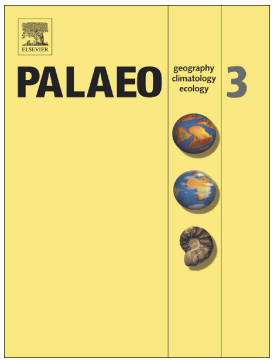

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Dental histology of late Miocene hipparionins compared with extant *Equus*, and its implications for Equidae life history

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Abstract

Hipparionins were a dominant element of the late Miocene faunas of Europe; however, their biology and ecology remain incompletely understood. In this paper, we explore the pace of life history of different-sized hipparionin horses, using dental histology, and compare it with extant equids. In doing so we consider (i) the size diversity of hipparionins, (ii) their generally smaller size compared to extant equids, and (iii) the allometric coupling between size and life history. In particular, we reconstruct the dental growth in lower first/second molars and in later-formed lower third molars for three hipparionin taxa: two dwarf species (*Hipparion periafricanum* and *H. gromovae*), and a larger species (*H. concudense*). We also analyze dental growth in an extant zebra (*Equus quagga*) for

comparative purposes. Our results reveal that, within each species, there are differences in enamel growth parameters between the first/second molars and third molars. These results illustrate the differences in the developmental timing of these teeth and the existence of a relationship between dental growth parameters with somatic growth. We also find that hipparionin teeth grow at slower rates and tend to erupt later in time than in extant *Equus*. Dwarf hipparionins, moreover, exhibit lower enamel extension rates than the larger species, but similar formation and eruption times. Considering the link between dental development and life history, these results suggest a slower life history strategy of selected hipparionins compared to extant equids, and a further slower pace of growth in the two dwarf forms compared to the larger taxon.

Keywords: paleohistology; enamel; growth rate; body size; hipparion; tooth growth

1. Introduction

The first hipparionins arrived in the Old World around 11 Ma, marking the start of the Vallesian European Land Mammal Age, and signaling one of the most important biochronological events of the Neogene in Europe (Bernor et al., 1996; Garcés et al., 2003, 1997). After their dispersal, hipparionins became dominant in European mammal associations and rapidly diversified into different forms in circum-Mediterranean areas, while they retained a more conservative body plan in central Europe (Alberdi, 1989; Bernor et al., 1996, 1990; Eisenmann, 1995; Woodburne, 1989). Besides their morphological diversity, hipparionin horses were highly diverse in size (Eisenmann, 1995; Ortiz-Jaureguizar and Alberdi, 2003), especially during the Turolian European Land Mammal Age (late Miocene) (Bernor et al., 1996, 1990). In the western Mediterranean late Turolian (MN13), for example, there were at least three sympatric hipparionins present (Alberdi, 1974; Alberdi and Alcalá, 1989; Ortiz-Jaureguizar and Alberdi, 2003; Pesquero, 2003): the

scarce and large *Hipparion primigenium truyolsi* [138 kg (Ortiz-Jaureguizar and Alberdi, 2003)], the small *H. gromovae* [59 kg (Ortiz-Jaureguizar and Alberdi, 2003)], and the dwarf *H. periafricanum* [23 kg (Ortiz-Jaureguizar and Alberdi, 2003)]. Different scenarios have been proposed to explain these size differences. On the one hand, based on the evolutionary size response model in Equini (Alberdi et al., 1995), the two small taxa have been hypothesized as representing species adapted to closed and forested areas (Ortiz-Jaureguizar and Alberdi, 2003). On the other hand, some authors have argued that these gracile and small-sized taxa were adapted to open habitats (Eisenmann, 1995; Pesquero, 2003), and that the small size represents an adaptation to energy economization due to the less nutritive xerophyte plants that typify open habitats (Forsten, 1978, 1968). Recently, however, hipparionin size shifts have been considered as a probable outcome of the evolution of life history strategies that indirectly affect body size (Orlandi-Oliveras et al., 2018) as has been previously proposed for island ecosystems (Köhler, 2010; Palkovacs, 2003).

Hipparionins have long been suggested to follow faster life histories than *Equus* based on population dynamics studies (Hulbert, 1982; Kurtén, 1953; Van Valen, 1964). Through the study of age classes defined by dental wear stages (Hulbert, 1982; Kurtén, 1953; Van Valen, 1964; Woodburne and MacFadden, 1982), and considering their smaller size (Van Valen, 1964), some authors proposed shorter life spans and earlier maturity ages in hipparionins compared to extant equids. Body mass frequently correlates with life history (Calder, 1984). However, this is not always the case since there are other factors on which life history traits depend and that are associated with an organism's ecology (Sibly and Brown, 2007; Stearns, 1992). Indeed, the pace of growth of hipparionins has recently been questioned based on the study of their molar eruption sequence interpreted within the context of Schultz's Rule (Domingo et al., 2018). Grounded on the idea that the

tooth replacement pattern is a valuable proxy of an animal's life history, Schultz's Rule states that the later eruption of the permanent molars compared to that of the replacement teeth (e.g. permanent premolars) is typically found in slow-growing taxa (Smith, 2000). Domingo et al. (2018), therefore, suggested that hipparionins might have followed a slow pace of growth due to their late third molar eruption, which occurs prior to the appearance of the fourth premolar in some extant equids (Easley et al., 2005; Hoppe et al., 2004; Joubert, 1972; Lkhagvasuren et al., 2013; Smuts, 1974). Recent studies, however, claim that the eruption sequence is more influenced by phylogeny than by life history in primates (Monson and Hlusko, 2018a) and artiodactyls (Monson and Hlusko, 2018b; Veitschegger and Sánchez-Villagra, 2016), challenging the predictive value of the Schultz's Rule. Within equids, the timing of third molar and fourth premolar eruption generally differs between extant and extinct representatives of the family, with basal equines, Miocene anchitheriines, and hipparionins showing a later m3 eruption (Domingo et al., 2018). However, in some taxa of the *Equus* genus the fourth premolar is the last to erupt (Easley et al., 2005; Hoppe et al., 2004; Joubert, 1972; Lkhagvasuren et al., 2013; Smuts, 1974) while in others it is the third molar (Grubb, 1981; McDonald, 1996; Penzhorn, 1982). This intrageneric variability suggests an adaptive component in the eruption pattern and takes issue with the idea of a phylogenetic meaning of this trait. The differences in the eruption sequence (Domingo et al., 2018), in dietary preferences (Semprebon et al., 2016), and in dental wear rates (Famoso and Davis, 2016; Hulbert, 1984) between extant equids and hipparionins make us reconsider the previous life history interpretations founded on population dynamics from the study of the dentition ontogeny (Hulbert, 1982; Kurtén, 1953; Van Valen, 1964). The deviations from the allometric coupling between life history and size (Sibly and Brown, 2007) also challenge the idea that faster life histories are necessarily associated with smaller sizes. Moreover, bearing in mind the hipparionin ecological and size diversity (Hayek et al., 1992; MacFadden et al., 1999; Ortiz-Jaureguizar and Alberdi,

2003; Tütken et al., 2013), we could further expect diverse life history adaptations within the group.

Dental histology has proved to be a valuable tool for inferring life histories of extinct species since tooth formation times and growth rates are closely related to the animal's pace of life history (Dean, 2006; Jordana et al., 2014; Schwartz et al., 2002). Additionally, the emergence sequence of the permanent dentition has shown to generally correlate with the organism's postnatal growth (Smith, 2000), and the eruption time of certain teeth is linked to key life history traits such as weaning and attainment of maturity (Dean, 2006; Dirks and Bowman, 2007; Engström et al., 1983; Hillson, 2005; Smith, 1991, 2000). In this way, the study of dental growth has allowed the reconstruction of the life strategy of many extinct mammals (Dirks et al., 2012, 2009; Jordana and Köhler, 2011; Schwartz et al., 2002; Smith, 2016). Current advances in dental histology of extant equids, moreover, have characterized the growth of hypsodont teeth of the genus *Equus* (Nacarino-Meneses et al., 2017), providing a solid framework for studying and inferring the life history of fossil equid taxa.

Here we aim to explore the life history strategies of hipparionin horses and compare them to those of extant equids, focusing on the differences linked to body size variations within the clade. The high diversity and abundance of Turolian hipparionins in the western Mediterranean provides an excellent opportunity to study their pace of life history in relation to their size. Therefore, we analyze for the first time the dental development in different-sized hipparionins to shed light on their life history strategies. Specifically, we reconstructed the tooth growth patterns of lower first/second molars and lower third molars of three hipparionin taxa (*Hipparion periafricanum*, *Hipparion gromovae,* and the larger *Hipparion concudense*) together with the first and third molar growth of an extant equid. Amongst wild extant equids, we have chosen *Equus quagga* as a model because unlike

the larger *Equus grevyi* or the longer-lived *Equus hemionus*, it does not show any extreme life history trait (Ernest, 2003; Grubb, 1981; Lkhagvasuren et al., 2013; Smuts, 1974). We have analyzed first and third lower molars as these are formed and erupt during different moments of ontogeny that are characterized by two major life history events, weaning and attainment of maturity, respectively. Furthermore, the eruption of the first molar has been shown to correlate with different life history traits, and thus it can be used as a predictor of the organisms' pace of life history (Dirks and Bowman, 2007; Smith, 2000; Veitschegger and Sánchez-Villagra, 2016). The dental mineralization sequence – intimately related to the enamel matrix formation – is the same between hipparionin horses and extant horses (Domingo et al., 2018; Hoppe et al., 2004): the first lower molar (m1) begins to mineralize before birth, followed by the m2, and later by the p2 and the p3. Although the p4 eruption occurs earlier in hipparionins, the p4 starts to mineralize before the m3 as it happens in extant *Equus* (Domingo et al., 2018; Hoppe et al., 2004). This similar onset of the tooth formation, and the same degree of overlap between extant equids and hipparionins (e.g. the third molar is still being formed when the m1 and the m2 are fully in wear), endorse our interpretations obtained from individual tooth growth reconstructions. Due to their diversity and abundance, hipparionins constitute a key mammalian group widely used in paleoecological studies, especially in those involving isotopes (Domingo et al., 2009; Matson and Fox, 2010; Nelson, 2005; Rey et al., 2013; Van Dam and Reichart, 2009). Besides the importance of dental growth pattern as a life history proxy, the understanding of hipparionin molar growth patterns provides an important basis for future paleoecological inferences from hipparionin dental analyses.

