 1.70	43.09 ± 20.36	75.14 ± 9.74	116.35 ± 18.17
	E. quagga	17.23 ± 1.05	15 ± 18.99	112.44 ± 23.99	199.42 ± 66.11
	Eu. hooijeri	15	165	110	50
M1/2	*Anchitherium*	11.01 ± 1.25 (*n* = 11)	117.0 ± 7.68 (n = 2)	78.4 ± 2.81 (*n* = 2)	26.4 ± 14.3 (n = 5)
P2	*Anchitherium*	11.25 ± 1.07 (*n* = 22)	148.0 ± 29.7 (n = 2)	72.4 ± 23.0 (n = 2)	24.3 ± 12.6 (*n* = 6)

3.1. Daily Secretion Rate (DSR) and hypsodonty index (HI)

The DSR values were obtained across the entire crown in two m1 and two m3 specimens, as well as in a single M1 and P2. The first lower molar (8/17/10421.HC.1) and the second lower molars (8/17/0212.HC.2, 8/17/10421.HC.2) were excluded due to poor histological visibility of incremental lines, which did not provide sufficient data for analysis. Although an increasing trend in DSR was observed from the cervical to the cuspal part in all the specimens but for the P2, the results revealed no differences among the different regions of the analysed molars (Table S2). In contrast, the mean DSR values differed among the molars, with the m1 exhibiting the highest value (13.19 μm/day) and differing significantly from the other three elements (*p*-value m1-m3 < 0.001; *p*-value m1-P2 < 0.001, *p*-value m1-M1 < 0.001, Table S3). The values of the other three elements ranged from 11 to 11.7 μm/day (Fig. 3a).

As the DSR values have been previously associated with hypsodonty in ungulates (Jordana et al., 2014), we calculated the HI for our specimens. The values obtained were 0.85 for the M1, 0.70 for the m1, and 0.60 for the m3. Then, we plotted the DSR values against the previously calculated HI for the m1 and m3, including data from Iberian *Hipparion* and *Equus* taken from previously published literature (Orlandi-Oliveras et al., 2019) (Fig. 3b). It was not possible to compare the other dental elements as no such data could be found in the literature.

The linear relationship between these two dental parameters can be observed (m1 $R^2 = 0.95$; m3 $R^2 = 0.73$), as can the fact that *Anchitherium*

