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Similarities and differences in the dental tissue proportions of the deciduous and permanent canines of Early and Middle Pleistocene human populations

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Abstract

The two- and three-dimensional assessment of dental tissues has become routine in human taxonomic studies throughout the years. Nonetheless, most of our knowledge of the variability of the enamel and dentine dimensions of the human evolutionary lineage comes from the study of permanent dentition, and particularly from molars. This leads to a biased view of the variability of these features. Due to their early formation and rapid development, the deciduous teeth allow more simplified inferences regarding the processes involved in the dental tissue development of each group. Therefore, their study could be very valuable in dental palaeohistology. In this research, we have explored the dental tissue proportions of the deciduous canines belonging to some human samples of the Early and Middle Pleistocene. The purpose of this was to discuss the meaning of the similarities and differences observed in their histological pattern, as well as to evaluate the degree of covariance with that observed in the permanent dentition of these populations. Our results show that, although there are some similarities in the dental tissue proportions between the deciduous and permanent canines of the study samples, the two dental classes do not provide a similar or comparable pictures of the dental tissue pattern present in the dentition of fossil hominins. Future works on the dental tissue patterns of the anterior and posterior dentition, including deciduous teeth, of fossil samples, may help to shed light on this hypothesis.

KEYWORDS

canines, dentine, enamel, microtomography

1 | INTRODUCTION

Dental anatomy reflects the result of the interaction between genetic inheritance, selective pressure for functional adaptations and other evolutionary processes. That is why the study of human dentition has become a very useful tool in taxonomic and phylogenetic studies. In

recent years, the increase in the number of studies undertaken in the field of dental traits of fossil hominin species is evidence of the enormous interest in dental anthropology among paleoanthropologists (e.g., Bermúdez de Castro, 1988; Martín-Torres et al., 2007, 2012; Smith et al., 2007, 2012; Zanolli et al., 2015). In particular, the two- and three-dimensional assessment of dental tissues in human taxonomic

studies it has become routine (Kono, 2004; Macchiarelli et al., 2013; Martín-Francés et al., 2018; Olejniczak et al., 2008a, 2008b; Skinner et al., 2015; Smith et al., 2012; Zanolli et al., 2015). The investigation of enamel thickness in permanent dentition has provided a vast amount of information about the life history, diet or the behaviour of modern and past populations, but also several authors have employed it to infer the taxonomic identity and phylogenetic relationships of the different species (Grine, 2005; Kono, 2004; Martin, 1983; Plavcan, 2000; Schwartz, 2000; Smith et al., 2005).

Most of our knowledge on the variability of the dental tissue proportions of the human evolutionary lineage derives from the study of the permanent dentition, and especially of the molars (Martín-Francés et al., 2018; Smith et al., 2012; Zanolli, 2014; Zanolli et al., 2015, 2018). These studies show a decreased trend in the enamel thickness of the posterior dentition of the early African representatives of the genus *Homo* (Beynon & Wood, 1986; Pan et al., 2016; Smith et al., 2012) as well as in early Asian *Homo erectus* (Smith et al., 2012; Xing et al., 2014; Zanolli et al., 2015, 2018) relative to Australopithecines (Beynon & Wood, 1986; Olejniczak et al., 2008b; Ramirez Rozzi, 1998; Skinner et al., 2015; Smith et al., 2012). This trend remained in earlier *Homo* species such as Neanderthals, fossil *H. sapiens* and modern humans (Olejniczak et al., 2008a; Smith et al., 2012; Zanolli & Mazurier, 2013). However, evidence extracted from the fossil record suggests that two trends in molar tissue proportions emerged during the transition between the Early and Middle Pleistocene that were maintained in later populations. On the one hand, a thick enamel is present in the posterior dentition of the Early Pleistocene *H. erectus* from Asia, as well as in the Early and Middle Pleistocene populations of Sierra de Atapuerca, a trait shared with later populations of fossil *H. sapiens* and modern human groups (Martín-Francés et al., 2018; Smith et al., 2012; Zanolli, 2014; Zanolli et al., 2015, 2018). Whereas, on the other hand, in *H. neanderthalensis*, there is a clear decrease in the enamel thickness (Kupczik & Hublin, 2010; Macchiarelli et al., 2006; Olejniczak et al., 2008a), a character presents only in the molars of some African Lower Pleistocene specimens from Eritrea (Zanolli et al., 2014).

The dimensions of the enamel and dentine of anterior teeth have been the subject of few studies (Bayle et al., 2009a, 2009b; Buti et al., 2017; García-Campos et al., 2019; Smith et al., 2012; Zanolli et al., 2014), thus precluding a general view of the changes occurring in this feature across hominid phylogeny. A good example of this would be the study carried out by García-Campos and colleagues in 2019. In this research, they analysed the dental tissue proportions of the permanent canines of the Early and Middle Pleistocene populations from the Sierra de Atapuerca (Spain) and the Neanderthal sample from Krapina site (Croatia). These authors could appreciate that the relative enamel thickness (3DRET) of canines belonging to Gran Dolina and Sima de los Huesos dental samples concur with the thinly enameled pattern observed in the Neanderthals (García-Campos et al., 2019). These results differ from those obtained by the same authors on the study of the posterior dentition from these Sierra de Atapuerca populations (Martín-Francés et al., 2018, 2020). This suggests that the evolution of dental tissues of the anterior and

posterior dentition might not evolve in the same way. However, the scarcity of data available in the literature relating to anterior teeth tissue patterns makes it difficult to answer this issue. The study of tissue patterns of other dental classes, in addition to permanent molars, may help to clarify if we can confidently infer the same enamel thickness "category" (thinly or thickly enameled) for hominid anterior teeth based on the measure obtained from their posterior dentition (or vice-versa). In the same way, the study of the deciduous dentition may help to clarify if a predictable deciduous-permanent pattern exists, if so, it is specific to each taxon.

On account of their early formation and rapid development, the deciduous teeth are less eco-sensitive and hence provide a more simplified version of the processes involved in the histological pattern of each group. Therefore, their study might be vital for the field of dental paleohistology. The proportion in which the different tissues are expressed in the human dentition is the result of a complex interaction of genetic, epigenetic and environmental factors (Brook, 2009). This interaction can occur at the molecular, cellular or even extracellular level, generating diverse macroscopic results that are reflected in the external and internal anatomy of the dentition of each group. However, each factor's possible effect on the dental tissues will depend on the moment the formation occurs in each dental class. The life cycle of dental organs commences in the fifth week of gestation, with the beginning of the formation of the primary dentition. Specifically, the development of the majority of deciduous teeth begins with the calcification of their dental crowns between the 13th and 16th weeks after conception and approximately ending three years later (Irish & Scott, 2018). In contrast, the mineralization of permanent teeth is entirely postnatal (from birth to 10 years of age), with each tooth taking between 9 and 25 years to form (Irish & Scott, 2018). As a result, the deciduous teeth are believed to have stronger genetic control than the permanent dentition, along with the fact that they are less influenced by variations in the environment or hormone levels variations (Hillson, 1996). Therefore, the study of primary teeth might simplify the interpretation of the tissue patterns observed in the dentition of the different taxa.

Despite these advantages, the assessment of the enamel and dentine dimensions of deciduous teeth, as well as their degree of covariance with those of permanent dentition, has received little attention (e.g., Bayle et al., 2010; Crevecoeur et al., 2010; Macchiarelli et al., 2006; Zanolli et al., 2010, 2012, 2014, 2017a). This leads to the tendency to assume that the patterns present in the permanent dentition can be extrapolated to the deciduous dentition. In a preliminary study carried out by Zanolli et al. in 2017, the degree of parallelism in the dental tissue proportions between the lower dm2 and M1 was examined in different hominid taxa. Although the results of this study were inconclusive, it proposed a new analytical tool: the "enamel thickness diphodontic index" (ETDI). In addition, these authors highlighted the potential of comparative studies on the covariance of traits between the permanent and deciduous dentition.

In this study, we have employed the technique of computed microtomography (micro-CT) to explore the dental tissue dimensions of the deciduous canines belonging to some human samples of the

Early and Middle Pleistocene. Similarly, in this research, we have also analysed two extensive collections of permanent and deciduous teeth belonging to present-day humans of European descent. Although the available sample of deciduous anterior dentition in the human fossil record is scarce, this study aims to contribute to fill the knowledge gap on the tissue pattern present in these dental classes. Our study was focused on discussing the meaning of the possible similarities and differences in the dental tissue proportions of the deciduous canines among the samples examined and on attempting to assess the degree of covariance between the permanent and deciduous dentitions.

2 | MATERIALS AND METHODS

For this study, permanent and deciduous canines (maxillary and mandibular) were analysed from several fossil samples of the Early and Middle Pleistocene, as well as from two modern human samples of European origin (Table 1). A description of each of the study samples is included below, differentiating the fossil samples from the modern human samples.

A total of 47 permanent and 11 deciduous fossil *Homo* teeth were studied here (Table 1). The sample comprises approximately one million years of hominid evolution, including collections from Spain, Portugal, Croatia, France, Belgium, Israel and Morocco.