2. Material and Methods

The extremely high-crowned molars of *Equus* are produced by delaying the root formation (Hoppe et al., 2004; Nacarino-Meneses et al., 2017), which leads to extended

dental growth periods. Eruption of the crown occurs gradually, and tooth wear starts before the roots are formed (Kirkland et al., 1996). For this reason, the entire tooth development in *Equus* must be reconstructed from compositions of molars with different wear degrees (Nacarino-Meneses et al., 2017). Based on differences in the pattern of growth during the crown formation of unworn and worn extant equid molars, Nacarino-Meneses et al. (2017) defined three crown developmental stages (CDS) that differed in growth rate. The first stage is characterized by a fast and linear growth (CDS I), followed by a significant decrease in growth rate (CDS II) and the attainment of an almost residual enamel extension rate in the last formed part of the cervix (CDS III). These CDS described in *Equus*, further agree with the morphological appearance of the molar. The transition from stage I to stage II matches the tooth eruption time, and that of CDS II to CDS III represents the macroscopic root appearance (Nacarino-Meneses et al., 2017). The hypsodont teeth of hipparionins, however, tend to finish the crown formation shortly after the tooth eruption, allowing the observation of little worn rooted teeth (Supplementary Material Fig. S1). The histological analyses of the unworn or slightly worn hipparionin teeth, thus, allows the reconstruction of the complete tooth growth.

2.1 Fossil material

In this study, we studied isolated lower molars from three different hipparionin species of the Spanish Turolian. The teeth of the dwarf hipparionins *H. periafricanum* and *H. gromovae* come from the El Arquillo fossil site (Teruel Basin, Spain), also known as Rambla de Valdecebro II. This fossil site is dated to the late Turolian (MN13) and establishes the reference fauna for the MN13 with an estimated age of 6.2 Ma (de Bruijn et al., 1992; Van Dam et al., 2001). The *H. concudense* molars were from Concud, a classical and rich fossil site from the same Teruel Basin. This locality is less than 10 km away from El Arquillo and is dated around 7 Ma on the middle Turolian (MN12) (Pesquero

et al., 2010) (Fig. 1). All fossil samples are accessioned in the Institut Català de Paleontologia (Barcelona, Spain) collections (IPS-91731, IPS-91732, IPS-91733, IPS-91734, IPS-91762, IPS-91763, IPS-40644, IPS-40665, IPS-40668, IPS-85504, IPS-85506, IPS-85508, IPS-86850, IPS-86851, IPS-87855, IPS-87856, IPS-87857, Table 1).

Based on sectioned material, we analyzed two lower first/second molars (m1/m2) and three lower third molars (m3) for the smallest Old World hipparionin *Hipparion periafricanum*, four lower m3 molars and two lower m1/m2 molars from the small-sized *Hipparion gromovae*, and four lower m3 molars and two lower m1/m2 molars from the large *Hipparion concudense* (Table 1). To the greatest extent possible, the less worn teeth were selected in order to retain the maximal growth record and make analogous comparisons. Following Sondaar (1961) and Pesquero (2003), we used tooth width and length measurements at 1 cm above the base of the tooth as a biometric criterion for interspecific differentiation between the two contemporaneous small species *H. gromovae* and *H. periafricanum* (Supplementary Material Fig. S2 and S3). Body mass estimations based on first phalanx measurements (Alberdi et al., 1995) were obtained from the literature (Ortiz-Jaureguizar and Alberdi, 2003; Pesquero and Alberdi, 2012). Hypsodonty indices calculated from measurements of m1/m2 molars were also compiled from literature (Cantalapiedra et al., 2017; Pesquero, 2003). Using the same procedure described in Cantalapiedra et al. (2017), we calculated the hypsodonty indices for m3 molars (Table 1).

2.2 Extant Equus *material*

Our sample also includes four first molars of extant *Equus quagga* from the Hagenbeck Zoo (Hamburg, Germany). These teeth represent different eruption and wear stages (Table 1), thus comprising the entire tooth development of *Equus* molars (Nacarino-Meneses et al., 2017). We also sectioned three third molars from *Equus quagga* depicting different eruption and wear stages (Table 1). These third molars came from the

Réserve Africaine de Sigean (Sigean, France), the Barcelona Zoo (Barcelona, Spain) and the Hagenbeck Zoo (Hamburg, Germany). The extant samples from the Hagenbeck Zoo are accessioned in the Centrum für Naturkunde of the Universität Hamburg (Hamburg, Germany), the Barcelona Zoo specimen is accessioned in the Museu de Ciències Naturals de Barcelona (Barcelona, Spain), and the specimen from Sigean is housed at the Institut Català de Paleontologia Miquel Crusafont (Barcelona, Spain).

2.3 Thin section preparation and study

The dental histological sections were prepared following the standard procedures of our laboratory (Jordana et al., 2014; Nacarino-Meneses et al., 2017). Each molar was embedded in epoxy resin (Araldite 2020) and sectioned buccolingually through the higher mesial cusps (protoconid and metaconid) using an IsoMet low-speed saw (Buehler). In some cases, highly curved third molars were previously cut in a transversal plane. At this stage, the exposed buccolingual surfaces were polished using a grinder polisher (Buehler, MetaServ 250) and glued to a glass slide using ultraviolet curing glue (Loctite 352). Thereafter, the glass-mounted samples were cut using a diamond saw (Buehler, Petrothin) up to a thickness of 300 µm and grounded to around 120-70 µm using the grinder polisher. Finally, each sample was dehydrated in alcohol gradients and immersed in a histological clearing agent (Histo-Clear II) prior to covering them with a DPX medium.

The resulting thin sections were studied under polarized light using a Zeiss Scope A1 microscope with an attached digital camera (AxioCam ICc5). To estimate the tooth growth parameters, the acquired images of the overall enamel band (Fig. 2A) were merged using Adobe Photoshop® and analyzed with ZEN 2011® (Carl Zeiss) and Image J software.

2.4 Histological analysis techniques and dental growth parameters

We used measurements and counts of enamel incremental markings to assess the tooth growth patterns of the species. As the dental growth parameters depend on the cusp and enamel side studied (Jordana and Köhler, 2011; Kierdorf et al., 2013), we decided to standardize and analyze the buccal enamel band of the protoconid in agreement with previous work on equid teeth (Nacarino-Meneses et al., 2017). This allows future comparisons between extinct and extant equids. We did not analyze the lingual enamel band of the metaconid due to the presence of more marked Hunter-Schreger bands that hindered the correct observation of incremental markings. Enamel band thickness of each tooth was measured perpendicular to the enamel-dentine junction at a homologous zone situated 1 cm above the root. In order to reconstruct the entire tooth growth curve, the length of the cuspal enamel lost in worn molars was estimated by considering both the profile of unworn teeth (Tafforeau et al., 2007) and the hypoconulid wear degree (Stirton, 1941). We used the mean enamel extension rate of the nearest area to extrapolate the time that the worn portion required to be formed.

In ungulates, the main short-period enamel incremental features are laminations, which have a circadian periodicity and run oblique from the enamel-dentine junction (EDJ) (Jordana and Köhler, 2011; Kierdorf et al., 2013; Smith, 2006; Tafforeau et al., 2007). The other common incremental features are the Retzius lines or striae of Retzius, which correspond to the successive positions of the ameloblast secretory front (Dean, 2000). These long-period marks represent variations in the secretory activity, hypothesized to be ruled by two independent intern cycles (Smith, 2006) or by a general chronobiological rhythm (Bromage et al., 2016). Due to their known cyclical and regular nature, both laminations and Retzius lines permit the calculation of the following tooth growth parameters: the enamel daily secretion rate (DSR), or the amount of enamel secreted by

an ameloblast per day; the enamel extension rate (EER), or the enamel growth rate along the EDJ; and the total crown formation time (CFT). Firstly, and following prior studies on ungulate teeth (Jordana et al., 2014; Jordana and Köhler, 2011), we calculated the DSR quantifying the distance between laminations through the course of the enamel prisms (Fig. 2B). The DSR values have been seen to increase from inner to outer enamel regions (Kierdorf et al., 2014; Metcalfe and Longstaffe, 2012; Smith, 2008), although in some cases no significant differences between enamel areas have been identified (Nacarino-Meneses et al., 2017). We made many random counts in different enamel zones within all the tooth length to gather all the variation and obtain a representative mean. Because we were not able to perform the same amount of DSR measures in all the crown regions and enamel zones due to fossil preservation, direct comparison between the mean obtained DSRs should be taken with caution. We calculated EER following the methodology described in Jordana & Köhler (2011): the time that takes to form the distance that separate two incremental lines through the EDJ (X, Fig. 2C) is determined by dividing the prism length that separates those two incremental features (Y, Fig. 2C) by the rate of daily enamel deposition (DSR). This calculation is based on the ideas that the enamel prisms mark the enamel growth path and that accentuated lines indicate the position of the developing enamel front at a determinate moment (Dean, 2000). Applying this methodology through the entire enamel band, we obtained EER values in different tooth portions. The EERs were calculated at the intersection of clearly visible accentuated Retzius or stress lines, rather than at regularly spaced points (Dirks et al., 2012). In order to permit analogous comparisons, we divided the enamel band of each tooth into three thirds (cuspal, middle and cervical). Finally, the CFT was estimated calculating the overall time required to form the enamel band.

Differences between groups within each dental growth parameter were tested with a degree of significance of $\alpha = 0.05$. Non-parametric Kruskall-Wallis tests were used to test for differences in the DSR values between species since normality was not always met. Differences in EER values between fossil species were compared using an ANCOVA model, as this considers the decrease in EER along the crown height. Prior to the ANCOVA analysis, we tested for the significance of the interaction between the categoric predictive variable (species) and the continuous covariate (crown height) in the two different subsets analyzed (first/second molars and third molars). Moreover, we tested for the homogeneity variances (Levene test). In both cases, when the extant *Equus* data was excluded, we did not find significant violations of the assumptions of the ANCOVA model (Supplementary Material Table S1-S8). The statistical tests and graphs were performed using IBM SPPS Statistics 20 ® and PAST v. 3.18 (Hammer et al., 2001).