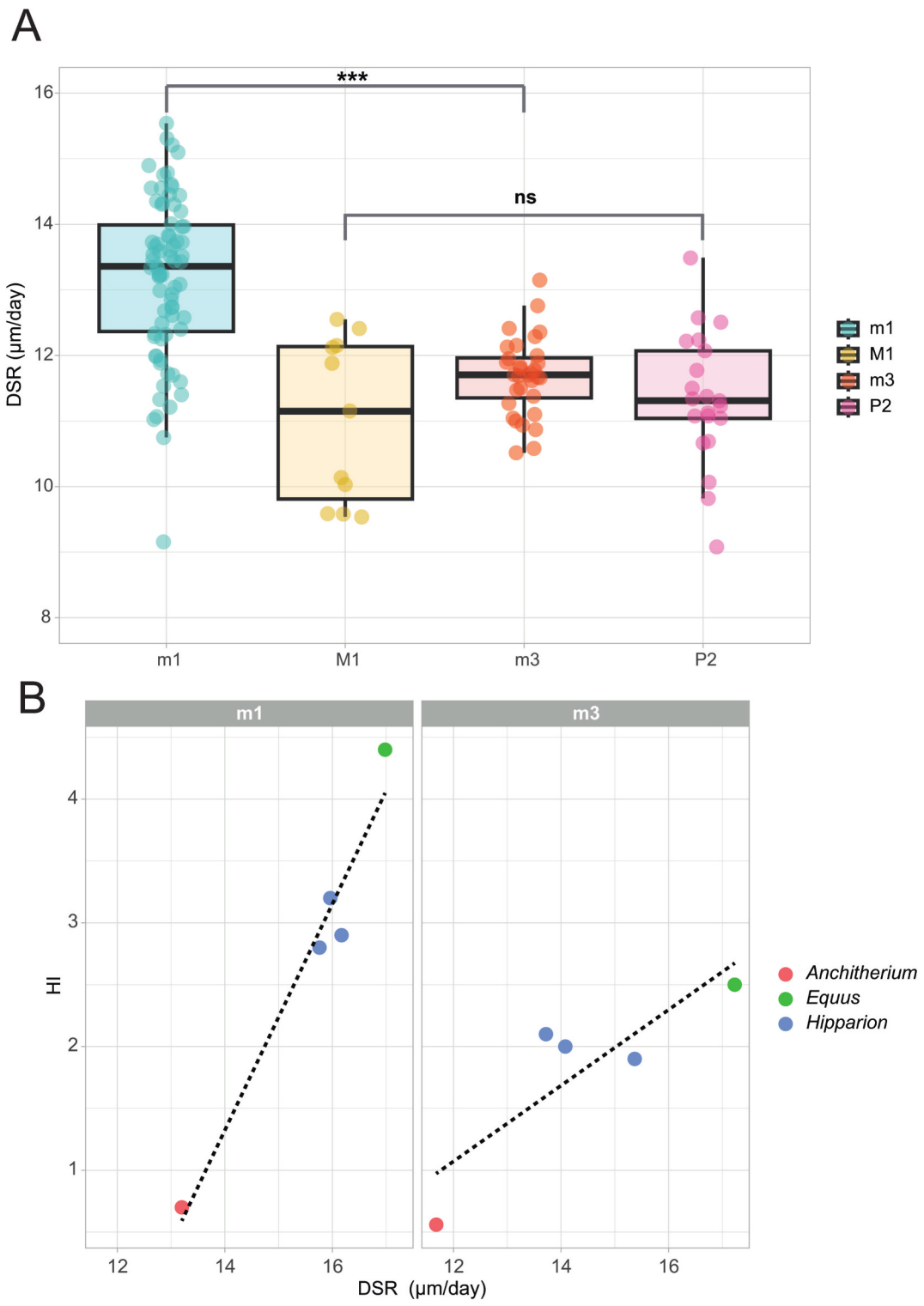

Fig. 3. Representation of the daily secretion rate (DSR) for (a) each tooth analysed (*** indicates significant differences among the groups (*p*-value: 0.007); ns indicates non-significant differences among the groups) and (b) the different equid species against the hypsodonty index (HI).

exhibits lower values than the other Iberian equids (Fig. 3b).

3.2. Enamel Extension Rate (EER) and Crown Formation time (CFT)

The EER was calculated along the crown of six molars (P2: 8/17/10348.HC, M1/2: 8/17/10308.HC; m1: 8/17/0212.HC.1 and 8/17/4013.HC; m3: 8/17/0212.HC.3 and 8/17/2548.HC). The EER exhibited a decreasing pattern from >100 µm/day at the cuspal part to about 20 µm/day at the cervical region in all the analysed molars (Table 1). The low EER values in the cervical region were statistically different to those in the cuspal region for all the molars (Table S4). In terms of comparison among teeth, the m3 showed the lowest EER values in all three enamel regions, while the other teeth exhibited similar rates (Table 1). However, statistical differences between the EER of m3 and the other teeth (when *p*-values are adjusted) were only found in the cervical third of the enamel (p-value<0.005; see Table S5).

The time taken for the formation of the entire crown varied slightly among the elements. While the upper elements (P2 and M1/2) took around 330 days to form approximately 17.000 and 18.000 μm of the crown (93% and 100% of the crown length following the EDJ) the m1 took around 300 days to form 16.000 μm of the crown (96% of the crown length), and the m3 required much more time (~400 days) to produce a shorter crown (~10.000 μm, 84% of the crown length) (Fig. 4). This outcome was expected given the low EER in the m3.

4. Discussion

Histological analysis of *Anchitherium* dental material provided information about enamel increments and rates of dental growth based on daily incremental lines, also known as laminations. The sub-daily markings representing infradian cycles of ameloblast activity of 8-12 h found in our sample have also been reported in several ungulate taxa, including suids (Kierdorf et al., 2019; Emken et al., 2021), cervids (Cuccu et al., 2025a), giraffids (Nacarino-Meneses et al., 2025), bovids (Kierdorf et al., 2013) and equids (Orlandi-Oliveras et al., 2019). The Retzius lines (i.e. periodic incremental marks including several days; Hillson, 2005) observed in the enamel of *Anchitherium* are primarily found in equids (Nacarino-Meneses et al., 2017; Orlandi-Oliveras et al., 2019; Nacarino-Meneses and Chinsamy, 2022), and have been also described in the outer enamel of suids (Kierdorf et al., 2019; Emken et al., 2021) and giraffids (Nacarino-Meneses et al., 2025). However, they have not been clearly defined in the enamel of other ungulates, such as cervids (Iinuma et al., 2004; Cuccu et al., 2025a) or bovids (Kierdorf et al., 2013). The non-periodic marks found in the enamel of the m1 and M1/2 could represent the neonatal line, given that the physiological stress of birth has been documented to affect dental growth (Fitzgerald and Rose, 2008), and these elements are the first to emerge in most equids (Hoppe et al., 2004; Domingo et al., 2018).

The quantitative information obtained from enamel parameters exhibited great variability depending on the level of study (tooth regions, dental element, equid clade).