The specimen of fossils contained members of *H. antecessor* species (HA, MNI = 93), individuals from Sima de los Huesos site (SH, MNI = 16), Neanderthals (NEA, MNI = 18) and fossil modern humans (FMH, MNI = 2). Even when the dimensions of the dental tissues of some of these teeth are available in the literature (Bayle et al., 2009a, 2009b; Bayle et al., 2010; Crevecoeur et al., 2010), to standardize measurement protocols, maximize the number of variables available for analysis and enable subsequent comparison, these permanent and deciduous canines were personally measured by the authors as is described below. Additionally, we included in our analysis the data of the permanent canines of the taxonomically attributed as fossil modern humans of Nahal-Oren (Israel), Hayonim (Israel), Dar es Soltane II (Morocco), Jebel Qafzeh (Israel) and Jebel Irhoud (Morocco) published by Buti et al. (2017) and Smith et al. (2012), who employed similar protocols to those are used here.

This work also comprises a recent modern humans sample (RMH) composed of 51 permanent and 72 deciduous dental canines belonging to individuals of European origin. This material comes from the anthropological collection of the Escuela de Medicina Legal de Madrid (Spain) (see García Campos, 2020; García-Campos et al., 2018a, 2018b, 2019, 2020) and the Ratón Pérez Collection stored at the Centro Nacional de Investigación sobre la Evolución Humana (see Martínez de Pinillos et al., 2021). A total of 101 individuals are represented in our study sample.

TABLE 1 Permanent and deciduous canines of fossil and extant hominins used in the investigation of 2D and 3D enamel thickness

Species	Site/Collection	N	Country	Reference
Permanent canines				
HA	Gran Dolina	3	Spain	García-Campos et al. (2019)
SH	Sima de los Huesos	21	Spain	García-Campos et al. (2019)
NEA	Krapina	11	Croatia	García-Campos et al. (2019)
FMH	Nahal-Oren	4	Israel	Buti et al. (2017)
	Hayonim	2	Israel	Buti et al. (2017)
	Dar es Soltane II	2	Morocco	Smith et al. (2012)
	Jebel Qafzeh,	3	Israel	Smith et al. (2012)
	Jebel Irhoud	1	Morocco	Smith et al. (2012)
RMH	Escuela de Medicina Legal de Madrid	51	Spain	García-Campos et al. (2019)
Deciduous canines				
HA	Gran Dolina	1	Spain	This study
SH	Sima de los Huesos	1	Spain	This study
NEA	L'Abri Suard	1	France	This study
	Roc de Marsal	2	France	This study
	Spy Cave	1	Belgium	This study
	Engis	1	Belgium	This study
	Abri Bourgeois-Delaunay	1	France	This study
FMH	Tossal de la Font	1	Spain	This study
	Lagar Velho	1	Portugal	This study
	La Madeleine	1	France	This study
RMH	El Ratón Pérez Collection	72	Spain	This study

Abbreviations: FMH, fossil recent modern human; HA, *H. antecessor*; NEA, *H. neanderthalensis*; RMH, recent modern humans; SH, Sima de los Huesos.

In some cases, a maxillary and a mandible canine from the same individual were measured, although only one antimeric per individual was taken into consideration. We discarded pieces with pathological damage that could affect the mineralized tissues. As a means of scoring the wear stages of the samples, the Molnar (1971) technique was followed. Only slightly worn teeth were included in this work, being those which a wear stage below or similar to 3, characterized by the absence of the apex in the incisal border, and where instead there is a point of dentine. Finally, the modern human samples were designed to obtain a similar representation of both sexes.

Fossil dental samples of Gran Dolina, Sima de los Huesos and Tossal de la Font were scanned using the Scanco Medical AG Micro-Computed Tomography 80, housed at the Centro Nacional de Investigación sobre la Evolución Humana (CENIEH) in Burgos (Spain). All scans were performed employing two 0.1 mm copper filters and applying the following parameters: voltage and amperage of 70 kV and 114 μ A, respectively. The resultant slice thickness ranged between 18 and 36 micrometers (μ m). Teeth included in mandibular or maxillary fragments were scanned using a Phoenix v/tome/x s (GE Measurement & Control), found in the same research centre. In this case, scans were performed with two 0.1 mm copper filters, 100–120 kV voltage and 110–140 μ A amperage, with the resulting isometric voxel ranging between 27 and 36 μ m. Synchrotron micro-CT data of the Krapina, L'Abri Suard, Roc de Marsal, Spy Cave, Engis, Abri Bourgeois-Delaunay, Lagar Velho and La Madeleine upper and lower canine teeth were obtained from NESPOS[®] microtomographic database (voxel size: 20–40 μ m). The modern human sample was scanned using a Phoenix v/tome/x s (GE Measurement and Control) microtomographic system held at the CENIEH and the CTP-Mlab microCT located in the Multidisciplinary Laboratory of the International Centre for Theoretical Physics (ICTP) of Trieste (Italy) (Tuniz et al., 2013). All scans were performed with two 0.1 mm copper filters, 100–120 kV voltage and 110–140 μ A amperage. The output images had a voxel size ranging between 17 and 21 μ m.

Subsequently, the image edition processing was performed using the Amira 6.0.0 software (Visage Imaging, Inc.). Dental tissues (enamel, dentine-pulp complex) were semi-automatically segmented using the Watershed Segmentation Tool and through manual editing. A Non-Local Means Filter was also applied. Small fractures and cracks were virtually filled in. Once the images were segmented and the different dental tissues separated, the dimensions of the enamel and dentine were then measured as follows.

In order to assess the histological crown area from a buccolingual plane, a virtual cross-section for each tooth was obtained following the methodology outlined in Benazzi et al. (2014) (Figure 1b). As some canines presented occlusal wear, crown reconstruction was carried out using open-source software (GIMP 2.8) and following the protocol proposed by Saunders et al. (2007) (Figure 1c). Lastly, 2D tissue measurements and indices described by Martin in 1983 (in detail in Table S1 and in Figure 1) were obtained using ImageJ software (v. 1.49i). The variables included in the study are: enamel cap area (Ae, mm²), coronal dentine and

pulp area (Acdp, mm²), the length of the enamel-dentine junction (EDJS, mm) and the bicervical diameter (BCD, mm). Subsequently, these variables were employed to compute the 2D average enamel thickness index (2DAET, mm); the 2D relative enamel thickness index (2DRET, scale free) and the relative coronal dentine and pulp complex area (Acdp/Ac, percentage scale). We also assessed the absolute crown strength (ACS, scale free) proposed by Schwartz et al. (2020).

As for evaluating the three-dimensional (3D) tissue proportions, we measured the variables and indices described by Olejniczak et al. (2008a, b). Before that, we isolated the crown from the root in each dental piece, considering the cervical line as the fundamental morphological feature to establish the lower limit between both structures (see García-Campos et al., 2018a, 2018b, 2019, 2020, and Figure 1e). We quantified the 3D variables described in detail in Table S2 and in Figure 1f. The variables included in the study are: the coronal volume (Vc, mm³), the volume of the enamel cap (Ve, mm³), the volume of the coronal dentine including the coronal pulp (Vcdp, mm³) and the surface area of the enamel-dentine junction (EDJS, mm²). Subsequently, these variables were employed to compute the 3D average enamel thickness index (3DAET, mm); the 3D relative enamel thickness index (3DRET, scale free) and the relative coronal dentine and pulp complex volume (Vcdp/Vc, percentage scale). Likewise, the ratio of enamel-thickness to dentine-thickness (3DRED, scale free) proposed by Yi et al. (2021).

Statistical analyses were performed using the SPSS software (v. 18.0, SPSS Science, Inc.). We could not apply a comparative statistical analysis to determine the magnitude of the differences described on account of the sample size. Instead of that, to evaluate the differences between the dental tissue patterns of the deciduous canines a box and whisker diagrams were created considering the data obtained in this study from the maxillary and mandibular canines of HA, SH, NEA and RMH samples. Likewise the diagrams included the data of the FMH permanent canines published by Buti et al. (2017) and Smith et al. (2012). Similarly, to evaluate possible similarities or differences in enamel thickness distribution patterns in deciduous canines of the different taxa, 3D enamel distribution maps were created. These maps were obtained by calculating the distance between two triangulated surfaces, the enamel-dentine junction and the outer enamel surfaces. For each vertex of one surface the closest point of the other surface was computed, and the results were visualized by spectral colours, with thicker enamel values (greater distance between surfaces) represented in red and thinner ones (smaller distance) in blue.

To assess the possible similarities and differences in the dental tissue proportions of the deciduous canines among the samples examined, Principal Component Analysis (PCA) was employed, with absolute and relative variables, and 2D and 3D measurements assessed, separately. The indices ACS and 3DRED were not included in the PCA to maximize the number of specimens investigated. Once the PCA results were obtained, the centroids of each group were located.