3. Results

Laminations represent the main incremental features observed in our sample. These are more appreciable on the central and inner enamel zones, although also identifiable between some striae of Retzius that outcrop at the outer enamel surface. In the inner enamel zone, laminations are less regularly spaced and more difficult to distinguish as some sub-daily incremental lines appear. Retzius lines are clearer in the outer enamel (Fig. 2C). These Retzius lines are also more discernible in the cervical region (Fig. 2C), where the angles between those and the EDJ are more obtuse (~ 10º) than in the middle or cuspal region (<5º). These observations agree with previous findings on equid enamel histology (Hoppe et al., 2004; Nacarino-Meneses et al., 2017).

3.1 Enamel daily secretion rates

In our sample, the mean daily secretion rates vary from ~14 to ~17 μm /day (Table 2). Contrary to hipparionins, *Equus quagga* shows different DSRs within the three different

tooth zones (cuspal, middle, cervical) considered from the reconstructed third molar ($p < 0.001$). These differences are only observed in the m3, since the m1 does not show significant variations on the DSR through the tooth length ($p = 0.08$). The first formed region of the third molar presents higher DSRs, while it decreases in the middle zone and reaches its minimum at the final formed root zone (Table 2). Considering this, the calculation of the other parameters in third molars of *Equus* has been done using the mean DSR of each stage, not the general mean of the taxon. When compared within hipparionin species, the DSRs of the first/second molars are significantly higher than those reported in third molars ($p < 0.001$) (Table 2). We do not find these significant differences between the DSR of m1 and m3 in extant *Equus* ($p = 0.34$), when we use the medial region DSR of the m3 as a general mean for the third molar.

The DSR is significantly different between species, both in the case of the analyzed m1/m2 ($p < 0.001$) and the m3 ($p < 0.001$). Among the first/second molars, the *H. concudense* m1/m2 exhibit the lowest DSR values with a mean value of 15.76 µm/day, which is significantly different from the higher secretion rates of *H. gromovae* ($p = 0.009$) and *Equus* ($p < 0.001$) (Table 2). The first/second molars of *H. periafricanum* have a mean DSR of 16 µm/day, which is significantly different ($p = 0.009$) to the higher rates of *Equus quagga* (~17 µm/day). The DSR of the third molars differ significantly between most species ($p < 0,001$), except for *H. concudense* and *H. periafricanum,* which present similar low DSRs (Table 2). In general, therefore, *H. gromovae* exhibit greater mean DSR values than the other two hipparionin species, both in the third and in the first/second molars, and this is only surpassed by the always higher DSRs of *Equus quagga*. This pattern is followed both in the m3 and the m1/m2, with lower values of DSR in the later formed third molars (Table 2). It should be noted that the same number of DSR measures within the crown regions and the enamel zones was not possible in our sample. We should, hence,

be prudent when interpreting the differences in DSRs found between species and tooth types. However, since the DSRs in the equids' first molars tend to do not vary significantly between regions (Nacarino-Meneses et al., 2017), we assume that our results are more reliable than if the same measures were performed on taxa that reported high variations on the DSRs, like pigs (Kierdorf et al., 2019, 2014).

3.2 Enamel extension rates

The first/second molars' ameloblasts differentiated along the EDJ at higher rates than in the third molars in all the studied taxa, particularly in the cuspal and medium zones (Table 2). Due to these differences, and in order to make interspecific comparisons, we separated the higher EER values of the m1/m2 from the lower EERs of the m3. We always observed higher EERs in the first formed enamel area, while the rates were lower towards the cervical region (Table 2, Fig. 3 and Supplementary Material Fig. S4). For example, the first third of the crown of the m1/m2 of *H. periafricanum* grow at a mean rate of 166.62 µm/day, while at the end of the enamel band the mean EER is 37.27 µm/day. This diminution is present in all taxa and in both tooth types, thus representing a general decrease in the tooth growth rate through the development of the tooth (Table 2, Fig. 3 and Supplementary Material Fig. S4). Taking into account this growth rate decrease, the intraspecific variation in EER within the crown height is high, thus hindering the growth rate comparisons between species. To evaluate the EER differences between species, therefore, we considered the crown height as a covariate affecting the EER. By doing this, interspecific differences between EER in fossil first/second molars and third molars are significant ($p < 0.001$). These differences are found between all groups of hipparionin teeth ($p \leq 0.001$), except for the m1/m2 of *H. gromovae* and *H. periafricanum,* which grow at similar rates ($p = 0.075$). In all cases, *H. concudense* teeth grow at higher rates, while the teeth of the dwarf species grow at a slower pace. Although not directly tested by the

ANCOVA model (see section 2.4), *Equus quagga* teeth develop at higher rates compared to the three hipparionin species (Table 2 and Fig. 3). The higher EER of extant *Equus* molars compared to hipparionins molars is comparably more pronounced in m1/m2 than in m3 (Table 2 and Fig. 3).

3.3 Growth reconstruction and crown formation times

To reflect the whole tooth growth pattern, we plotted the crown height against the crown formation time (CFT) for each species tooth type (Fig. 4). Extant *Equus* tooth development has been plotted considering the entire tooth growth reconstruction using differently worn teeth (Nacarino-Meneses et al., 2017) (see section 2). All the reconstructed tooth growth curves fit well with von Bertalanffy growth models (Fig. 4 and Supplementary Material Fig. S5). Compared to recently erupted *Equus* teeth (Nacarino-Meneses et al., 2017), we confirm that unworn or slightly worn hipparionin teeth register most of the tooth development, since asymptotic size is being reached (Fig. 4). Some residual cervical enamel, however, could be further deposited although without increasing the tooth height substantially. Steeper slopes than those for the *H. gromovae* and *H. periafricanum* teeth indicate the higher growth rates of the *Equus* and *H. concudense* molars (Fig. 4). We also identify a higher EER in m1/m2 compared to m3 in the steeper slopes of the first molars within each species (Supplementary Material Fig. S5). These intraspecific EER differences between m3 and m1/m2 are more pronounced in the *Equus quagga* molars than in hipparionin horses, as first molars show comparably much higher crown extension rates (Fig. 3 and Supplementary Material Fig. S5).

The complete growth period or CFT is explored in the teeth that have not lost cuspal growth register and that have started forming roots (Table 3). Despite having the higher crowns, the m1/m2 of *H. concudense* take 500 days to be formed, a similar time to that of the smaller species' teeth (Table 3 and Fig. 4). When we compare the third molars

total CFT, these generally take more time to form than first/second molars. Within species, the m3 of *H. gromovae* and *H. periafricanum* require similar time to form (around 600 days), whereas the *H. concudense* m3 spans 100 days more to finish formation (Table 3). The total crown formation time of the composite *Equus* tooth is much higher, taking 785 days in the case of the first molar and 1750 days in the third. However, these *Equus* CFTs are the product of differently worn teeth compositions, as they keep growing after eruption and delay their root closure, depositing residual enamel (Nacarino-Meneses et al., 2017). Analogous comparisons of the formation time in *Equus* and *Hipparion* teeth that present a similar ontogenetic stage (recently erupted and little worn, Table 1: *Equus quagga* IPS-92341 and *Hipparion concudense* IPS-91763) show that, although the *H. concudense* tooth (IPS-91763) lost the final part of enamel due to preservation, it took more time (367 days) to be formed than the extant *Equus* tooth (200 days) (Fig. 5). Growth rate differences between these two teeth are also evident (Fig. 5).

Aside from the total growth span, eruption times can be inferred considering that the different crown developmental stages are identifiable in the reconstructed growth curve (Nacarino-Meneses et al., 2017). Hence, here we estimated tooth eruption from the transition from the first CDS to the second CDS (arrowheads, Fig. 4), following Nacarino-Meneses et al. (2017). That is, from the change of an almost linear growth (CDS I) over a reduced growth rate (CDS II) that represents intermediate rates of growth (Nacarino-Meneses et al., 2017). Based on the *Equus quagga* m1 growth curve, we can identify this point at around 230 days, thus indicating the eruption of the first molar. For the *Equus quagga* m3, on the other hand, we estimate a time of eruption of around 500 days after the start of the formation (Fig. 4). As the first molars of extant zebras erupt around 9 months after birth (270 days) (Smuts, 1974), our estimation for the m1 seems reliable. In zebras, the third molar erupts at the age of 3 years and 3 months (Smuts, 1974) (39 months).

Assuming that zebras' m3 starts to develop at a similar age as when the third molar of *Equus caballus* starts to mineralize [21 months of age (Hoppe et al., 2004)], the estimated eruption time of 500 days (~16.5 months) (Fig. 4) also agrees quite well. The decrease in growth rate is more progressive in hipparionin teeth than in *Equus* (Fig. 4), thus, the transitions between the CDSs in the hipparionin growth curves are less clear. Nevertheless, a change in the slope of the curves is identifiable (arrowheads, Fig. 4) and might match the eruption time as in the extant equid model. Applying this methodology, we infer a later eruption time in hipparionins' fist/second molars compared to extant *Equus* [300 days to 350 days of formation (Fig. 4)]. The hipparionin third molars, on the other hand, likely erupted around 500 days after the beginning of their formation, similar to extant *E. quagga* third molars. Besides using the equid tooth growth model by Nacarino-Meneses et al. (2017), we can further endorse the inferences of the eruption times based on radiographic evidence of hipparionin eruptive sequences (Domingo et al., 2018). Judging from the radiographs provided in Domingo et al. (2018), the enamel band of the mesial lobe of an hipparionin m1 close to eruption measures ~53 mm, and that of a fully erupted one ~60 mm. Considering the tooth growth curve of the larger hipparionin species (Fig. 4), these teeth may have taken ~300 to ~350 days to be formed, respectively. In the third molars, an unerupted – still in crypt – hipparionin m3 measures ~45 mm and a totally erupted m3 measures ~55 mm, which represent ~350 and ~600 days of formation respectively. Thereafter, our inferred eruption times in hipparionin lower first/second molars and third molars are in agreement with the estimated CFTs of the teeth that have erupted or are close to eruption.