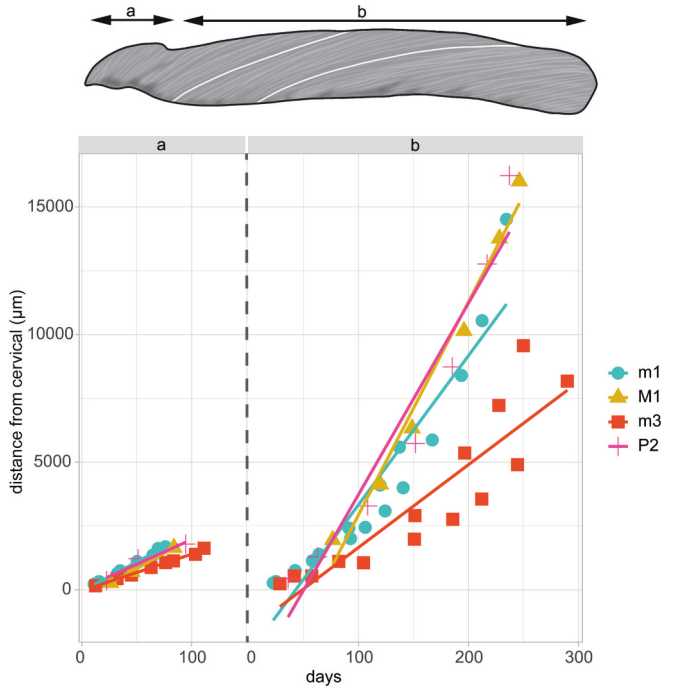

Fig. 4. Representation of the time required to form the enamel crown of the different teeth analysed.

4.1. Variability between tooth regions

The uniform DSR along the crowns in all *Anchitherium* teeth aligns with findings of Nacarino-Meneses et al. (2017) and Orlandi-Oliveras et al. (2019) for hipparionine horses and *Equus* species. Nacarino-Meneses and Chinsamy (2022) also documented uniformity in DSR along the crown of an African hipparionine horse, albeit they did note differences in DSR in the inner, middle, and outer parts of the enamel, but only in the first molars. Despite this slight variability, equids appear to exhibit a stable secretory rhythm at the intra-tooth level. This may be a characteristic particular to the Equidae, as it has been demonstrated that other Miocene brachydont taxa different from *Anchitherium* (e. g. the cervid *Procervulus*) do show variable DSR values along the crown (Cuccu et al., 2025a).

Unlike DSR, the EER values exhibited more variation, as they dropped significantly along the crown (approximately 6 times faster in the cuspal parts of the crown) of all analysed *Anchitherium* specimens. These outcomes are in accordance with the results obtained for other ungulates (e.g. Kierdorf et al., 2019), including equids (Nacarino-Meneses et al., 2017; Orlandi-Oliveras et al., 2019; Nacarino-Meneses and Chinsamy, 2022).

4.2. Variability between dental elements

Interpreting the variation of DSR among dental elements is challenging. As in our case, some authors have reported lower secretion rates in the m3 than in the m1 (Orlandi-Oliveras et al., 2019; Cuccu et al., 2025a). Since the m1 forms at an earlier ontogenetic stage than the m3 (Dean, 2006; Domingo et al., 2018), it has been suggested that the decrease in ameloblast secretory activity throughout ontogeny could explain lower rates (Nacarino-Meneses et al., 2025). In addition, Orlandi-Oliveras et al. (2019) attributed the depletion to the different tooth morphology, as the m1 is more hypsodont than the m3, based on the correlation found by Jordana et al. (2014) between the DSR and the HI. While both hypotheses are plausible for the analysis of lower molars, our findings for upper teeth did not agree with any of them, as the M1/2 showed DSR values significantly similar to the m3, despite forming earlier in ontogeny (Hoppe et al., 2004; Dixon, 2011; Domingo et al., 2018). Having this in mind, it is possible that the upper elements also experience a decrease in DSR coincident with ontogenetic depletion, but their DSR values are not concurrent with those of the corresponding lower molars. However, —despite the limited sample size— our data do not support this assumption (at least in *Anchitherium*) since, according to Domingo et al. (2018) —and assuming that maxillary teeth emerge at the same time than the mandibular ones (Seo et al., 2017)—, P2 is formed and erupted later in ontogeny than M1 and M2 and, according to our results, they exhibit similar DSR values. Regarding the DSR-HI correlation, the M1 exhibited a higher HI than the m1 in *Anchitherium*; however, its DSR was lower, being similar to that of the m3 and not matching the correlation found by Jordana et al. (2014).

The similar EER values found among *Anchitherium* lower and upper dental elements differed greatly from the results obtained for other taxa, as Dirks et al. (2009) documented higher EERs in the lower molars of two archaic ungulates. Alternatively, Orlandi-Oliveras et al. (2019) and Nacarino-Meneses and Chinsamy (2022) documented significantly lower EER for the m3 in equids, which was interpreted as ameloblast differentiation depleting over ontogeny, as the m1 forms at early stages when growth rates are fast, whereas the m3 erupts at later stages when growth rates decline and maturity is reached (Dean, 2006; Hillson, 2005). Accordingly, the similar EER observed in this study suggests that *Anchitherium* exhibited sustained growth rates throughout ontogeny, with a subtle decrease towards maturity.

4.3. Variability between equid clades

At a family level, a clear pattern emerges when analysing the DSR.