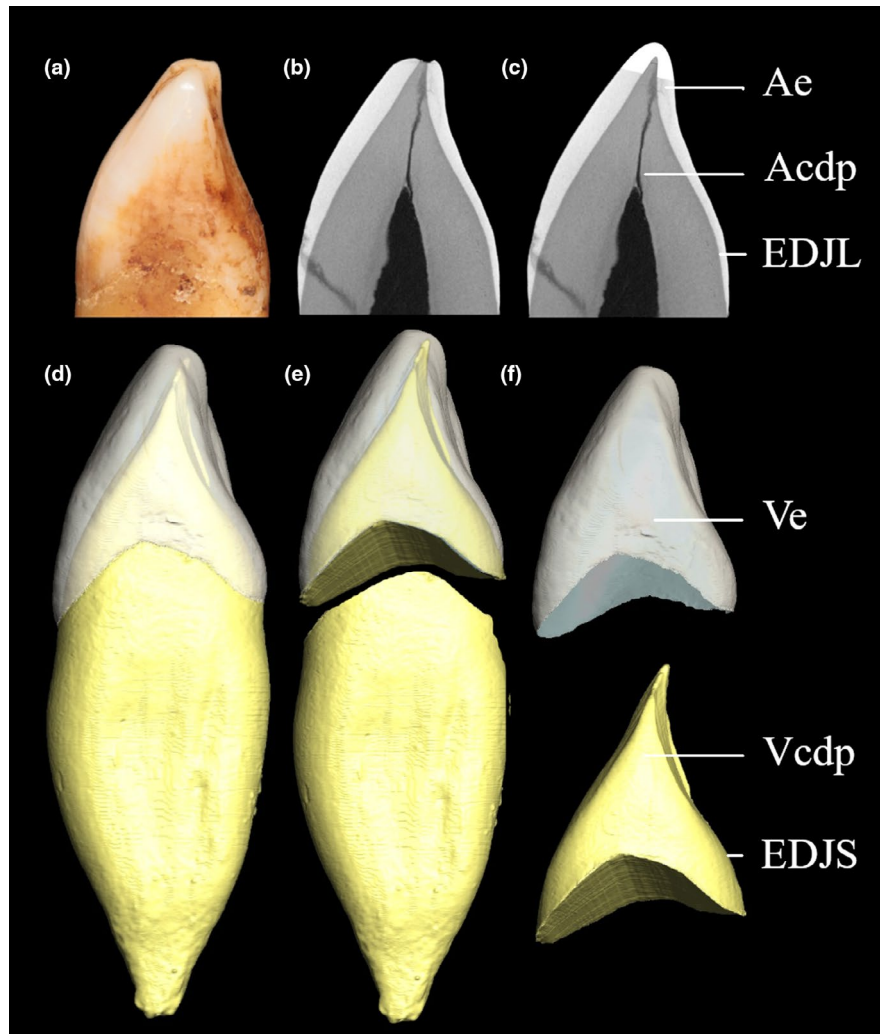


FIGURE 1 (a) Mesial view of an individual lower canine included in the study sample. (b) The virtual cross-sections were obtained following the method of Benazzi et al. (2014) and the crown reconstruction (c) was performed by applying the Saunders et al. (2007) methodology. In the virtual volumetric model (d), the crown was isolated from the root following the protocol described by García-Campos et al. (2019) (e). In that way, only the dentine and pulp contained in the enamel cap are considered as part of the coronal dentine-pulp complex (f). Ae: enamel cap area; Acdp: coronal dentine and pulp area; EDJL: length of the EDJ; Ve: volume of the enamel cap; Vcdp: coronal dentine and pulp volume; EDJS: EDJ surface area

To attempt to evaluate the degree of similarity between permanent and deciduous dentition tissue pattern, following Zanolli et al. (2017a) the “enamel thickness diphodontic index” (ETDI) was calculated as: μ_c/μ_C . To do that, the mean values of the indices 2DAET, 3DRET and ACS, as well as those of the indices 3DAET, 3DRET and 3DRED were calculated.

Finally, in order to estimate the intra- and inter-observer error, a subsample of seven teeth was analysed by two researchers (C.G.C. and M.M.M.). Each tooth was measured three times by both observers, who independently orientated, segmented and isolated each specimen, and assessed each variable and index following the methods described above. The degree of error was estimated by calculating the inter- and intra-observer mean differences for each crown component measurement. The average intra-observer error was 1.59% (0.69%–2.48%), whereas the average inter-observer error was 2.43% (1.72%–3.15%). All values are within the accepted range.

3 | RESULTS

3.1 | Dental tissue proportions of the deciduous canines

The box and whisker diagrams that compare the values of the different enamel thickness indices and those of the relative dimensions of the crown dentine-pulp complex obtained from the deciduous canines of the study samples are shown in Figures 2 and 3. Correspondingly, the mean and standard deviation of each of the variables are shown in Tables S3–S6.

As can be observed in Figures 2 and 3, the maxillary and mandibular deciduous canines of *H. neanderthalensis* (NEA) present a 2-D and 3-D enamel thickness whose mean values are lower than those observed in modern human populations (RMH). These differences are visible in both the mean enamel thickness (2DAET,

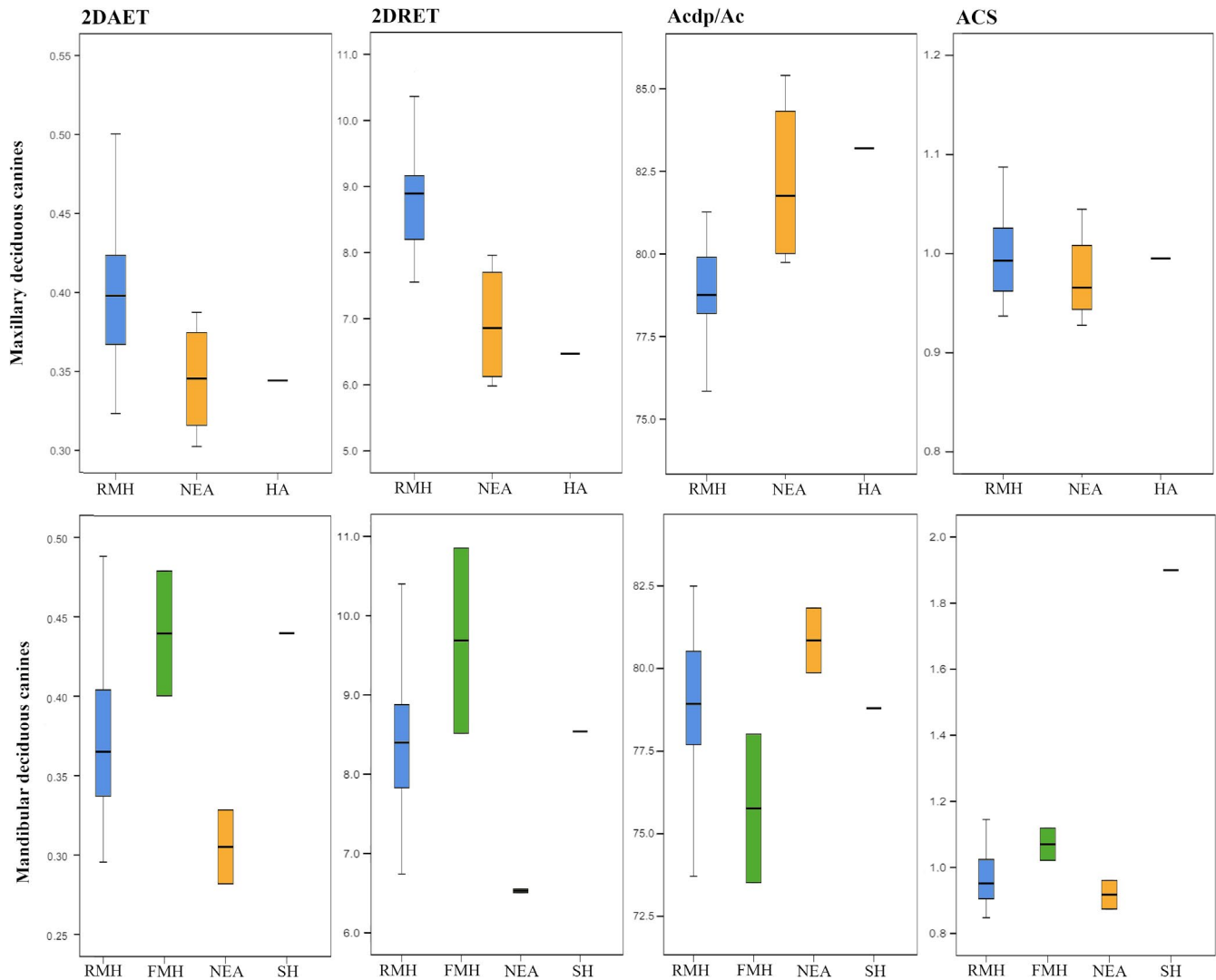


FIGURE 2 Standard box and whisker plot of the deciduous canine 2D tissue proportions (wear stages 1–3). These plots display the interquartile range (25th–75th percentiles: boxes), 1.5 interquartile ranges (whiskers) and the median values (black line). In the graph the following subsamples are represented: recent modern humans (RMH, in blue), fossil recent modern human (FMH, in green), *H. neanderthalensis* (NEA, in orange), Sima de los Huesos (SH) and *H. antecessor* (HA)

3DAET) and the relative enamel thickness (2DRET, 3DRET) as well as in the ratio of enamel-thickness to dentine-thickness (3DRED). However, they are not reflected in the absolute crown strength index (ACS). In contrast, the crown dentine-pulp complex of the primary dentition of Neanderthals shows relatively higher mean values (Acdp/Ac, Vcdp/Vc) than those observed in recent modern humans. These differences between *H. neanderthalensis* and modern humans could already be appreciated even in the deciduous canines of the earliest representatives of *H. sapiens*. The sample of deciduous canines of fossil modern humans (FMH) is represented by two specimens: LaVe1 from Lagar Velho (Portugal) and Mad4 from La Madeleine (France). The tissue pattern of these teeth is characterized by 2-D and 3-D enamel thickness mean values (2DAET, 3DAET, 2DRET, 3DRET, 3DRED) higher than those present in European modern populations. Likewise, their crown dentine-pulp complex (Acdp/

Ac, Vcdp/Vc) has relatively smaller dimensions than that of the modern groups.