4. Discussion

Our study represents the first comprehensive analysis of hipparionin tooth growth through dental histology. The addition of extant *Equus quagga* teeth, moreover, allows us

to compare the growth patterns and to identify differences in the tooth formation between extant and extinct representatives of the Equinae subfamily. Dental growth variations between different sets of permanent teeth have hitherto not been explored in ungulate mammals. Here, we analyze and compare, for the first time in ungulates, two different sets of permanent teeth, thus characterizing the growth of dental tissues during two distinct ontogenetic stages. A few other studies have addressed the differences in growth parameters between tooth loci, but these have been focused on hominoids (Guatelli-Steinberg et al., 2012; Shellis, 1984; Smith, 2016; Smith et al., 2007). Besides the growth characterization of the hipparionin teeth, widely used in many paleoecological studies (Domingo et al., 2009; Matson and Fox, 2010; Nelson, 2005; Rey et al., 2013; Van Dam and Reichart, 2009), our approach also allows us to make further inferences of the life history of the studied taxa. The assessment of the dental growth patterns, thus, led us to infer differences in the lifestyles of the different-sized hipparionin taxa. Moreover, the comparison between hipparionin and *Equus* molars has highlighted significant tooth growth differences that can be both involved in life history differences and in the formation of the higher crowned teeth of *Equus*.

4.1 Dental growth parameters: Daily secretion rate

We identified that the dominant incremental markings in hipparionin enamel are laminations, which is in agreement with previous findings on ungulate teeth (Jordana and Köhler, 2011; Kierdorf et al., 2013, 2014; Nacarino-Meneses et al., 2017; Tafforeau et al., 2007). The daily secretion rates obtained through quantification of the lamination spacing (Table 2) are congruent with those determined in the aforementioned studies focused on ungulate species. Nevertheless, our estimated DSR values ranging from 14 to 17 μm/day do not correspond to previous values of 5 μm/day calculated in domestic horses (Hoppe et al., 2004). As formerly pointed out by other authors (Kierdorf et al., 2013, 2014), and

already addressed in previous studies with equid teeth (Nacarino-Meneses et al., 2017), we consider those undervalued preliminary measures a result of the misinterpretation of laminations and sub-daily incremental marks.

In our sample, we identified higher DSRs in the hipparionin first/second molars compared to third molars (Table 2). Although the mean DSRs can be skewed to some extent by the unbalanced counts between the different enamel and crown portions, the higher DSR values in m1/m2 compared to those of the m3 are commonly found within all zones. The few previous studies that compared daily enamel secretion rate between hominoid tooth types did not detect variations between the molars' DSRs (Smith, 2016; Smith et al., 2007). In a similar way, we do not identify DSR differences between the *Equus quagga* tooth types. In our case, we interpret the lack of differences in *Equus* tooth types as being a result of the lumped measures, the lower number of observations done in the *Equus* first molars (n = 45) compared to the third molars (n = 138), and to the significant DSR decrease observed in the consecutive developmental stages of the *Equus* third molars. Similarly, a decrease in the enamel secretion rate at the end of the third molar formation has been observed in human teeth (Guatelli-Steinberg et al., 2012). We suggest that these lowered secretion rates on the *Equus* third molars might be related to a general depletion of tissue depositional rates after the eruption of the third molar (transition from CDS I to CDS II *sensu* Nacarino-Meneses et al. (2017), as this event is broadly correlated with the attainment of skeletal maturity (Dean, 2006). Thus, the lower DSRs of hipparionin third molars compared to the first/second molars can also be linked to the differences in the formation timing. In ungulates, however, the DSR parameter has shown to be more correlated with the tooth morphology (i.e. hypsodonty degree) than to the pace of life history or somatic growth (Jordana et al., 2014). This would agree with the differences observed in the DSRs between the hipparionin tooth types, since third molars

are lower crowned and less hypsodont than first/second molars (Table 1 and 3). The DSR, moreover, can also be related to the enamel band thickness, since the amount of enamel secreted per day (i.e. DSR) and the ameloblast secretory lifespan determine the final width of the enamel (Kierdorf et al., 2014). Accordingly, we measured the linear enamel thickness in our sample (Supplementary Material Fig. S6). Despite their lower DSRs, the third molars tend to have somewhat thicker enamel bands than the first/second molars. This result suggests that enamel thickness differences between tooth types are more influenced by the secretory lifespan of the ameloblasts (Kierdorf et al., 2014) or the total CFT (Kierdorf et al., 2019) than by the DSR. Regarding intergeneric comparisons, the higher DSR values of extant *Equus*' molars compared to those of hipparionins are expected considering the proposed relationship between hypsodonty and DSR (Jordana et al., 2014) but also considering the *Equus*' thicker enamel bands (Supplementary Material Fig. S6). However, interspecific differences in the hipparionin DSR do not seem to be directly linked to differences in hypsodonty (Table 1), nor to differences in enamel thickness (Supplementary Material Fig. S6). For example, the smallest and most hypsodont *H. periafricanum* presents low DSRs comparable to those of the less hypsodont *H. concudense*, which has considerably thicker enamel bands. Therefore, the ameloblast secretory lifespan and the CFTs may have more important roles than DSR in the formation of thicker enamel bands (Kierdorf et al., 2019) and more hypsodont teeth (Nacarino-Meneses et al., 2017; Witzel et al., 2018). Hence, the small differences in the hypsodonty between these high-crowned hipparionins might be of little importance in terms of DSR changes. We suggest that the relationship between hypsodonty and DSR (Jordana et al., 2014) could be more easily observed in large-scale comparisons between more brachydont to more hypsodont species. Alternatively, the differences identified here could represent interspecific variations in the quotient between enamel and dentine, a characteristic that together with hypsodonty determine tooth durability (Famoso et al.,

2013; Jordana et al., 2012; Winkler and Kaiser, 2015). Thus, we interpret that DSR is probably influenced by many other factors besides hypsodonty, such as tooth morphology, phylogeny (Dirks et al., 2012), enamel thickness (Kierdorf et al., 2014), tooth formation timing or even general somatic growth. The interplay of many of these factors might determine the overall DSR of the tooth. Further studies addressing the relationship between this parameter and the disposal and proportion of enamel could shed light on this topic.

4.2 Dental growth parameters: Extension rate

Various isotopic studies have already inferred hipparionin tooth growth in height thanks to the identification of cyclic variations in δ13C and δ18O signal (Matson and Fox, 2010; Nelson, 2005; Van Dam and Reichart, 2009). These estimations represent mineralization rates rather than matrix secretion rates. However, since the enamel starts to mineralize soon after matrix deposition, the extension rates estimated from isotopic studies tend to resemble those calculated using histology (Metcalfe and Longstaffe, 2012). Accordingly, the tooth growth rates calculated from the isotopic cyclicity in hipparionin teeth agree well with the enamel extension rates calculated here from incremental growth marks. For example, van Dam and Reichart (2009) estimated a growth rate of 40 mm/year (= 109.60 μm/day) for the upper third molar of *H. concudense* from Concud. In our study, we obtained a similar mean EER of 95.54 μm/day in the middle region of the lower third molar from the same species population, supporting the link between formation and mineralization rates, which has also been corroborated in extant equids' molars (Nacarino-Meneses et al., 2017).

In general, the proper estimation of a representative EER mean in high-crowned teeth is difficult since there is a general decrease of this parameter through the course of tooth formation (Fig. 3). This declining in the tooth growth pace from the cusp to the cervix

is a reflection of a non-linear dental growth pattern (Bendrey et al., 2015), and has already been described in other mammalian groups (Guatelli-Steinberg et al., 2012; Jordana and Köhler, 2011; Kierdorf et al., 2013; Shellis, 1984) and in extant equids (Nacarino-Meneses et al., 2017). Due to the larger crowns' heights, the variation in the EER values through the enamel band is especially high in hypsodont ungulate teeth and should be considered when making comparisons. Moreover, apart from the within-tooth variation, the extension rates of the first/second molars and the third molars differed in our sample, being always higher in the first/second molars of all taxa. Similarly, Shellis (1984) found that in humans the first formed deciduous teeth grow eight times faster than third molars do, and higher crown extension rates have been calculated in first molars compared to third molars in other hominoids (Smith, 2016; Smith et al., 2010, 2007). Higher EERs might be influenced by the need to form the tooth within a shorter CFT (Kierdorf et al., 2014), as tooth crowns in m1/m2 tend to take less time to be formed than those of m3 (Table 3 and Supplementary Material Fig. S5). Moreover, we interpret the higher EER values of the m1/m2 compared to those of the m3 as a reflection of the higher growth rates of the organism. Mammalian first molars are formed and erupt earlier in the ontogeny, while third molars finish their formation when skeletal maturity is being reached (Dean, 2006; Hillson, 2005; Hoppe et al., 2004). For this reason, we can expect greater EERs in m1/m2 since there are more activated ameloblasts due to the higher general somatic growth than posteriorly in ontogeny, when m3 are being formed. The fact that the deposition of bone lamella is coupled with the Retzius line formation (Bromage et al., 2009), and that there is an endogenous rhythm controlling both functions (Bromage et al., 2016), endorses the relationship between enamel growth rates with bone growth and, then, the general somatic growth. The same idea has recently been suggested due to the discovery of lower EER in mammaliamorphs that experienced an earlier reduction of the overall skeletal growth rates (O'Meara et al., 2018). The influence of growth hormones (GH and IGF-1) and their

receptors in the differentiation and proliferation of the odontogenic cells involved in the formation of teeth (Symons and Seymour, 2000; Young, 1995; Zhang et al., 1992) further supports this link.

Besides the variations in intraspecific somatic growth rates during ontogeny, the differences EERs between taxa might also be indicative of differences in the organism's pace of growth (Jordana et al., 2014). The estimation of this parameter, therefore, might allow further interpretations to be made on the biology of animals, although caution should be taken due to the variation of these parameters between and within tooth types. Considering the differences between the third and first/second molars, and the decreasing of EER through the enamel band, we could identify higher EER in *E. quagga* compared to all hipparionin horses, and higher EER in the larger hipparionin species compared to the dwarfed taxa. Within a sample of 21 ruminant species and taking into account their phylogenetic relationships, Jordana et al. (2014) found a significant correlation between the residuals of the age at first reproduction – a proxy of the pace of life history corrected by body size – and the tooth enamel extension rate. Hence, lower EER values have shown to be indicative of a slower pace of growth than those expected from body size scaling (Jordana et al., 2014). Additionally, slower extension rates have also been associated to a later attainment of foraging independence in cebids (Hogg and Walker, 2011) and to the prolonged growth periods of modern humans compared to *Homo erectus* and earlier hominins (Dean et al., 2001). The influence of the EER in the formation of a tooth at the required time for its eruption, might be the reason underlying the EER relationship with the timing of life history events, since the tooth development is strongly tied to the mammals' life cycle (Smith, 2000, 1991). In terms of EER, therefore, we can infer a slower pace of growth in the analyzed hipparionin species in comparison to extant equids. Moreover, the

extension rates of the dwarf late Turolian Spanish forms further suggest a slower pace of growth compared to *H. concudense*.