Our estimated DSR values of 13.19 μm/day (m1) and 11.68 μm/day (m3) are the lowest among the secretion rates calculated for more derived equids, including hipparionines and *Equus* (Nacarino-Meneses et al., 2017; Orlandi-Oliveras et al., 2019; Nacarino-Meneses and Chinsamy, 2022). When plotting these DSR values against the HI, a positive relationship was observed in both the m1 and m3, which somewhat agrees with Jordana et al. (2014). This pattern, may reflect the evolution of the tooth morphology within the equid family (MacFadden, 1994, 1997), characterised by a relatively little dental morphological change throughout the Eocene and Oligocene (MacFadden, 1988; MacFadden and Hulbert, 1988), and the acquisition of hypsodont teeth around the Middle Miocene (MacFadden, 2005; Strömberg, 2006) in response to the development of open environments (i.e. evolution of grasslands) and subsequent dietary change towards grazing (MacFadden, 1997).

The strong correlation between DSR and HI when comparing different equid genera, coincides with the results published by Jordana et al. (2014), that documented the correlation comparing specimens from different ruminant genera. However, this correlation seem not to occur at lower taxonomic levels, since Orlandi-Oliveras et al. (2019) reported that the DSR found in different *Hipparion* species was not directly linked to hypsodonty. Our results revealed that the correlation does not occur when comparing dental elements either (see section 4.2) —although this could be conditioned by the limited sample size for some of the dental elements. Other authors analysing differences in DSR between species (Dirks et al., 2012) claimed that the DSR could be regulated by interactions among tooth morphology, body size, and life history. The disparity in these studies highlights the need for further research into the factors affecting the secretion activity of the ameloblasts at different levels (along the crown, among elements, among species, among genera, etc.), which would improve the interpretation of dental growth and development.

When we compared the EER among groups, we found that it increased over time. The most basal group, the anchitherines, showed the slowest rates, followed by the hipparionines (Orlandi-Oliveras et al., 2019; Nacarino-Meneses and Chinsamy, 2022), while the most recent equid taxa, including several *Equus* species, showed the fastest rates (Nacarino-Meneses et al., 2017; Orlandi-Oliveras et al., 2019). It is noteworthy that the increase in EER over time within the equid family also coincided with the development of hypsodonty. O'Meara et al. (2018) observed this trend when comparing enamel growth rates in non-hypsodont and hypsodont mammals. However, as Orlandi-Oliveras et al. (2019) found no such relationship in different European hipparionine horses, this assumption that EER increases with hypsodonty might only occur at a higher taxonomic level, where the differences in hypsodonty are more accused.

Assuming the link between dental development and life history assessed in primates (Smith et al., 1994, Schwartz et al., 2002, Dirks and Bowman, 2007; Bromage et al., 2009) and ungulates (Jordana and Köhler, 2011; Jordana et al., 2014), the EER results obtained here point towards a slower pace of growth in *Anchitherium* in relation to the other equids examined. This characteristic is also related to slow life-history traits, such as a long lifespan, late age at weaning, and prolonged growth period (Ricklefs, 2008), which is consistent with our results of slow and sustained growth rates throughout ontogeny. In a broader perspective, the variation in EER among different equid taxa indicates that the development of faster life histories progressed gradually in parallel with the evolutionary history of the group (at least from the Miocene onwards). Due to the current lack of studies in other equid taxa, it is not possible to know with certainty how life history evolved further back in time. Nevertheless, given the trend observed in this work, we could consider the possibility that early equid representatives had shown even slower life histories and, thus, a general progression of the family towards gradually faster life histories.

In contrast, since it is well known that life history is shaped by environmental changes, —specifically in terms of resource availability and intrinsic mortality (Stearns, 1992; Gaillard et al., 2000)—, another hypothesis could be that the trend towards a fast life history documented here had its origin in the Miocene triggered by changes in the climate and, hence, in the environment. A double-peak warming event (Miocene Climatic Optimum, ~16.9–14.7 Ma) was followed by an abrupt drop in temperatures, declining pCO2 levels and expansion of the Antarctic ice sheet (Miocene Cooling Transition, 14.7–13.8 Ma) (Methner et al., 2020; Steinthorsdottir et al., 2021). As a result, this led to the restructuring of terrestrial ecosystems, with a transition to more arid environments with higher degree of openness and seasonality (Janis, 1993; Zachos et al., 2001; DeMiguel et al., 2010). This change would imply higher unpredictability (Southwood, 1988), a factor that promotes faster life histories (e.g. as early onset of maturity, short lifespan, small adult body size, small offspring and high reproductive rates; Reynolds, 2003). Accordingly, Miocene events may have constituted a pivotal role in the evolutionary history of equids, explaining the shift towards faster life histories.