In this study, we also analysed the Early and Middle Pleistocene samples from the Sierra de Atapuerca. The Sima de los Huesos dental sample, is represented by the deciduous mandibular canine AT-90, which has a wear degree 4 (Molnar, 1971) with an oblique wear platform. This allowed us to evaluate the 2D variables of this tooth, although not the 3D ones. The values attained for this deciduous piece show a 2D tissue pattern (2DAET, 2DRET, Acdp/Ac) closer to that observed in the RMH sample of European origin. It highlights the considerable value of the ACS index obtained for this tooth, which is greater than that obtained in the remaining samples analysed. On the one hand, discrepancies are observed between the values from the 2D and 3D measurements obtained from the deciduous maxillary canine found in level TD6-2 from Gran Dolina (ATD6-14). While the 2D enamel thickness values (2DAET and 2DRET), as

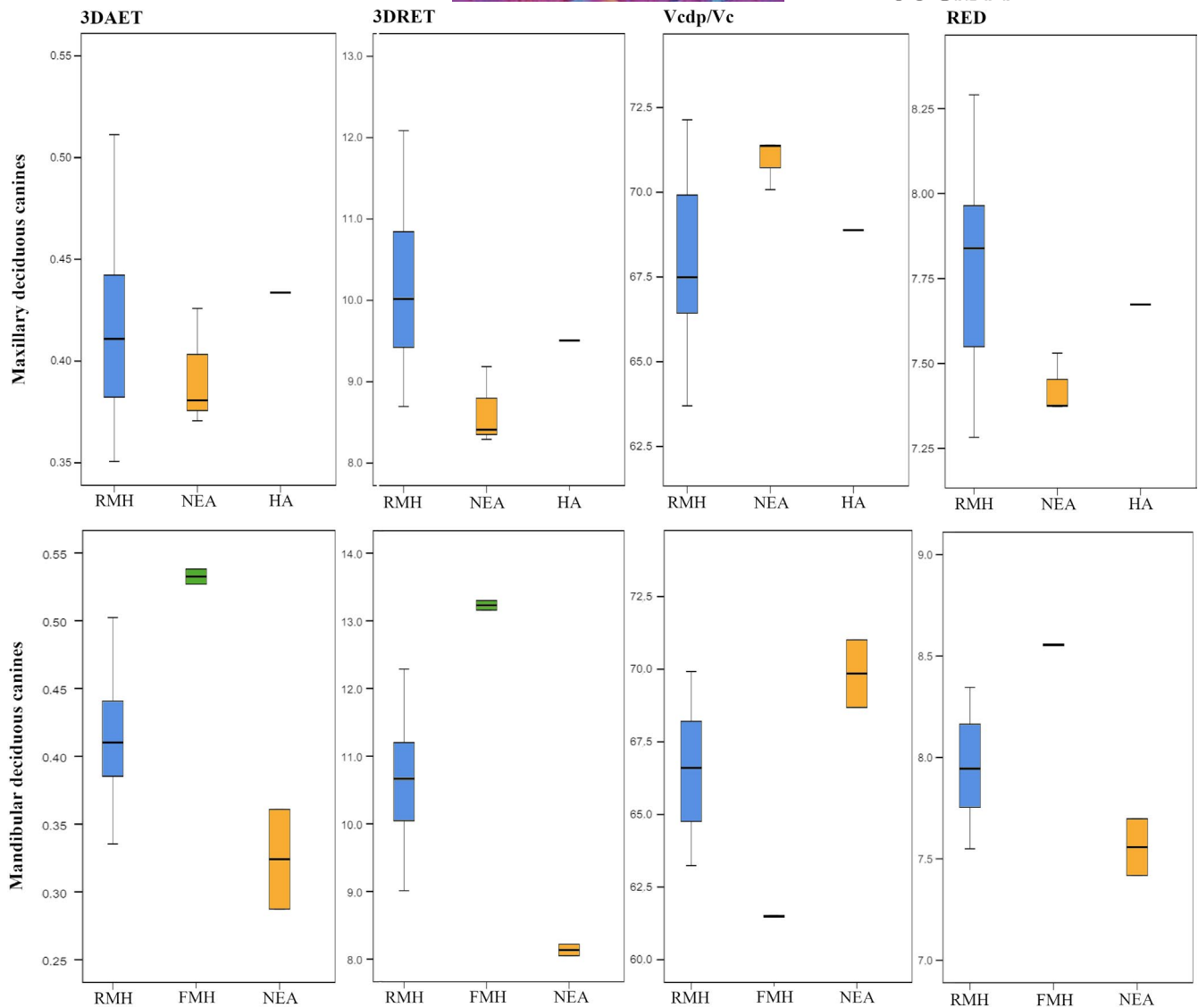


FIGURE 3 Standard box and whisker plot of the deciduous canine 3D tissue proportions (wear stages 1–3). For more information see the caption of the Figure 2

well as that of the $Acdp/Ac$ index, match the pattern observed in *H. neanderthalensis*, the values obtained from the 3D indices (3DRET, 3DRED, $Vcdp/Vc$) are located in an intermediate position between Neanderthals and the recent modern humans. In the case of the 3DAET index, the value acquired in ATD6-14 is even within the range of variation of RMH.

Lastly, the 3D enamel thickness distribution maps are shown in Figures 4 and 5, which reflect patterns of enamel distribution and in which the thickest enamel is represented in red and the thinnest in blue. Overall, these maps show that the enamel cap is thicker on the buccal aspect in both maxillary and mandibular canines in all populations examined. Specifically, it is thicker on the cuspal half of the crown, whereas in the cervical portion it is relatively thinner. Fossil and recent modern human canines show the highest values of all the study samples and present an additional thickening of the enamel in the mesial and distal aspects. While on the other hand, Neanderthals displays the lowest

enamel thickness figures among the fossil samples. Lastly, the HA and SH teeth display an intermediate pattern that is closer to that of *H. sapiens*.

3.2 | Similarities and differences between the pattern observed in the permanent and deciduous canines

The outcome of the principal component analyses performed on the sample of permanent canines (A and C) and deciduous canines (B and D) can be found in Figures 6 and 7. Graphs A and B represent the results obtained from the analyses of the absolute variables (PCAabs): Ae, $Acdp$ and EDJL (Figure 6); Ve, $Vcdp$ and EDJS (Figure 7). Whereas Graphs B and D show the results obtained from the analyses of the relative variables (PCAre): 2DAET, 2DRET and $Acdp/Ac$ (Figure 6); 3DAET, 3DRET and $Vcdp/Vc$ (Figure 7).

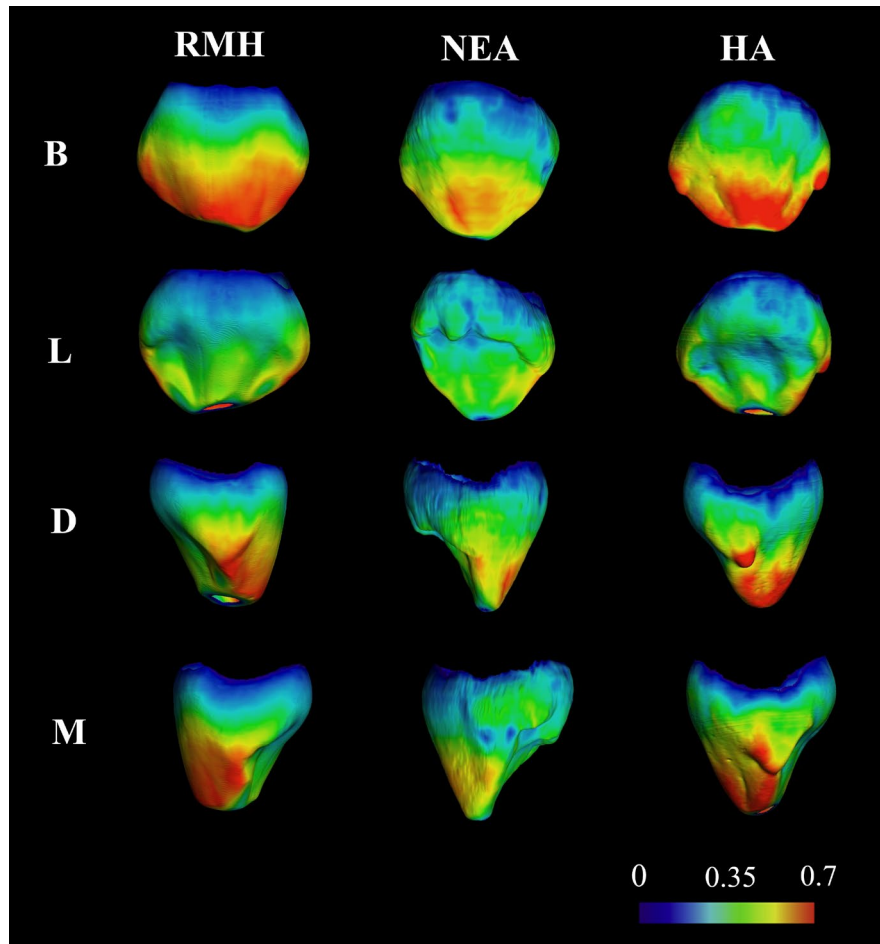


FIGURE 4 3D enamel thickness distribution maps of upper canines (wear stages 1–3). This figure represents the 3D maps obtained through the computing of the distance between two triangulated surfaces (EDJS and OES) in which, for each vertex of one surface, the closest point on the other surface is computed. The results are visualized using spectral colours, where the largest distance (thickest enamel) is represented in red and the shortest (thinner enamel) in blue. Colour scale: 0–0.7 mm. From left to right the subsample represented comprises: RMH (RP36); NEA, (Roc de Marsal 1); and HA (ATD6–14). All the teeth represented are left canines; right canines have been mirrored. Views: buccal (B), lingual (L), distal (D) and mesial (M)