Some authors have considered the existence of an allometric coupling of the EER with total crown height (Dirks et al., 2012) or body mass (O'Meara et al., 2018). Taking this into account, the lower EERs of the hipparionins compared to extant equids, and the lower EER of dwarf hipparionins compared to larger representatives, could represent the consequence of the allometric scaling of this parameter. The same relationship between tooth size and EER was also identified in humans by Guatelli-Steinberg et al.(2012), estimating higher EERs in those teeth with larger enamel dentine junction lengths. On the other hand, however, the formation of larger canines in male gorillas has been related to longer formation times rather than to higher growth rates (Schwartz and Dean, 2001). Smith (2016) also found that taller fossil teeth from larger extinct pongines had lower EERs compared to those of extant orangutans. Similarly, Jordana and Köhler (2011) identified lower EERs in the higher crowned insular *Myotragus balearicus*, which was a slow-growing mammal with a delayed life history schedule compared to extant caprines (Jordana et al., 2012; Köhler, 2010; Köhler and Moyà-Solà, 2009). In our case, *H. periafricanum* and *H. gromovae* were relatively higher crowned – more hypsodont – than the larger hipparionin species from the late Miocene (Pesquero, 2003). Thus, to form those more hypsodont teeth, we should expect higher EERs compared to less hypsodont species (*H. concudense*), especially if we consider that they would have advanced the general life history schedule due to their reduced size and the theoretical size coupling with life history traits (Calder, 1984; Stearns, 1992). Our results suggest the opposite scenario, namely more hypsodont dwarf hipparionin species exhibiting lower EER, which we further relate to a slower life history than the expected from their size.

4.3 Formation and eruption times

The previous life history interpretations are not only supported by the EER values, but also by the estimated formation and eruption times, two parameters that are strongly correlated with the life history of an organism (Dean, 2006; Macho and Williamson, 2002; Smith, 2000, 1991).

Regarding the formation times, we generally identified higher CFTs in third molars compared to those of first molars. Similarly, CFTs in human third molars are much higher than in the case of other teeth (Shellis, 1984), as has also been observed in other hominoids where the first molars form faster and in less time than third molars (Smith et al., 2010, 2007). The longer formation times in the third molars might be related to their lower EER. When comparing between taxa, the overall crown formation times in *Equus* are much higher than in hipparionins, since *Equus* CFTs are the result of a composite growth reconstruction as they extend the molar formation. When we compare growth curves and, thus, observe the total crown formation times of individual teeth of similar developmental stages, we observe higher CFTs in hipparionin teeth compared to *Equus* (Fig. 5), and in some cases higher CFTs in the small hipparionins compared to the larger species (Fig. 4). Macho and Williamson (2002) found a significant correlation between the crown formation times corrected by body mass and the relative gestation length in African bovids. Although they arguably overestimated the CFTs due to the misinterpretation of sub-daily striations with daily increments (Kierdorf et al., 2014, 2013), the similar repeat intervals that they reported relating these features across the studied taxa (Macho and Williamson, 2002) might maintain the proportionality of the correction and thus the significance of the correlation. Hence, taking in mind the high relative CFTs obtained in hipparionins, and especially the two dwarf taxa, we might infer higher relative gestation

lengths, a characteristic of a slower pace of life history compared to *Equus* and to larger hipparionins.

However, a more direct life history proxy not related to the continuous growth of extant equid teeth is the timing of dental eruption (Smith, 2000). On the one hand, the first molar eruption is considered as an indicator of the weaning event (Dirks and Bowman, 2007; Smith, 2000, 1991), and the age of eruption is an informative estimate of postnatal growth rate, with faster-growing animals erupting their first molars earlier in life (Smith, 2000). The age of first molar eruption, moreover, has been shown to positively correlate with important life history traits such as longevity or female sexual maturity (Veitschegger and Sánchez-Villagra, 2016). On the other hand, the onset of the third molar eruption has been shown to be correlated with skeletal maturity (Dean, 2006; Engström et al., 1983). Data relative to the weaning and maturity attainment of extant equids coincide with those of the first and third molar eruption times (Table 4). This endorses our interpretations based on the hipparionin eruption times. In extant foals, the first molar starts to be formed close to the birth event, and begins mineralization two weeks after (Hoppe et al., 2004). Taking into account the growth curve reconstruction, we identified the emergence time of extant *Equus* molars (Nacarino-Meneses et al., 2017) at ~230 days (Fig. 4), which agrees quite well with that reported in the literature (Smuts, 1974). Assuming a similar age of the first molar formation onset in hipparionins, we can infer later eruption times compared to extant equids. By this, we roughly estimate the weaning age of the hipparionins analyzed in a span from 300 to 350 days of age (10-12 months).

The absolute eruption age of the third molar is difficult to establish because we do not know at which age hipparionins started to form the third molar. However, the hipparionin tooth mineralization pattern has shown to be analogous to that of extant *Equus* (Domingo et al., 2018). From the growth curves, we estimated a similar third molar

eruption time of ~500 days after the onset of the formation in all analyzed taxa. If we conservatively assume that hipparionin third molars begin to form as in *Equus,* where third molar mineralization starts at the age of 21 months (Hoppe et al., 2004), the hipparionin third molars should have erupted around the age of ~37.5 months. Considering the allometric coupling between size and life history, we would expect that the smaller species followed faster life history strategies and, thus, they would have matured earlier (Calder, 1984). However, we observe how hipparionins, and especially the dwarf Spanish species, had a similar maturity age to extant equids. In this sense, we could infer a slower lifestyle in hipparionin horses bearing in mind the similar timing despite the smaller size (Sibly and Brown, 2007). Our results on weaning and maturity schedules, together with the hipparionin smaller size compared to extant equids, further support our previous interpretation of slower life histories. Conforming to the Schultz's Rule (Smith, 2000), moreover, the hipparionin eruption sequence have already indicated a slow-growing strategy (Domingo et al., 2018). Besides the slower pace of life history compared to *Equus,* our results also suggest that the dwarf taxa from the Spanish late Turolian followed slower lifestyles than the larger middle Turolian species; because although they were smaller, which predicts faster life history from body mass scaling (Calder, 1984), they followed similar life history schedules.

4.4 Hypsodonty and life history interpretations

The more hypsodont *Equus* molars erupt earlier compared to those of hipparionins, probably related to a faster ontogenetic development. In a similar way, the South American notoungulates with ever-growing teeth exhibited an earlier eruption of permanent molars compared to premolars, which has also been linked to an accelerated life history (Gomes Rodrigues et al., 2017). The higher wear exposure due to earlier eruption of the permanent molars would have been compensated by the observed extended dental growth span and

a late root formation. After the earlier *Equus* molar eruption, therefore, the subsequent slower growth and extended formation time helps to counterbalance the height loss due to wear (Nacarino-Meneses et al., 2017). Moreover, the higher EERs related to earlier formation, and the extended crown formation times in *Equus*, may be involved in the higher hypsodonty indices exhibited within the genus. As a result, extant equids could have increased the endurance of their masticatory apparatus and, thus, incremented their potential life span (Damuth and Janis, 2011; Jordana et al., 2012; Veiberg et al., 2007). Hipparionins, on the other hand, would have erupted their permanent molars later than extant equids, thus having a prolonged potential tooth life span as teeth began to wear later. Moreover, instead of increased crown heights, hipparionin horses relied on occlusal enamel-band complexity as a way to expand their tooth durability (Famoso et al., 2016; Famoso and Davis, 2016).

Previous inferences on the life history of the Hipparionini tribe suggested an earlier maturity attainment and shorter life span compared to modern equids (Hulbert, 1982; Kurtén, 1953; Van Valen, 1964). These first surveys were based on age-structure and population dynamics studied from attritional equid fossil populations by means of their dentition ontogeny (Woodburne and MacFadden, 1982). Therefore, to define and characterize discrete age stages for the reconstruction of the demographic profile, these studies depended on models of age determination for extant species and their eruption patterns (Kurtén, 1953; Spinage, 1972). For this reason, the inferences of accelerated life history in hipparionins, and especially the interpretations of potential life span, strongly relied on the lower crown height of these taxa compared to present-day equids (Hulbert, 1982) and on the smaller size of some species (Van Valen, 1964). However, as we have seen, hipparionins do not follow the same formation, nor eruption pattern as extant equids (Domingo et al., 2018), and probably they also had different wear rates (Hulbert, 1984)

due to their different dietary niches (Semprebon et al., 2016). Hence, there are arguments in support of the thesis that hipparionins were slow-growing mammals, as has also been recently proposed from their molar eruption pattern based on the Schultz's Rule (Domingo et al., 2018).

More recent approaches using bone paleohistological analyses have provided a new framework to test for the life history adaptations of this diverse and successful group (Martinez-Maza et al., 2014; Orlandi-Oliveras et al., 2018). Thus, the study of the bone histology of *Hipparion concudense* has provided evidence of the attainment of skeletal maturity during the third year of life (Martinez-Maza et al., 2014), coinciding with our inferred age of third molar eruption. Although Martinez-Maza et al. (2014) pointed out similar life history patterns between *H. concudense* and extant equids, they suggested that *H. concudense* developed over a shorter time span. Their interpretation was based on an estimated age of sexual maturity at three years old in *H. concudense*, while most extant equids reach reproductive maturity during or after the fourth year of life (Martinez-Maza et al., 2014). However, the identification of absolute sexual maturity attainment from bone histology analysis is an issue still under debate (Marín-Moratalla et al., 2013; Nacarino-Meneses et al., 2016), as Martinez-Maza et al. (2014) already indicated, and the results should be considered carefully. Furthermore, wild equid populations can exhibit a wide range of variability in sexual maturity attainment since there are many possible factors influencing it (Choquenot, 1991; Penzhorn and van der Merwe, 1988; Smuts, 1976a, 1976b; Westlin-van Aarde et al., 1988). The dental histology parameters and eruption times calculated here, however, point towards a slower life strategy in hipparionins compared to extant equids. Considering their size, the Spanish dwarf taxa would have followed an even slower lifestyle than the larger hipparionins, as was also proposed by Orlandi-Oliveras et al. (2018) from the study of *H. gromovae* bone histology. This slower

pace of growth has been related to a different ecology of the smaller species compared to the larger hipparionin forms (Orlandi-Oliveras et al., 2018). In African bovids, Macho and Williamson (2002) also found that the small-sized taxa tend to follow slower life history strategies due to their relatively longer gestation lengths. In this case, small ruminants showed different habitat preferences than the larger ones, browsing in more wooded habitats. Future paleodietary reconstructions using micro or mesowear techniques (Calandra and Merceron, 2016; Kaiser and Solounias, 2003) on the teeth of these dwarf hipparionins could shed light on their diet and habitat preferences.