5. Conclusion

This work presents a comprehensive analysis of the dental histology of *Anchitherium*, revealing details about the growth and development of the teeth of this Miocene equid for the first time. The exhaustive study of the enamel microstructure of different *Anchitherium* dental elements revealed a consistent DSR along the crown, presence of Retzius lines, significantly higher DSR in the first lower molar and similar EER in upper and lower dental elements. In addition, the analysis of both upper and lower elements in this work seems to indicate that the method of analysing only the lower dentition, as employed in many studies, may not provide the complete framework to make inferences and, hence, should be taken with caution. This will be the case of works attributing the drop in DSR in third lower molars to an ontogenetic depletion, since it seems not to be supported when upper elements are included in the sample (at least in *Anchitherium*). All of these outcomes have raised numerous questions about the factors affecting enamel secretion in ungulates and, hence, highlighted the need for comprehensive studies including both complete upper and lower dental rows in different ungulate taxa.

Finally, when only considering the family Equidae, we perceived a correlation between DSR and HI, which increase over time, coinciding with the acquisition of hypsodont teeth. In parallel, the EER values within the group suggested a slow life history for *Anchitherium* and a trend of faster life history with time that could have possibly been triggered by the Miocene to more open environments. These results have deepened our knowledge of equid dental development and life history, contributing to a better understanding of the evolution of this iconic group of ungulates.

CRediT authorship contribution statement

Teresa Calderón: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Andrea Cuccu:** Writing – review & editing, Visualization, Methodology, Funding acquisition, Conceptualization. **Jorge Morales:** Writing – review & editing, Resources. **Beatriz Azanza:** Writing – review & editing, Funding acquisition, Conceptualization. **Daniel DeMiguel:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

None of the authors has any conflicts of interest to disclose concerning the study “Quantitative.

histological analysis of dental variability in *Anchitherium*: insights into growth dynamics and.

dental development".

Acknowledgements

We would like to express our sincere appreciation to *Palaeogeography, Palaeoclimatology, Palaeoecology* on the occasion of its 60th anniversary and acknowledge its long-standing commitment to advancing palaeontological research through the publication of high-quality scientific contributions. In addition, we deeply thank M. T. Alberdi for her assistance with the identification and classification of the dental material. We are also grateful to the two anonymous reviewers for their valuable comments. This research is part of the projects PID2023-151089NB-I00 and PID2024-160207NB-I00 funded by the MCIU/AEI/10.13039/ 501100011033/FEDER, UE, with funding also by the Government of Aragón (Research Group ref. E33_23R), the "Juan de la Cierva Formación" postdoctoral program (ref. JDC2022-050216-I) to T. Calderón, and a predoctoral fellowship (ref. PRE2021-096969) to A. Cuccu.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.palaeo.2026.113557.

Data availability

The authors confirm that all data necessary for supporting the scientific findings of this paper have been provided.