The results of the principal component analysis performed on the sample of deciduous canines provided similar results to those of the univariate descriptive statistics. When only the absolute variables were used in the analysis (PCAabs), a large part of the variability present in the sample analysed was explained by the first two components (2-D variables: 92.36%; 3-D variables: 98.13%). The PCAabs show two point clouds with a low degree of overlap that defines the RMH and NEA samples (Figures 6b and 7b). The point cloud representing Neanderthal deciduous canines is located in the lower right quadrant due to their larger tooth size, especially that of their dentine-pulp complex (see Table 2). On the one hand, the PCAabs places the FMHs close to the point cloud of the RMHs or at its upper right limit (Figures 6b and 7b). It shows that FMH deciduous teeth are larger than those of recent humans, particularly in the size of their enamel component. On the other hand, PCAabs locates the deciduous canine found at Sima de los Huesos within the upper right quadrant, at an intermediate point between the RMH and NEA point clouds (Figure 6b). Finally, the deciduous canine ATD-14 is grouped

in the lower right quadrant together with the Neanderthal sample (Figures 6b and 7b), and even fall within the *H. neanderthalensis* point cloud in the case of 2-D measurements (Figure 6b).

The relative dimensions of dental tissues (PCAreI) were able to explain a higher percentage of the variability observed within the deciduous dental sample of the PCA (2-D variables: 98.69%; 3-D variables: 99.03%) than the absolute variables. If we control for the effect of tooth size, the overlap between the main two point clouds (RMH, NEA) increases (Figures 6b and 7b). In this case, the NEA deciduous canines appear to be located in the upper left quadrant due to their smaller enamel thickness values (2DAET, 3DAET, 2DRET and 3DRET) and larger relative dentine component size (Acdp/Ac, Vcdp/Vc). As in the PCAabs, the FMHs deciduous canines were placed close to the RMH point cloud (Figures 6b and 7b), although shifted to the right quadrant, indicating the presence of greater enamel thickness (Table 2). On the other hand, the 2-D histological pattern of the canine of AT-90 was placed in the top left quadrant, at the utmost limit of the point cloud of the RMH sample (Figure 6d). Finally, the

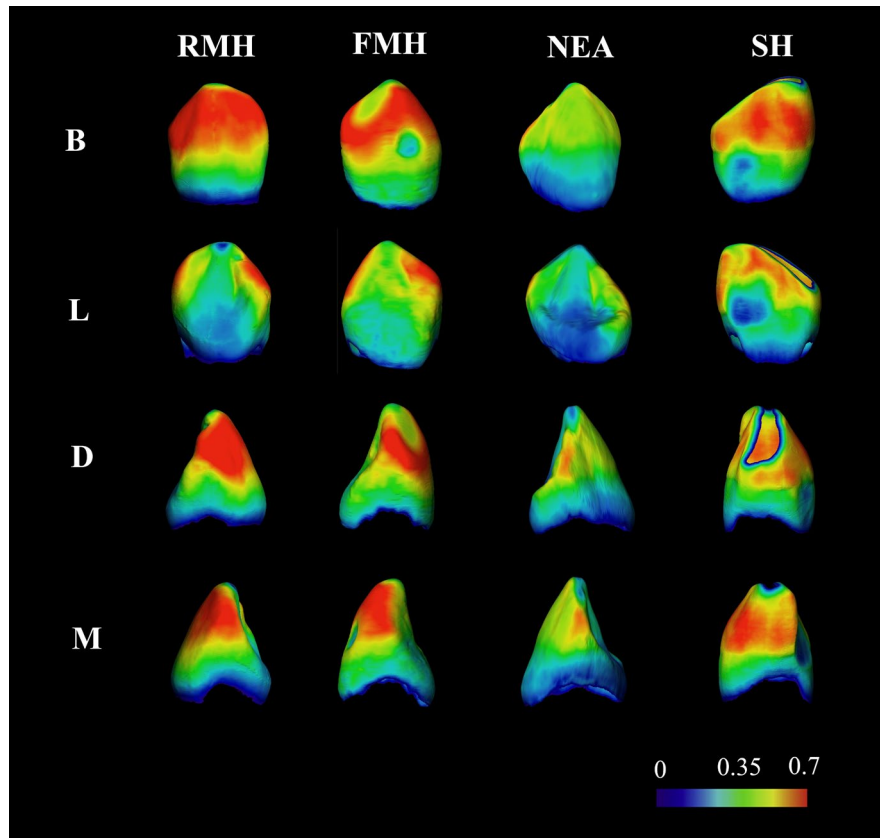


FIGURE 5 3D enamel thickness distribution maps of lower canines (wear stages 1–3). This figure represents the 3D maps obtained through the computing of the distance between two triangulated surfaces (EDJS and OES) in which, for each vertex of one surface, the closest point on the other surface is computed. The results are visualized using spectral colours, where the largest distance (thickest enamel) is represented in red and the shortest (thinner enamel) in blue. Colour scale: 0–0.7 mm. The subsample represented comprises from left to right: RMH (RP31); FMH (La Madeleine 4); NEA (S45) and SH (AT-90). All the teeth represented are left canines; right canines have been mirrored. Views: buccal (B), lingual (L), distal (D) and mesial (M)

2-D measurements (Figure 6d) located the HA deciduous canine in the upper left quadrant of the PCA, next to the NEA point cloud, although with higher values for the second component. However, when considering the 3-D variables (Figure 7d), ATD6-14 was placed in the upper right quadrant, at the upper limit of the point cloud of RMHs.

The results found in the enamel thickness diphodont index (ETDI) assessed with the 2DAET, 2DRET, ACS, 3DAET, 3DRET and 3DRED values (Table 3) show that samples had a lower range of variation for the 3-D indices than the 2-D ones. The 2DAET-ETDI has values that range between 0.45 and 0.5 in the maxillary canines, and between 0.43 and 0.67 in the mandibular ones, whereas the 3DAET-ETDI values span between 0.44 and 0.50 in the maxillary canines and between 0.54 and 0.55 in the mandibular canines. The same results are found in the RET-ETDI (maxillary canines, 2DRET-ETDI: 0.59–0.70 and 3DRET-ETDI: 0.60–0.63; mandibular canines, 2DRET-ETDI: 0.61–0.95 and 3DRET-ETDI: 0.57–0.59). Equivalently, while ACS-ETDI has a broader variation range in the maxillary canines (0.48–0.58) and narrower in the mandibular ones (0.54–0.55), the 3DRED-ETDI values are very similar in all groups (maxillary canines: 0.83–0.85; mandibular canines: 0.82–0.85).

4 | DISCUSSION

Despite the potential usefulness of studying the deciduous dentition, the variation in dental tissue proportions of the extinct and extant hominin primary teeth has hardly been studied (e.g., Bayle et al., 2010; Crevecoeur et al., 2010; Macchiarelli et al., 2006; Zanolli et al., 2010, 2012, 2014, 2017a). In 2017, Dr. Clément Zanolli and colleagues highlighted this fact in a preliminary study that aimed to assess the degree of parallelism existing in tissue proportions between lower dm2 and M1s belonging to different hominin taxa. Although this study did not obtain conclusive results, it proposed a new analytical tool: the enamel thickness diphodontic index, and highlighted the potential of comparative studies on the covariance of traits present in the permanent and deciduous dentition (Zanolli et al., 2017a). In the present study, deciduous and permanent canines belonging to the fossil populations of Gran Dolina (HA) and Sima de los Huesos (SH), as well as to the members of the taxa of *H. neanderthalensis* (NEA), fossil modern humans (FMH) and recent modern humans (RMH), were analysed and compared. The dental tissue proportions of each group were evaluated using the computerized microtomographic techniques at both two-dimensional (areas and lengths) and three-dimensional (volumes and surfaces) level.