5. Conclusions

Our analyses of the dental histology of hipparionin teeth allow the characterization of their growth and comparisons both between different-sized taxa and with an extant equid model. In all cases, hipparionin teeth grow and develop differently from those of the genus *Equus*. Variations are also found between hipparionin species and between the two tooth types analyzed within each taxon. We have associated the higher enamel secretion and extension rates of the first/second molars compared to the third molars with differences in the formation timing, although the variation in the secretion rates could also be influenced by the crown height or other characteristics related to tooth morphology. The higher enamel extension rates of the first/second molars are a reflection of the fast somatic growth of early ontogenetic stages, while the third molars are formed close to maturity attainment. Moreover, third molars generally take more time to be formed and to erupt than first/second molars, which is probably linked to their lower extension rates. Regarding the differences between groups, we found lower daily secretion rates in hipparionin teeth in comparison to *E. quagga*. We hypothesize that these distinct secretion rates, together with the disparity between hipparionin species, are the consequence of the interplay of

numerous factors, such as hypsodonty, enamel thickness, secretory ameloblast lifespan, and overall tooth morphology.

Due to the link between pace of life history and dental growth development, we could also draw inferences on the life history of the studied taxa. The parameters that are more related to the pace of growth are the enamel extension rate and the formation time. The formation and eruption of first and third molars are further linked to key life history events such as weaning and maturity attainment, respectively. As a result, we were able to compare the hipparionin life history strategies with an extant equid model, and also to make comparisons between the two dwarf forms and the larger one. Hipparionin teeth grow more slowly and erupt relatively later than those of extant equids, suggesting a slower pace of growth. In addition, the dwarf hipparionins exhibit lower extension rates but similar formation and eruption schedules than the larger species, which considering their smaller size indicates that they had slower life histories. These results point toward a slow-growing strategy in hipparionin horses, as has recently been suggested from dental eruption patterns (Domingo et al., 2018), and debunk previous hypotheses of general shorter life spans and earlier maturity. Dwarfed hipparionin species from the late Turolian of Spain, moreover, do not follow the size scaling of the life history schedule but show a similar life history strategy as larger hipparionins, which is slower than predicted from the body mass.

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Data availability

Relevant data is shown within the paper and in Supplementary Material. Raw data related to this article can be found at http://dx.doi.org/10.17632/h2ywzbcs59.1, an open-source online data repository hosted at Mendeley Data.

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Table and Figure Captions

Table 1. Teeth analyzed in the present study. Mean body mass (BM) and hypsodonty indices (HI) are provided per each species.

Body mass data from [a] Pesquero and Alberdi (2012), [b] Ortiz-Jaureguizar and Alberdi (2003), and [c] Cantalapiedra et al. (2017). Hypsodonty indices calculated for first/second molars are taken from [d] Pesquero (2003) and [c] Cantalapiedra et al. (2017). [e] Hypsodonty indices for third molars were calculated from our sample using the less worn specimens.

Table 2. Dental growth parameters – daily secretion rate (DSR) and enamel extension rate (EER) – estimated through dental histology analyses. DSR values of *Equus* m3 are shown separately due to the significant differences in the DSR values within the different areas of *Equus* third molar. The division of *Equus quagga* molars for the EER considers the complete tooth development and corresponds to the different CDS defined by Nacarino-Meneses et al. (2017).

Table 3. Crown heights through the enamel band and crown formation times of the most complete teeth calculated through the dental histology analysis. *Equus quagga* calculations (*) relative to the total reconstructed molar growth.

Table 4. Eruption dates of first and third molars contrasted with life history information in two extant equid species.

Fig. 1. Map of the area around the city of Teruel (Aragon, Spain). The fossil sites of El

Arquillo (1) and Concud (2) are indicated with a star. (single column).

Fig. 2. A: Protoconid buccal enamel band of a *Hipparion periafricanum* first/second molar (IPS-91733) sectioned longitudinally and observed under polarized light. B: Detail of the enamel band depicted in (A) illustrating the method used to calculate the daily secretion rate (DSR). The distance between laminations through the prism path (dashed line) is divided by the number of laminations (arrows). Dashed arrow represents the prisms' direction. C: Detail of the cervical zone in (A), showing the methodology used for the enamel extension rate (EER) determination. "X" represents the increase in crown height between two incremental features (black lines) and "Y" indicates the distance following the enamel prism between those two enamel marks. Black arrows point to Retzius lines that outcrop the outer enamel, and "EDJ" depicts the enamel dentine junction. (2 column image, color)

Fig. 3. Enamel extension rate values (EER) plotted against the crown height from the first formed zone (cuspal, left size) to the last formed region (cervical, right side). Left chart shows EERs for first/second molars and right chart for third molars. Note the different scales between the two charts. (2 column image)

Fig. 4. Molar growth curves obtained from plotting the crown height through the enamel band (mm, y axis) against the crown formation time (days, x axis), from the cusp to the root area. Left plots show mean curves for first/second molars while right plots the third molar mean curves. Upper graphs represent the growth curves of all the species analyzed, while lower graphs only show hipparionin teeth. *Equus* growth curves obtained from the composition of differently worn teeth, which are represented by different grey scale dots. Note that the plot axes are not equally scaled. Dashed lines represent the 95% confidence intervals and the arrows the inferred eruption times. (1.5 column image)

Fig. 5. Growth curves of two molars of comparable developmental stage (*Equus quagga* IPS-92341 and *Hipparion concudense* IPS-91763). The curve represents the growth from the cusp to the root. Scale shown in the teeth images correspond to 1 cm. (single column image, color)

TABLE 1

	Species	Tooth	BM (kg)	HI	Code	Locality	Age	Wear
m1/m2	*H. concudense*	m1/m2	160[a]	2.8[d]	IPS-91762	Concud	middle Turolian (MN12)	Slight
	H. concudense	m1/m2			IPS-91763	Concud	middle Turolian (MN12)	Slight -
	H. gromovae	m1/m2	59[b]	2.9[d]	IPS-91731	El Arquillo	late Turolian (MN13)	Slight
	H. gromovae	m1/m2			IPS-91732	El Arquillo	late Turolian (MN13)	Slight
	H. periafricanum	m1/m2	29[b]	3.2[d]	IPS-91733	El Arquillo	late Turolian (MN13)	Slight
	H. periafricanum	m1/m2			IPS-91734	El Arquillo	late Turolian (MN13)	Slight +
	Equus quagga	m1	250[c]	4.4[c]	IPS-92341	Hagenbeck Zoo	Extant	Slight -
	Equus quagga	m1			IPS-92342	Hagenbeck Zoo	Extant	Null
	Equus quagga	m1			IPS-92345	Hagenbeck Zoo	Extant	Crypt
	Equus quagga	m1			IPS-92346c	Hagenbeck Zoo	Extant	Moderate
m3	*H. concudense*	m3	160[a]	2.0[e]	IPS-40644	Concud	middle Turolian (MN12)	Null
	H. concudense	m3			IPS-40665	Concud	middle Turolian (MN12)	Slight
	H. concudense	m3			IPS-40668	Concud	middle Turolian (MN12)	Slight
	H. concudense	m3			IPS-87855	Concud	middle Turolian (MN12)	Null
	H. gromovae	m3	59[b]	1.9[e]	IPS-85506	El Arquillo	late Turolian (MN13)	Slight -
	H. gromovae	m3			IPS-85508	El Arquillo	late Turolian (MN13)	Slight
	H. gromovae	m3			IPS-86851	El Arquillo	late Turolian (MN13)	Slight +
	H. gromovae	m3			IPS-87856	El Arquillo	late Turolian (MN13)	Slight +
	H. periafricanum	m3	29[b]	2.1[e]	IPS-85504	El Arquillo	late Turolian (MN13)	Slight -
	H. periafricanum	m3			IPS-86850	El Arquillo	late Turolian (MN13)	Slight +
	H. periafricanum	m3			IPS-87857	El Arquillo	late Turolian (MN13)	Moderate
	Equus quagga	m3	250[c]	2.5[e]	IPS-104358	Réserve Africaine	Extant	Crypt

						Sigean		
	Equus quagga	m3			MZB-94-1229	Barcelona Zoo	Extant	Slight +
	Equus quagga	m3			IPS-92346e	Hagenbeck Zoo	Extant	Moderate

(footnote) Body mass data from [a] Pesquero and Alberdi (2012), [b] Ortiz-Jaureguizar and Alberdi (2003), and [c] Cantalapiedra et al. (2017). Hypsodonty indices calculated for first/second molars are taken from [d] Pesquero (2003) and [c] Cantalapiedra et al. (2017). [e] Hypsodonty indices for third molars were calculated from our sample using the less worn specimens.