References

Adobe Inc, 2019. Adobe Photoshop.
Alberdi, M.T., Rodríguez, J., 2012. *Anchitherium* Meyer, 1844 (Perissodactyla, Equidae) de Sansan. Museum National d'Histoire Naturelle.
Bromage, T.G., 1991. Enamel incremental periodicity in the pig-tailed macaque: a polychrome fluorescent labeling study of dental hard tissues. Am. J. Phys. Anthropol. 86 (2), 205–214. https://doi.org/10.1002/ajpa.1330860209.
Bromage, T.G., Lacruz, R.S., Hogg, R., Goldman, H.M., McFarlin, S.C., Warshaw, J., Dirks, W., Perez-Ochoa, A., Smolyar, I., Enlow, D.H., Boyde, A., 2009. Lamellar bone is an incremental tissue reconciling enamel rhythms, body size, and organismal life history. Calcif. Tissue Int. 84 (5), 388–404.
Cantalapiedra, J.L., Prado, J.L., Hernández Fernández, M., Alberdi, M.T., 2017. Decoupled ecomorphological evolution and diversification in Neogene-Quaternary horses. Science 355 (6325), 627–630. https://doi.org/10.1126/science.aag1772.
Cuccu, A., Calderón, T., Azanza, B., DeMiguel, D., 2025a. First insights into the life history of the early Miocene deer *Procervulus ginsburgi* from Spain. J. Anat. 247, 842–855. https://doi.org/10.1111/joa.14220.
Cuccu, A., Calderón, T., Azanza, B., DeMiguel, D., 2025b. Hard tissue thin-sectioning techniques in vertebrate paleohistology: review, synthesis and improvements. Methods Ecol. Evol. 16 (1), 66–83. https://doi.org/10.1111/2041-210X.14469.
Daams, R., Freudenthal, M., 1981. Aragonian: the stage concept versus Neogene mammal zones. Scr. Geol. 62, 1–17.
de Buffrénil, V., de Ricqlès, A.J., Zylberberg, L., Padian, K., 2021. Vertebrate Skeletal Histology and Paleohistology. CRC Press.
Dean, M.C., 1987. Growth layers and incremental markings in hard tissues; a review of the literature and some preliminary observations about enamel structure in Paranthropus boisei. J. Hum. Evol. 16 (2), 157–172.
Dean, M.C., 2000. Incremental markings in enamel and dentine: what they can tell us about the way teeth grow. In: Teaford, M.F., Smith, M.M., Ferguson, M.W.J. (Eds.), Development, Function and Evolution of Teeth. Cambridge University Press, Cambridge, pp. 119–130. https://doi.org/10.1017/CBO9780511542626.009.
Dean, M., 2006. Tooth microstructure tracks the pace of human life-history evolution. Proc. R. Soc. B Biol. Sci. 273 (1603), 2799–2808. https://doi.org/10.1098/rspb.2006.3583.
DeMiguel, D., Azanza, B., Morales, J., 2010. Trophic flexibility within the oldest Cervidae lineage to persist through the Miocene Climatic Optimum. Palaeogeogr. Palaeoclimatol. Palaeoecol. 289, 81–92. https://doi.org/10.1016/j.palaeo.2010.02.010.
Dirks, W., Bowman, J.E., 2007. Life history theory and dental development in four species of catarrhine primates. J. Hum. Evol. 53 (3), 309–320. https://doi.org/10.1016/j.jhevol.2007.04.007.
Dirks, W., Anemone, R.L., Holroyd, P.A., Reid, D.J., Walton, P., 2009. Phylogeny, life history and the timing of molar crown formation in two archaic ungulates, *Meniscotherium* and *Phenacodus* (Mammalia, 'Condylarthra'). Front. Oral Biol. 2009 (13), 3–8. https://doi.org/10.1159/000242381.
Dirks, W., Bromage, T.G., Agenbroad, L.D., 2012. The duration and rate of molar plate formation in *Palaeoloxodon cypriotes* and *Mammuthus columbi* from dental histology. Quat. Int. 255, 79–85. https://doi.org/10.1016/j.quaint.2011.11.002.
Dixon, 2011. Dental anatomy. In: Dixon, Schumacher (Eds.), Equine Dentistry, Easly. Elsevier, pp. 51–77.
Domingo, M.S., Cantero, E., García-Real, I., Chamorro Sancho, M.J., Martin Perea, D.M., Alberdi, M.T., Morales, J., 2018. First radiological study of a complete dental ontogeny sequence of an extinct equid: implications for Equidae life history and taphonomy. Sci. Rep. 8 (1), 8507. https://doi.org/10.1038/s41598-018-26817-3.
Emken, S., Witzel, C., Kierdorf, U., Frölich, K., Kierdorf, H., 2021. Characterization of short-period and long-period incremental markings in porcine enamel and dentine—results of a fluorochrome labelling study in wild boar and domestic pigs. J. Anat. 239 (5), 1207–1220. https://doi.org/10.1111/joa.13502.
Emken, S., Witzel, C., Kierdorf, U., Frölich, K., Kierdorf, H., 2023. Wild boar versus domestic pig—Deciphering of crown growth in porcine second molars. J. Anat. 242 (6), 1078–1095. https://doi.org/10.1111/joa.13838.
Fitzgerald, C.M., Rose, J.C., 2008. Reading between the lines: Dental development and subadult age assessment using the microstructural growth markers of teeth. In: Katzenberg, M.A., Saunders, S.R. (Eds.), Biological Anthropology of the Human Skeleton. Wiley Liss, pp. 237–263.
Forsten, A., 1991. Size trends in holarctic anchitherines (Mammalia, Equidae). J. Paleontol. 65 (1), 147–159. https://doi.org/10.1017/S0022336000020266.
Gaillard, J.M., Festa-Bianchet, M., Yoccoz, N.G., Loison, A., Toïgo, C., 2000. Temporal variation in fitness components and population dynamics of large herbivores. Annu. Rev. Ecol. Syst. 31 (1), 367–393. https://doi.org/10.1146/annurev.ecolsys.31.1.367.
Hernández-Ballarín, V., Peláez-Campomanes, P., 2017. Updated biochronology of the Madrid Basin: species distribution and implications for the Middle Miocene rodent faunas of Spain. Hist. Biol. 30 (3), 305–316. https://doi.org/10.1080/08912963.2016.1276580.
Hillson, S., 2005. Teeth. Cambridge University Press.
Hogg, R., 2018. Permanent record: the use of dental and bone microstructure to assess life history evolution and ecology. In: Croft, D., Su, D., Simpson, S. (Eds.), Methods in paleoecology: Reconstructing Cenozoic Terrestrial Environments and Ecological Communities. Springer, pp. 75–98. https://doi.org/10.1007/978-3-319-94265-0_6.
Hoppe, K.A., Stover, S.M., Pascoe, J.R., Amundson, R., 2004. Tooth enamel biomineralization in extant horses: implications for isotopic microsampling. Palaeogeogr. Palaeoclimatol. Palaeoecol. 206 (3–4), 355–365. https://doi.org/10.1016/j.palaeo.2004.01.012.
Iinuma, Y. M., Tanaka, S., Kawasaki, K., Kuwajima, T., Nomura, H., Suzuki, M., Ohtaishi, N. 2004. Dental incremental lines in sika deer (*Cervus nippon*); polarized light and fluorescence microscopy of ground sections. J. Vet. Med. Sci., 66(6), 665–669. https://https://doi.org/10.1292/jvms.66.665.
Janis, C.M., 1993. Tertiary mammal evolution in the context of changing climates, vegetation, and tectonic events. Annu. Rev. Ecol. Evol. Syst. 24, 467–500. https://doi.org/10.1146/annurev.ecolsys.24.1.467.
Janis, C.M., 2007. The horse series. Icons Evol. 257–280.
Jordana, X., Köhler, M., 2011. Enamel microstructure in the fossil bovid *Myotragus balearicus* (Majorca, Spain): implications for life-history evolution of dwarf mammals in insular ecosystems. Palaeogeogr. Palaeoclimatol. Palaeoecol. 300 (1–4), 59–66. https://doi.org/10.1016/j.palaeo.2010.12.008.
Jordana, X., Marín-Moratalla, N., Moncunill-Solé, B., Köhler, M., 2014. Ecological and life-history correlates of enamel growth in ruminants (Artiodactyla). Biol. J. Linn. Soc. 112 (4), 657–667. https://doi.org/10.1111/bij.12264.
Kierdorf, H., Kierdorf, U., Frölich, K., Witzel, C., 2013. Lines of evidence–incremental markings in molar enamel of Soay sheep as revealed by a fluorochrome labelling and backscattered electron imaging study. PLoS One 8 (9), e74597. https://doi.org/10.1371/journal.pone.0074597.
Kierdorf, H., Breuer, F., Witzel, C., Kierdorf, U. 2019. Pig enamel revisited–Incremental markings in enamel of wild boars and domestic pigs. J. Struct. Biol., 205(1), 48–59. https://https://doi.org/10.1016/j.jsb.2018.11.009.
Klementiev, A.M., Sizov, A.V., 2015. New record of anchithere (*Anchitherium* aurelianense) in the Miocene of Eastern Siberia, Russia. Russ. J. Theriology 14 (2), 133–143.
Lemmers, S.A., Dirks, W., Street, S.E., Ngoubangoye, B., Herbert, A., Setchell, J.M., 2021. Dental microstructure records life history events: a histological study of mandrills (*Mandrillus sphinx*) from Gabon. J. Hum. Evol. 158, 103046. https://doi.org/10.1016/j.jhevol.2021.103046.
MacFadden, B.J., 1988. Fossil horses from "*Eohippus*" (*Hyracotherium*) to *Equus*: rates of dental evolution revisited. Biol. J. Linn. Soc. 35 (1), 37–48.
MacFadden, B.J., 1994. Fossil Horses: Systematics, Paleobiology, and Evolution of the Family Equidae. Cambridge University Press.
MacFadden, B.J., 1997. Origin and evolution of the grazing guild in New World terrestrial mammals. Trends Ecol. Evol. 12, 182–187. https://doi.org/10.1016/S0169-5347(97)01049-5.
MacFadden, B.J., 2005. Fossil Horses–Evidence for Evolution. Science 307, 1728–1730. https://doi.org/10.1126/science.1105458.
MacFadden, B.J., Hulbert, R.C., 1988. Explosive speciation at the base of the adaptive radiation of Miocene grazing horses. Nature 336 (6198), 466–468.
Macho, G.A., Williamson, D.K., 2002. The effects of ecology on life history strategies and metabolic disturbances during development: an example from the African bovids. Biol. J. Linn. Soc. 75 (2), 271–279. https://doi.org/10.1046/j.1095-8312.2002.00013.x.
Methner, K., Campani, M., Fiebig, J., et al., 2020. Middle Miocene long-term continental temperature change in and out of pace with marine climate records. Sci. Rep. 10, 7989. https://doi.org/10.1038/s41598-020-64743-5.