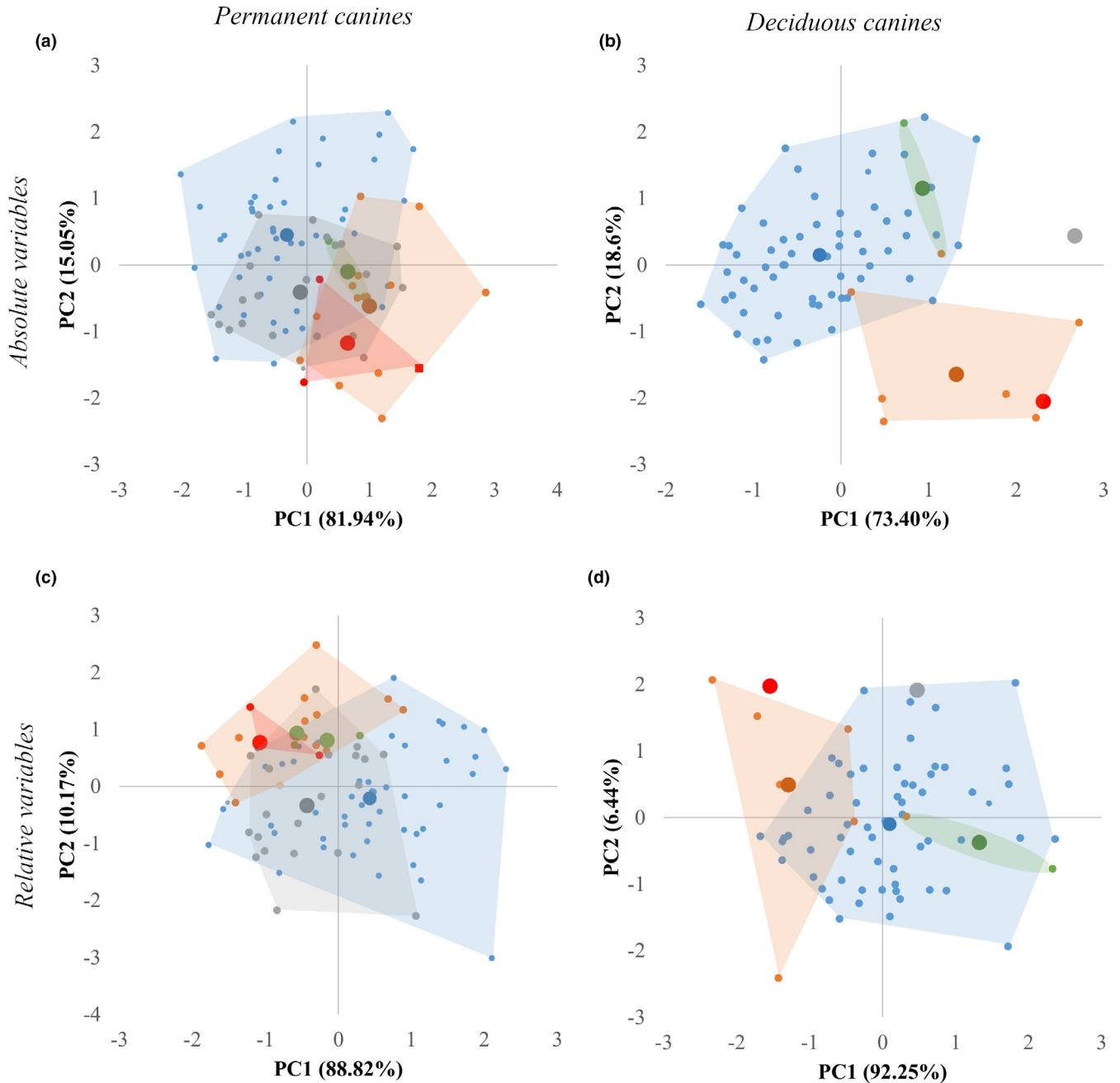


FIGURE 6 Principal Component Analysis (PCA) applied to the 2D dental tissue proportions of the permanent and deciduous canines (maxillary and mandibular) from *Gran Dolina* (in red), *Sima de los Huesos* (in grey), *H. neanderthalensis* (in orange), fossil modern human (in green) and the modern human (in blue) samples. Each scatter shows the first two components of the PCA (PC1 and PC2). The centroid of each subsample is represented by a bigger point. In each PCA the following variables are represented: (a) the absolute variables measured on the permanent canines; (b) the absolute variables measured on the deciduous canines; (c) the relative variables measured on the permanent canines; and (d) the relative variables measured on the deciduous canines

Various indices on enamel thickness were also assessed, including the traditional indices proposed by Martin in 1983 and adapted by Olejnickzak et al. in 2008a, as well as the recently described ACS (Schwartz et al., 2020) and 3DRED (Yi et al., 2021) indices. The aim was to describe the possible similarities and differences present in the histological pattern of the deciduous canines of each of the samples analysed, as well as to evaluate the degree of covariance between deciduous and permanent dentitions.

4.1 | Tissue pattern of deciduous canines and its similarities and differences with that of permanent teeth

Enamel thickness has been widely employed in paleoanthropology to infer the taxonomic assignment and the phylogenetic relationships between different fossil populations (e.g., García-Campos et al., 2019; Olejnickzak et al., 2008a; Smith et al., 2012). Numerous

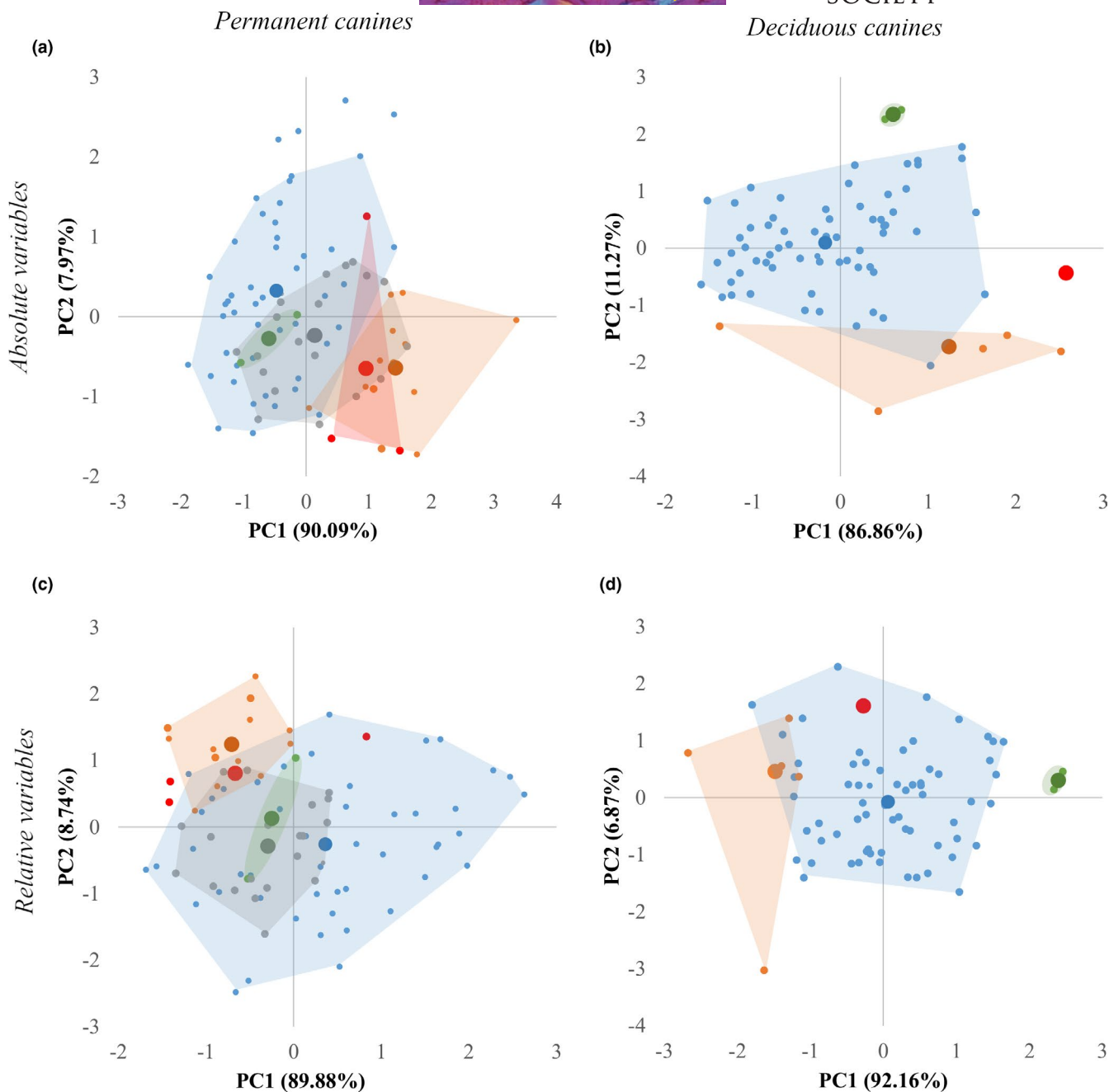


FIGURE 7 Principal Component Analysis (PCA) applied to the 3D dental tissue proportions of the permanent and deciduous canines. For more information see the caption of the Figure 6

studies have shown that *H. neanderthalensis* dentition presents a 2-D and 3-D enamel thickness whose mean values are lower than those observed in modern human populations, while the dimensions of the dentine-pulp complex are absolutely and relatively lower in the latter (e.g., Bayle et al., 2009a, 2009b; Buti et al., 2017; García-Campos et al., 2019; Macchiarelli et al., 2006; Olejniczak et al., 2008a; Smith et al., 2012). In particular, previous works have described these differences in the maxillary and mandibular deciduous canines of Neanderthals and recent modern human populations (Bayle et al., 2010; Crevecoeur et al., 2010; Macchiarelli et al., 2006).