TABLE 2

	Species	**Overall DSR** (µm/day)	**Cuspal EER** (µm/day)	**Middle EER** (µm/day)	**Cervical EER** (µm/day)
m1/m2	*H. concudense*	**15.76** ±0.96 (N = 90)	**211.50** ± 27.25 (N = 6)	**177.08** ± 24.71 (N = 6)	**82.52** ± 38.96 (N = 9)
	H. gromovae	**16.27** ±1.15 (N = 104)	**144.73** ± 28.05 (N = 7)	**131.32** ± 29.63 (N = 6)	**55.75** ± 35.85 (N = 16)
	H. periafricanum	**15.96** ±1.37 (N = 85)	**166.62** ± 33.08 (N = 7)	**121.14** ± 8.11 (N = 5)	**37.27** ± 19.84 (N = 15)
	Equus quagga	**16.98** ±1.63 (N = 45)	**441.69** ± 152.37 (N = 10)	**316.18** ± 146.18 (N = 13)	**78.37** ± 73.25 (N = 30)
m3	*H. concudense*	**14.08** ±1.73 (N = 78)	**150.39** ± 45.50 (N = 9)	**95.54** ± 18.87 (N = 12)	**41.30** ± 22.43 (N = 15)
	H. gromovae	**15.37** ±1.78 (N = 93)	**119.09** ± 10.51 (N = 7)	**95.36** ± 14.25 (N = 11)	**43.06** ± 20.59 (N = 15)
	H. periafricanum	**13.72** ±1.70 (N = 36)	**116.35** ± 18.17 (N = 6)	**75.14** ± 9.74 (N = 8)	**43.19** ± 20.36 (N = 12)
	Equus quagga	Cuspal: **18.11** ±1.02 (N = 25) Middle: **17.23** ±1.05 (N = 40)	**199.42** ± 66.11 (N = 8)	**112.44** ± 23.99 (N = 13)	**25.00** ± 18.99 (N = 44)

		Cervical: **15.92** ±1.78 (N = 73)			

TABLE 3

	Species	Tooth	Code	**Crown height** (mm)	**CFT** (days)
m1/m2	*H. concudense*	m1/m2	IPS-91762	**64.47**	**523**
	H. gromovae	m1/m2	IPS-91731	**53.39**	**610**
	H. gromovae	m1/m2	IPS-91732	**49.97**	**511**
	H. periafricanum	m1/m2	IPS-91733	**44.10**	**504**
	H. periafricanum	m1/m2	IPS-91734	**45.29**	**498**
	Equus quagga	m1	Composition	**125.94***	**785***
m3	*H. concudense*	m3	IPS-87855	**57.38**	**701**
	H. gromovae	m3	IPS-85506	**46.71**	**584**
	H. periafricanum	m3	IPS-85504	**41.51**	**591**
	Equus quagga	m3	Composition	**98.71***	**1750***

TABLE 4

Species	Event	Age (months)	Reference
Equus caballus	**First molar eruption**	8-12	(Hoppe et al., 2004)
	Weaning age (feral)	8-9	(Waran et al., 2008)
	Third molar eruption	42-48	(Hoppe et al., 2004)
	Femoral epiphysis fusion	36-42	(Silver, 1969)
	Breeding (Przewalski's horse)	36-48	(Monfort et al., 1994)
Equus quagga	**First molar eruption**	9-12	(Smuts, 1974)
	Weaning age	8-12	(Pluháček et al., 2007)

	Third molar eruption	39	(Smuts, 1974)
	Adult body mass attainment	36	(Smuts, 1975)
	Sexual physiological maturity	42	(Smuts, 1976a)

Highlights

- The life history of Miocene hipparionins is reconstructed using dental histology
- Equid first/second molars grow differently than later-formed third molars
- Hipparionin teeth grew at slower rates and erupt relatively later than in *Equus*
- Tooth growth in hipparions suggests slower pace of growth compared to extant equids
- The analyzed dwarf hipparionins followed slower life histories than larger species

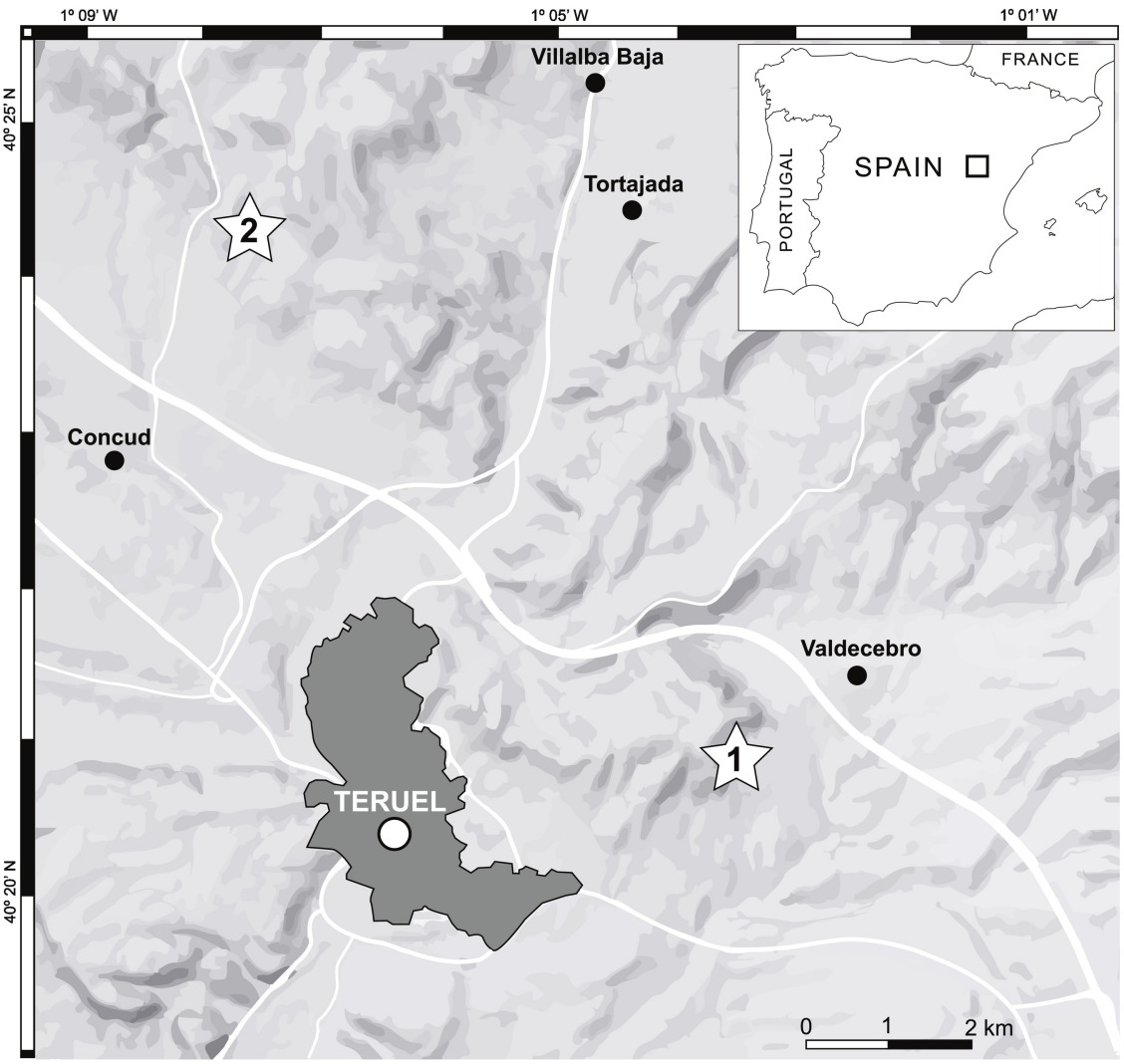

1° 09' W
1° 05' W
1° 01' W
40° 25' N
40° 20' N
Villalba Baja
Tortajada
Concud
Valdecebro
TERUEL
1
2
FRANCE
SPAIN
PORTUGAL
0
1
2 km

Figure 1

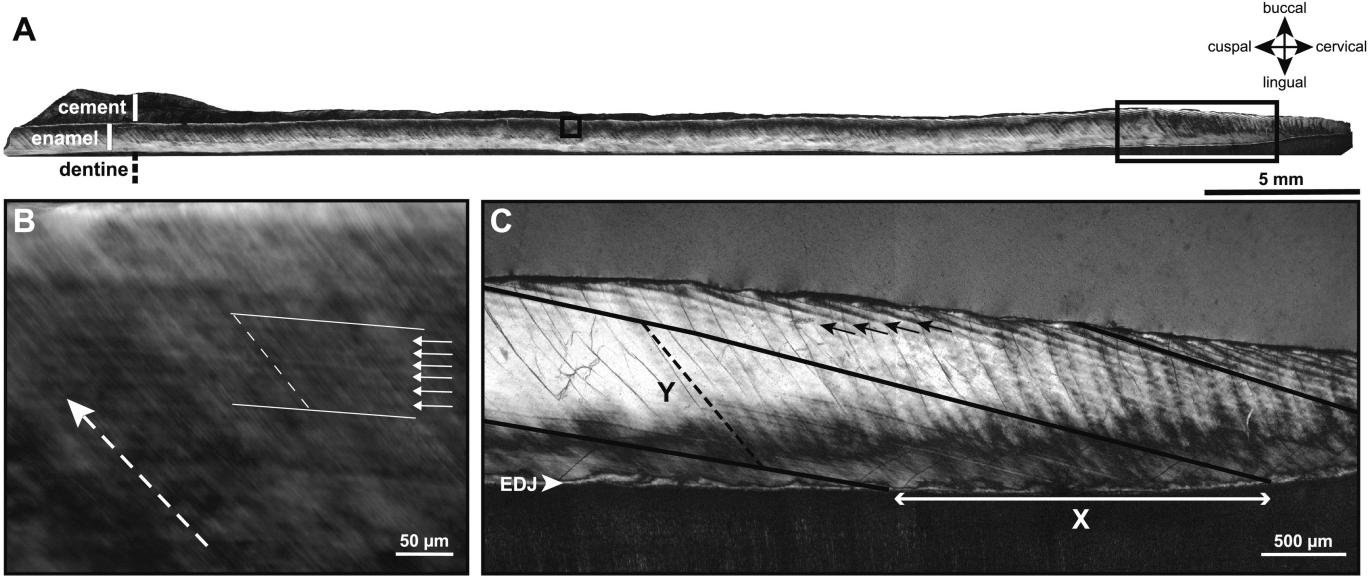
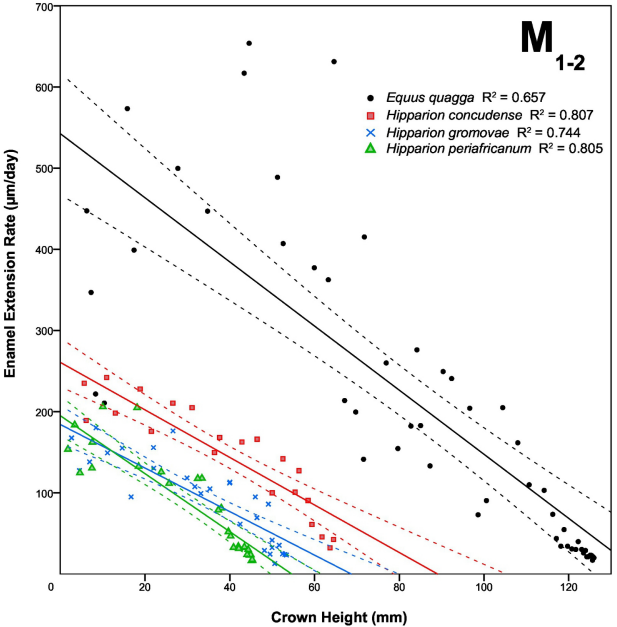