Nacarino-Meneses, C., Chinsamy, A., 2022. Mineralized-tissue histology reveals protracted life history in the Pliocene three-toed horse from Langebaanweg (South Africa). Zool. J. Linnean Soc. 196 (3), 1117–1137. https://doi.org/10.1093/zoolinnean/zlab037.

Nacarino-Meneses, C., Jordana, X., Orlandi-Oliveras, G., Köhler, M., 2017. Reconstructing molar growth from enamel histology in extant and extinct *Equus*. Sci. Rep. 7 (1), 15965. https://doi.org/10.1038/s41598-017-16227-2.

Nacarino-Meneses, C., Jannello, J.M., Chinsamy, A., 2025. Life history data derived from the dental histological analysis of *Giraffa camelopardalis*: Implications for the palaeohistology of extinct giraffids. J. Anat. 247 (3–4), 869–884. https://doi.org/10.1111/joa.14191.

O'Meara, R.N., Dirks, W., Martinelli, A.G., 2018. Enamel formation and growth in non-mammalian cynodonts. R. Soc. Open Sci. 5 (5), 172293. https://doi.org/10.1098/rsos.172293.

Orlandi-Oliveras, G., Nacarino-Meneses, C., Köhler, M., 2019. Dental histology of late Miocene hipparionins compared with extant *Equus*, and its implications for Equidae life history. Palaeogeogr. Palaeoclimatol. Palaeoecol. 528, 133–146. https://doi.org/10.1016/j.palaeo.2019.04.016.