This research results contributed to support the conclusion of prior studies on differences in the deciduous and permanent canines of *H. sapiens* and *H. neanderthalensis* (e.g., Bayle et al., 2009a, 2009b; Buti et al., 2017; García-Campos et al., 2019; Macchiarelli et al., 2006; Olejniczak et al., 2008a; Smith et al., 2012). As has been observed, the deciduous canines of Neanderthals seem to exhibit a pattern characterized by the presence of a larger coronal dentine-pulp complex (Acdp, Vcdp, Acdp/Ac, Vcdp/Vc) and a lower two-dimensional (2DAET, 2DRET and ACS) and three-dimensional (3DAET, 3DRET, 3DRED) enamel thickness, relative to extant modern human populations. These differences are also

TABLE 2 Principal component analysis scores obtained from the permanent and deciduous canines included in the present work

		Ve	Vcdp	EDJS	3DAET	3DRET	Vcdp/Vc	Ae	Acdp	EDJL	2DAET	2DRET	Acdp/Ac
Figure 6a	PC1							0.82	0.94	0.95			
	PC2							0.57	-0.26	-0.24			
Figure 6b	PC1							0.76	0.91	0.89			
	PC2							0.64	-0.24	-0.31			
Figure 6c	PC1										0.89	0.98	-0.96
	PC2										0.46	-0.16	0.26
Figure 6d	PC1										0.94	0.99	-0.96
	PC2	0.91	0.97	0.97									
Figure 7a	PC1	0.41	-0.19	-0.20									
	PC2	0.87	0.96	0.96									
Figure 7b	PC1	0.49	-0.22	-0.23									
	PC2				0.91	0.98							
Figure 7c	PC1				0.41	-0.11							
	PC2				0.93	0.99							
Figure 7d	PC1				0.35	-0.07							
	PC2												

present in the permanent canines of both taxa, as has been observed in this study and in the previous works (Buti et al., 2017; García-Campos et al., 2019). Likewise, the 3D enamel thickness distribution maps show a distinct dental tissue pattern in both species. The deciduous canines from *H. neanderthalensis* show lower enamel thickness values than were measured in the remainder fossil and recent modern human samples, which is reflected in a less intense red colour in Figures 4 and 5. It can also be observed that in modern humans the areas with greater enamel thickness values are more widely spread through the buccal surface of the crown, and even reach the mesial and distal margin ridges. These results concur with those obtained by Buti et al. (2017), and later by García-Campos et al. (2019), from the assessment of the permanent canines of Neanderthals and modern humans. On the other hand, in the Principal Component Analysis (PCA) shows two well-distinguished point clouds with a low degree of overlap which represent both taxa (Figures 6 and 7).

These research results also have allowed us to appreciate that the differences between *H. neanderthalensis* and modern humans could already be present even in the deciduous canines of the earliest representatives of *H. sapiens* (fossil modern humans, FMH). The tissue pattern of LaVe1 (Lagar Velho, Portugal) and Mad4 (La Madeleine, France) is characterized by 2D and 3D enamel thickness mean values even higher than those present in European modern populations and their crown dentine-pulp complex has relatively smaller dimensions than that of the current groups. All these variables illustrate the differences between Neanderthals and the first representatives of our species (FMH). These results concur with those obtained by Bayle et al. (2009a, 2009b). However, they do not agree that been described in previous works on the permanent dentition of fossil *H. sapiens*. Buti et al. (2017) observed that early modern humans' permanent canines ($N = 7$) are within the range of variation of Neanderthals for all crown tissue components. Similar results were obtained by Smith et al. in 2012. They concluded that variations across the permanent dentition are less pronounced between Neanderthals and fossil *H. sapiens* ($N = 6$) than between Neanderthals and recent *H. sapiens*. The results of the PCA also reflect the differences between primary and secondary dentition tissue proportions of FMH (Figures 6 and 7). Although in all cases the permanent and deciduous canines of FMHs are within or near the point cloud of recent modern humans, the secondary canines are in an intermediate position between Neanderthals and current populations, whereas the primary ones are placed in the opposite extreme of the *H. neanderthalensis* points cloud (especially in the 3-D measurements, Figure 7). Curiously, the 3-D enamel thickness distribution maps show that the pattern of FMH canines resembles that observed in modern humans in both their permanent (Buti et al., 2017) and their deciduous teeth (Figures 4 and 5).

Regarding the deciduous canines of Sima de los Huesos (AT-90) and Gran Dolina-TD6.2 (ATD6-14), the results seems to be more ambiguous. Sima de los Huesos, on the one hand, is represented by the deciduous mandibular canine AT-90, whose wear

TABLE 3 Enamel thickness diphodont index assess with the 2DAET, 2DRET, ACS, 3DAET, 3DRET and 3DRED values obtained from the permanent and deciduous canines analysed

	2DAET	2DRET	ACS	3DAET	3DRET	3DRED
<i>Maxillary canines</i>						
RMH	0.50	0.70	0.58	0.50	0.62	0.83
NEA	0.40	0.59	0.48	0.44	0.60	0.83
HA	0.45	0.61	0.51	0.49	0.63	0.85
<i>Mandibular canines</i>						
RMH	0.43	0.61	0.55	0.44	0.57	0.82
NEA	0.47	0.72	0.54	0.41	0.59	0.85
SH	0.67	0.95	0.54	–	–	–

degree did not allow us to evaluate the 3-D variables of this tooth. However, the values attained for AT-90 show a 2-D tissue pattern closer to that observed in the RMH sample (Figures 2 and 6). These results agree with those obtained in this study from the analysis of the permanent dentition of the SH population (SI Tables S3 and S5, and Figure 6) but nevertheless, they do not concur with the conclusions obtained in García-Campos et al. (2019). In 2019, García-Campos and colleagues analysed the tissue proportions of the secondary canines of Sima de los Huesos and Gran Dolina dental samples and compared them with a sample of recent modern humans (of African and European origin) and the Neanderthal from Krapina. These authors were able to appreciate that Sima de los Huesos hominins present on average the thinly enameled pattern characteristic of Neanderthal populations in their permanent canines, although with values close to the lower limit of the variation range of recent modern humans (García-Campos et al., 2019). Gran Dolina is represented by the deciduous maxillary canine ATD6-14. Both 2D and 3D dental tissue proportions of this canine place it in an intermediate position between *H. neanderthalensis* and *H. sapiens*. However, García-Campos et al. (2019) observed that *H. antecessor* shares with Sima de los Huesos and Neanderthals the three-dimensional dental tissue proportions of its permanent canines, which have been corroborated by the results of the present study (Table S5 and Figure 6). Finally, the 3D enamel thickness distribution maps shows that Sima de los Huesos and *H. antecessor* deciduous teeth display an intermediate morphology. These results contrast with those obtained from permanent dentition, in which the enamel thickness pattern of distribution of both populations are more similar to that observed in *H. neanderthalensis* (García-Campos et al., 2019; Martín-Francés et al., 2018).

It is important to note that the small size of the study sample from Sima de los Huesos and Gran Dolina, composed of one tooth from each site, might not be representative of these populations. However, other issues should be taken into account that may explain these results. The first is that the enamel thickness is not distributed homogeneously in canine crowns (Buti et al., 2017; García-Campos et al., 2019, and this study). It has been described that, in contrast with modern humans, in *H. antecessor*, Sima de los Huesos and Neanderthal permanent canines, the enamel cap

is thicker on the distal aspect compared to the mesial one, and particularly thick at the level of the distal marginal ridge (García-Campos et al., 2019 and this study). This study has also revealed these differences in the pattern of enamel thickness distribution between the deciduous canines of *H. sapiens* and those of the remaining fossil species studied (Figures 4 and 5). Thus, it is plausible that the 2D measurements taken from a buccolingual longitudinal plane of the canine crown may not accurately reflect the differences between the dentition of these taxa. It should be also noted that the composition of the comparative recent modern human samples is not the same in all studies. In the study by García-Campos et al. (2019), a permanent canine sample of modern humans included individuals from Europe and Africa, whereas in this study, only a sample of permanent canines of European origin was evaluated. The purpose of this was to make the permanent and deciduous canines samples were as similar as possible in order to contrast them. The permanent canines of African populations tend to have a greater enamel thickness than European populations (Feeney et al., 2010; García-Campos et al., 2017), so the employment of only European samples might be underestimating the values of the enamel thickness present in the modern human groups, thus minimizing the differences with the Sima de los Huesos and Gran Dolina samples.