A
buccal
cuspal
cervical
lingual
cement
enamel
dentine
5 mm
B
50 µm
C
Y
EDJ
X
500 µm

Figure 2


M1-2
Equus quagga R² = 0.657
Hipparion concudense R² = 0.807
Hipparion gromovae R² = 0.744
Hipparion periafricanum R² = 0.805
Enamel Extension Rate (µm/day)
Crown Height (mm)
0
20
40
60
80
100
120
100
200
300
400
500
600
700

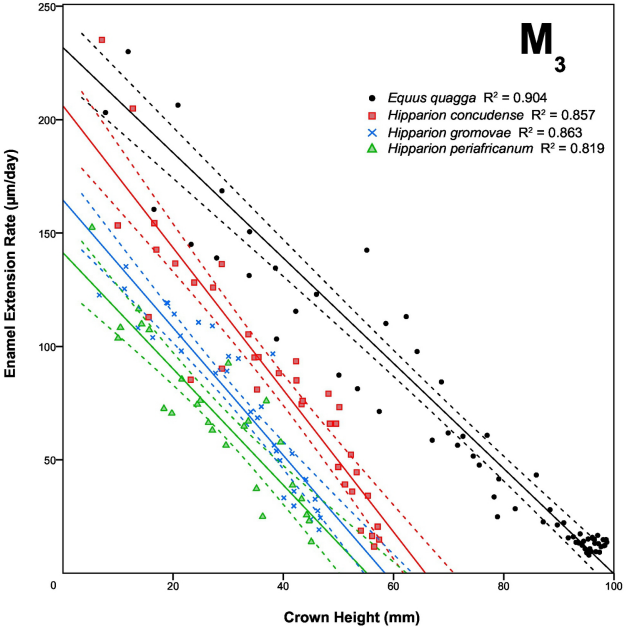

M3
Equus quagga R² = 0.904
Hipparion concudense R² = 0.857
Hipparion gromovae R² = 0.863
Hipparion periafricanum R² = 0.819
Enamel Extension Rate (µm/day)
Crown Height (mm)
0
20
40
60
80
100
50
100
150
200
250

Figure 3

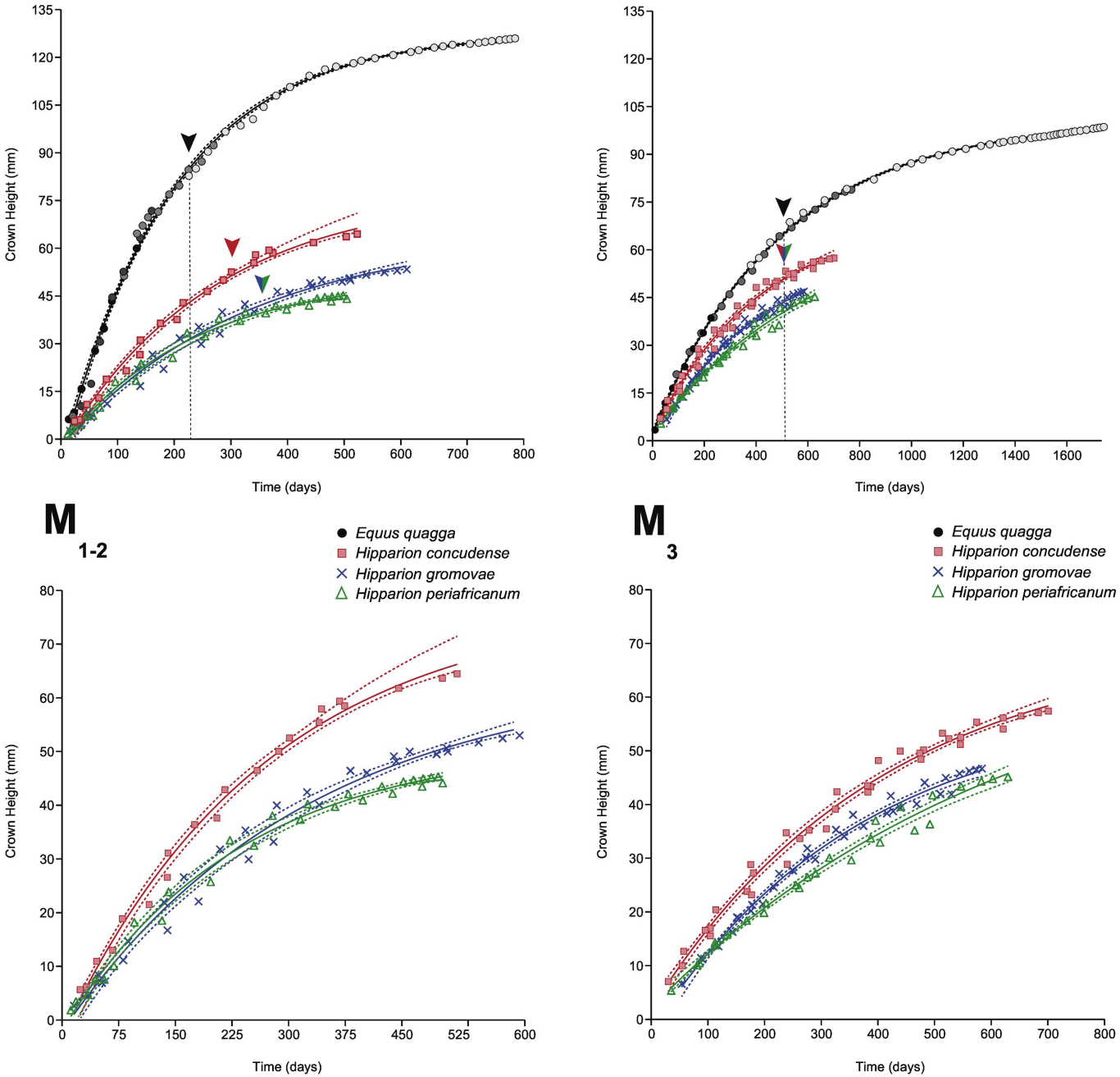

M
1-2
M
3
Equus quagga
Hipparion concudense
Hipparion gromovae
Hipparion periafricanum
Crown Height (mm)
Time (days)

Figure 4

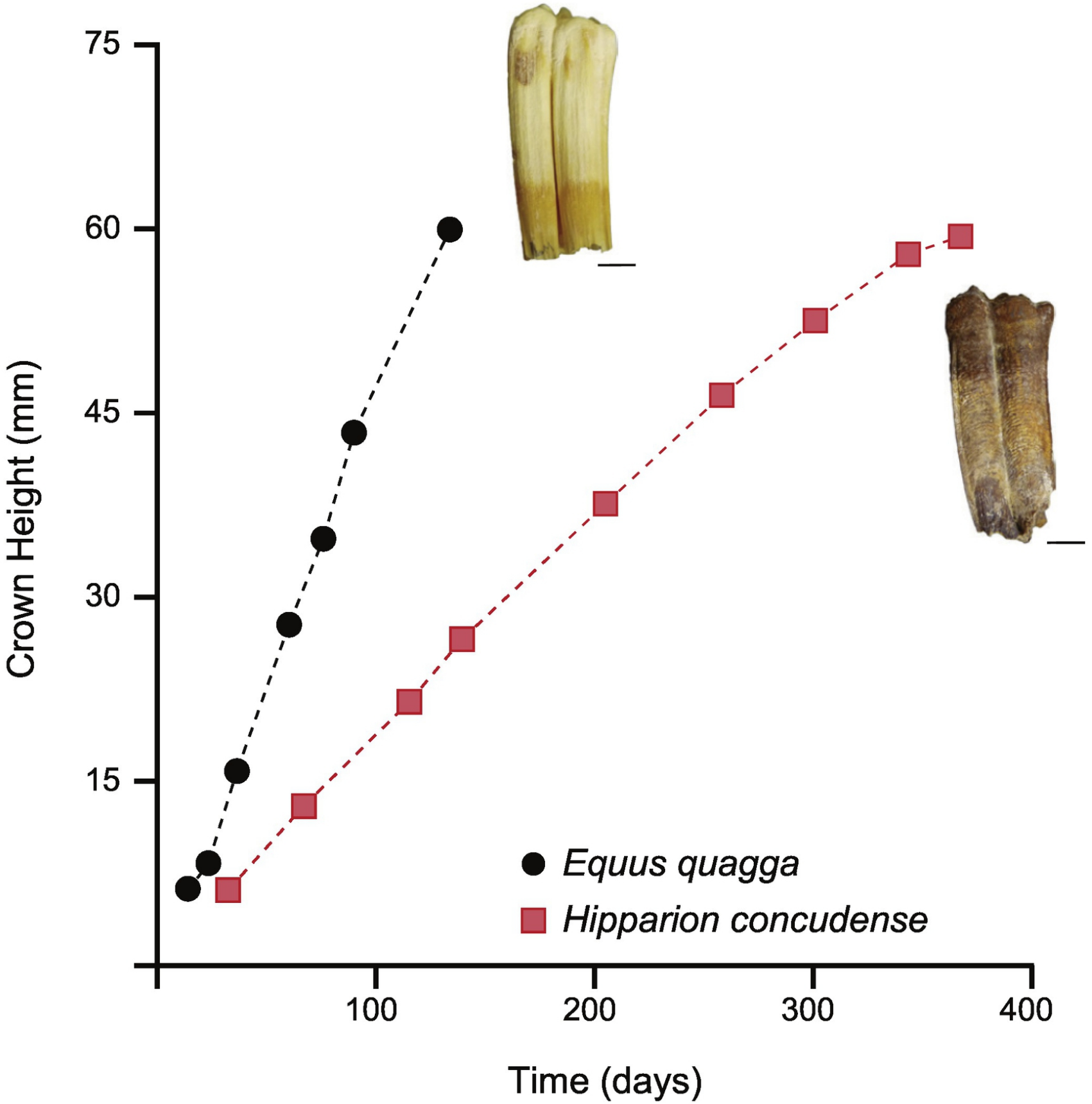

75
60
45
30
15
Crown Height (mm)
100
200
300
400
Time (days)
Equus quagga
Hipparion concudense

Figure 5