Padian, K., Lamm, E.T., 2013. Bone Histology of Fossil Tetrapods: Advancing Methods, Analysis, and Interpretation. University of California Press.

Pickford, M., 2013. *Conohyus simorrensis* (Lartet, 1851) (Suidae, Mammalia) from the Middle Miocene of Carpetana (Madrid, Spain). Span. J. Palaeontol. 28 (1), 91–102. https://doi.org/10.7203/sjp.28.1.17834.

Posit team, 2024. RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA.

Reynolds, J.D., 2003. Life Histories and Extinction Risk. Macroecology. Blackwell Publishing, Oxford, UK, pp. 195–217.

Ricklefs, R.E., 2008. The evolution of senescence from a comparative perspective. Funct. Ecol. 22, 379–392. https://doi.org/10.1111/j.1365-2435.2008.01420.x.

Sánchez, I.M., Salesa, M.J., Morales, J., 1998. Revisión sistemática del género *Anchitherium* Meyer 1834 (Equidae; Perissodactyla) en España. Estud. Geol. 54, 39–63. https://doi.org/10.3989/egeol.98541-2204.

Schwartz, G.T., Samonds, K.E., Godfrey, L.R., Jungers, W.L., Simons, E.L., 2002. Dental microstructure and life history in subfossil Malagasy lemurs. Proc. Natl. Acad. Sci. 99 (9), 6124–6129. https://doi.org/10.1073/pnas.092685099.

Seo, H., Kim, J., Seomun, H., Hwang, J.J., Jeong, H.G., Kim, J.Y., Cho, S.W., 2017. Eruption of posterior teeth in the maxilla and mandible for age determination of water deer. Arch. Oral Biol. 73, 237–242. https://doi.org/10.1016/j.archoralbio.2016.10.020.

Siliceo, G., Morales, J., Antón, M., Salesa, M.J., 2020. New fossils of Amphicyonidae (Carnivora) from the middle Miocene (MN6) site of Carpetana (Madrid, Spain). Geodiversitas 42 (15), 223–238. https://doi.org/10.5252/geodiversitas2020v42a15.

Smith, B.H., 1991. Dental development and the evolution of life history in Hominidae. Am. J. Phys. Anthropol. 86 (2), 157–174. https://doi.org/10.1002/ajpa.1330860206.

Smith, T.M., 2006. Experimental determination of the periodicity of incremental features in enamel. J. Anat. 208 (1), 99–113. https://doi.org/10.1111/j.1469-7580.2006.00499.x.

Smith, B.H., Crummett, T.L., Brandt, K.L., 1994. Ages of eruption of primate teeth: a compendium for aging individuals and comparing life histories. Yearb. Phys. Anthropol. 37, 177–231. https://doi.org/10.1002/ajpa.1330370608.

Southwood, T.R.E., 1988. Tactics, strategies and templets. Oikos 52, 3–18. https://doi.org/10.2307/3565974.

Stearns, S.C., 1992. The Evolution of Life Histories. Oxford University Press.

Steinthorsdottir, M., Coxall, H.K., De Boer, A.M., Huber, M., Barbolini, N., Bradshaw, C. D., Burls, N.J., Feakins, S.J., Henderiks, J., Holbourn, A.E., et al., 2021. The miocene: the future of the past. Paleoceanogr. Paleoclimatol. 36. https://doi.org/10.1029/2020PA004037 e2020PA004037.

Strömberg, C.A.E., 2006. Evolution of hypsodonty in equids: testing a hypothesis of adaptation. Paleobiology 32 (2), 236–258. https://doi.org/10.1666/0094-8373(2006)32[236:EOHIET]2.0.CO;2.

Tafforeau, P., Bentaleb, I., Jaeger, J.J., Martin, C., 2007. Nature of laminations and mineralization in rhinoceros enamel using histology and X-ray synchrotron microtomography: potential implications for palaeoenvironmental isotopic studies. Palaeogeogr. Palaeoclimatol. Palaeoecol. 246 (2–4), 206–227. https://doi.org/10.1016/j.palaeo.2006.10.001.

Tleuberdina, P., Forsten, A., 2001. *Anchitherium* (Mammalia, Equidae) from Kazakhstan, Central Asia. Geobios 34 (4), 449–456. https://doi.org/10.1016/S0016-6995(01)80008-5.

Witzel, C., 2014. Echoes from birth – mutual benefits for physical and forensic anthropology by applying increment counts in enamel of deciduous teeth for aging. Anthropol. Anz. 71, 7e103. https://doi.org/10.1127/0003-5548/2014/0386.

Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292, 686–693. https://doi.org/10.1126/science.1059412.

Zanolli, C., Bondioli, L., Manni, F., Rossi, P., Macchiarelli, R., 2011. Gestation length, mode of delivery, and neonatal line-thickness variation. Hum. Biol. 83 (6), 695–713. https://doi.org/10.3378/027.083.0603.