Finally, this study has employed the enamel thickness diphodontic index (ETDI) to assess the degree of similarity in the dental tissue proportions between primary and secondary canines of the modern human and fossil samples analysed. The ETDI index consists of dividing the mean value obtained for each variable in the deciduous teeth by the value obtained in their permanent equivalents. Thus, the more similar values exhibit both dental classes, the closer to 1 is the result obtained; similarly, they will move away from this value as the differences increase. This index was first employed by Zanolli et al. (2017a) to investigate the degree of covariation in enamel proportions between 25 pairs of mandibular dm2 and M1 of nine extant and fossil hominids. In this work, the authors raised the following questions: "Can we confidently predict an enamel thickness category for a hominid deciduous crown based on the measure of the permanent tooth (or vice versa)? Does a predictable deciduous-permanent pattern exist for tooth enamel thickness in hominids? If so, is

it taxon-specific?" (page 2, Zanolli et al., 2017a). Regarding the first question, as could be appreciated in molars by Zanolli et al. (2017a), the results of the enamel thickness diphyodont index (ETDI), assessed hereby employing the values obtained of the bi-dimensional and three-dimensional enamel thickness of canines show ETDI ratios <1.0 in all cases. This concurs with what would be expected. Permanent dentition crowns are equipped with a thicker coating of enamel than their homologues in the primary dentition since they have to be able to resist a higher and more prolonged dental wear. However, in contrast with molars, the ETDI values of canines were lower than 0.8, except in the 3DRED index, which may be relevant as it might indicate bigger differences between the c1 and C1 concerning molars. Therefore, based on the above and the results obtained from the ETDI, we could assert that deciduous and permanent canines would not provide a similar and/or comparable picture of the enamel thickness pattern of each taxon. There is an exception, the 3DRED index. The values obtained for the ETDI of the 3DRED index in the mandibular and maxillary canines were very similar in all fossil samples assessed, ranging between 0.82 and 0.85, which may provide a predictable deciduous-permanent pattern for tooth enamel thickness in hominids.

The average enamel thickness (AET) and relative enamel thickness (RET) (Kono, 2004; Martin, 1983) are the two classic indices employed to assess the enamel thickness. Nevertheless, in recent years, alternative indices have been proposed to study worn teeth, like the lateral RET (e.g., Benazzi et al., 2011; Kono and Suwa, 2008; Zanolli et al., 2018); to estimate tooth crown resistance to fracture, as the absolute crown strength estimated on 2D sections (Schwartz, McGrowsky, and Strait, 2020); or to avoid the reduction of the spatial resolution caused by the voxel size variation, as the ratio of enamel-thickness to dentine-thickness (3DRED) (Yi et al., 2021). Because of the absence of standardized measurement protocols elaborated for canines, in this study we only have employed the last two indices. Although the results for the ACS index, proposed by Schwartz, McGrowsky and Strait (2020), are ambiguous and difficult to interpret, the 3DRED (Yi et al., 2021) has emerged as a useful index in studies on dental paleohistology. 3DRED offers comparable results to those obtained from the 3DRET index, but it has the advantage for being more robust to voxel size variation to the latter (Yi et al., 2021). This could be an important advantage when working with fossil samples scanned in different institutions and under different conditions. Furthermore, 3DRED shows less degree of correlation with 3DAET than 3DRET, which indicates that it is an independent variable (Yi et al., 2021). Moreover, as observed in the results of this study, this index could offer an additional advantage: 3DRED seems to be more stable than 3DAET or 3DRET in the deciduous and permanent dentition of the different taxa, which would allow us to establish predictable deciduous-permanent patterns. Future work on other dental samples or using other dental classes could help to corroborate this aspect.

4.2 | Possible biological implications of the differences observed between dental tissue proportions of deciduous and permanent canines

Different hypotheses have been proposed to explain the differences between the dental tissue patterns of *H. neanderthalensis* and *H. sapiens*. According to some studies, the proportion in which the different tissues are expressed in the human dentition has an important genetic regulation and is strongly influenced by the interaction of different hormones (e.g., Alvesalo, 2009; Guatelli-Steinberg et al., 2008; Pentinuro et al., 2017; Ribeiro et al., 2012, 2013; Townsend & Alvesalo, 1985).

On the one hand, in recent years, some researchers have focused on the differences found in the Neanderthal genome contrasted with those of modern human groups (e.g. Green et al., 2010; Lopez-Valenzuela et al., 2012; Zanolli et al., 2017b). The first work published comparing the genotypes of both taxa revealed that modern humans differ from Neanderthals by substituting of several nucleotides in the miRNA (Green et al., 2010). In particular, in 2012 López-Valenzuela and colleagues observed a polymorphism in miRNA-1304. The ancestral form of this polymorphism, found in the genome of non-human primates and in the genome of *H. neanderthalensis*, reduced gene expression associated with enamelin and amelotin by 50%. On the contrary, the derived miRNA-1304, present in modern humans, seems to not have an effect. Similarly, Zanolli et al. (2017b) analysed 13 enamel and dentine extracellular matrix proteins, comparing the genomic sequences among Neanderthals, Denisovans and modern humans. These authors observed that only three morphotypes appeared in all three taxa, while the rest were specific to Neanderthals and Denisovans and were in very low frequencies (10%) in modern humans. Therefore, the conclusions reached by these authors seemed to reveal the existence of differences between the genes involved in the regulation of the enamel and dentine dimensions of the Neanderthals and modern humans, which might thus be affecting the proportion in which both tissues are expressed in their dentition.

On the other hand, the influence of hormones on odontoblastic activity has been broadly studied (Dempsey et al., 1999; Guatelli-Steinberg et al., 2008; Ribeiro et al., 2012, 2013; Zilberman & Smith, 2001). The presence of receptors for growth hormone (GH) in the dental lamina may provide a clue about the process through which hormones could be influencing the development of different dental tissues (Zhang et al., 1997). This hormone is the primary regulator of postnatal growth and development (Giustina & Veldhuis, 1998), and has a strong influence on the development of oral tissues (Slootweg, 1993). GH could be behind the apparent association between the changes produced in the dimensions of the coronal dentine and the variations in craniofacial morphology described by several authors (Kupczik & Hublin, 2010; Lieberman, 2001). In accordance, some studies linked the increase in the size of the anterior dentition of Neanderthals with an increase in the dentine component and their

craniofacial skeleton, indicating that both tissue, dentine and bones, might be have similar responses to biological signals (Hublin, 1998; Trinkaus, 1987). This might also influence the calculation of enamel thickness, since most of the indices employed for its assessment present a negative correlation with the dimensions of the coronal dentin-pulp complex.

Although there are still many unknowns about the complex adaptive system that regulates the human dental tissue development, it seems to be clear that the underlying factors affect each tooth class differently depending on their formation times. The primary dentition, including deciduous canines, is formed earlier in the individual's life so these teeth are presumably under stronger genetic control as well as being less influenced by the environment or variations in hormone levels than the permanent dentition. All that may be behind the differences observed in this study in the dental tissue pattern of deciduous and permanent canines of the samples assessed. Likewise, it could also explain why the differences between dm1 and M2 are less marked than between c1 and C1. It is important to keep in mind that canines are teeth that are particularly sensitive to the variations in sex hormones levels produced throughout an individual's life (e.g., Dempsey et al., 1999; Ribeiro et al., 2012, 2013; Zilberman & Smith, 2001). Different studies have described how changes in concentrations of steroid hormones influence the dimensions of the dentin component of permanent canines (Zilberman & Smith, 2001). So much so that the proportions in which different dental tissues are present in permanent canines have been successfully used for sex estimation, not only in modern humans but also in fossil samples (García-Campos et al., 2018a, 2018b, 2020, 2021). Therefore, the dental tissue proportions of the deciduous and permanent canines of fossil and modern human populations might have a genetic origin. However, hormones may also play a relevant role in permanent dentition, especially in the case of the canines, the most dimorphic tooth in primates (Plavcan, 2012), which might be behind the differences observed between both dental classes.

5 | CONCLUDING REMARKS

In conclusion, the results obtained in this study show that although there are some similarities in the dental tissue proportions between the deciduous and permanent canines of the study samples, the two dental classes do not provide similar or comparable pictures of the dental tissue pattern of these populations. Once again, it can be seen that the 2-D measurements offer a biased view of the pattern present in the different taxa, preventing an accurate appreciation of the existing differences (Benazzi et al., 2014; García-Campos et al., 2018a; Olejniczak et al., 2008a). Although the results yielded by the "enamel thickness diphodontic index" did not provide an unequivocal picture, it proves as a useful exploratory tool to assess the variation within the deciduous and permanent dentition. Likewise, 3DRED index has proven to be worthwhile in dental paleohistology studies, since it

offers comparable results to 3DRET but also additional advantages to the classic enamel thickness indices. In particular, 3DRED might be more stable than 3DAET or 3DRET in the deciduous and permanent dentition of the different taxa, which would allow the establishment of predictable deciduous-permanent patterns. Future works on the dental tissue patterns of the anterior and posterior deciduous dentition should be developed employing other fossil samples, in the same way future proteomics and paleogenetics research may help to shed light on these aspects and perhaps help to support or discard the hypotheses put forward in this paper.

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AUTHOR CONTRIBUTIONS

C.G-C designed the study and acquisition of data. C.G-C and M.M-M performed the statistical analysis. C.G-C, M.M-M and J.M.B.C drafting of the manuscript. M.M-T, L.M-F, M.M-P and J.L.A critically revised the manuscript. L.M-F, M.M-P, M.M-T, J.M.B.C and J.L.A assisted in the creation and management of anthropological collections.

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