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Middle paleolithic human deciduous incisor from Grotta del Cavallo, Italy

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First published: 01 July 2016 https://doi.org/10.1002/ajpa.23044Citations: 11

Abstract

Objectives

In this contribution, we present a morphological description and comparative morphometric analysis of Cavallo D, a human tooth unearthed from the Mousterian FIII sublayer of Grotta del Cavallo (Apulia, Italy).

Materials and Methods

We used microCT data to provide a detailed morphological description and morphometric analysis of the Cavallo D human tooth based on traditional diameter measurements and 3D enamel thickness. Moreover, new AMS radiocarbon dating of charcoals from layers FII was carried out.

Results

Morphological features observed in Cavallo D align the tooth to Neandertals. Similarly, the large size of the tooth (e.g., BL diameter) and the relatively thinner enamel thickness are typical Neandertal traits. ^{14}C datings of layer FII attribute the tooth to a time range of 45,600–42,900 cal BP (at 68% level of probability).

Discussion

Up to now, the Rdi1 Cavallo D represents the most recent Neandertal human remain in southern Italy related to a radiocarbon dated stratigraphy. Moreover, since deciduous teeth have been less investigated than the permanent ones, this contribution brings new data to increase our knowledge on the variability of the Neandertal deciduous dentition

1 Introduction

Grotta del Cavallo (40°9′18.85″N, 17° 57′37.27″E) is a well-known Paleolithic site located on the rocky coast of the Bay of Uluzzo, near Nardò (Apulia, Southern Italy). Excavations started in the 60s by A. Palma di Cesnola and are now directed by L. Sarti.

The eight-meter deep stratigraphy is divided in four main chrono-cultural units: Mousterian, strata N-F; archaic Upper Paleolithic (Uluzzian), strata E-D; final Upper Paleolithic and Mesolithic, stratum B; sparse Neolithic, stratum A. Three human teeth have been discovered by A. Palma di Cesnola: a Neandertal lower left second deciduous molar (Ldm2; Cavallo A) from the Mousterian L stratum; two early modern humans, an upper left first deciduous molar (Ldm1; Cavallo B) and an upper left second deciduous molar (Ldm2; Cavallo C), from the Uluzzian

stratum (respectively layer EIII and EII-EI) (Benazzi et al., 2011, 2012; Palma di Cesnola & Messeri, 1967).

In 2013, a new human tooth (hereafter called Cavallo D) was identified among faunal remains collected during Sarti excavations (1987) in the Mousterian FIII sublayer. Recent excavations in the Mousterian strata revealed many paleosurfaces with hearths and concentrations of lithic artifacts and faunal remains. Cavallo D was found in the sublayer FIIIb, a thin paleosurface (6–10 cm), comprising extended areas of combustion and scattered lithic and bone remains. The lithic industry has been assigned to the local final Typical Mousterian rich in scrapers, which precedes the terminal Denticulate Mousterian of layers FII-FI. The Mousterian strata are topped by a thin layer of volcanic ash (Fa) followed by the Uluzzian sequence (Romagnoli et al., 2015).

As Cavallo D was retrieved in the Mousterian layer F, which is just at the bottom of the Uluzzian layer EIII, the taxonomical discrimination of the tooth Cavallo D and an accurate dating of layer F are pivotal to the current debate about the demise of Neandertals and the arrival of anatomical modern humans in Europe (Benazzi et al., 2011). Therefore, in this contribution we present a morphological description and comparative morphometric analysis of Cavallo D, as well as new ^{14}C dating of layer F.

2 Materials and Methods

2.1 Morphological description

An assessment of non-metric traits was made based on standards outlined by the Arizona State University Dental Anthropology System, ASUDAS (Turner, Nichol, & Scott, 1991). However, since the ASUDAS has been devised for modern human permanent dentition, we emphasize that a comparative study of non-metric dental traits in the deciduous teeth is warranted. Age at death was estimated using Alqahtani, Hector, and Liversidge (2010), whereas wear stage was based on Molnar (1971).

2.2 Morphometric analysis

For metric traits, we measured the fundamental crown and cervical diameters, as well as the three-dimensional (3D) enamel thickness. Mesio-distal (MD) and labio-lingual (LL) crown diameters of Cavallo D were then compared to a sample of Neandertals (N), Upper Paleolithic *Homo sapiens* (UPHS), and recent *Homo sapiens* (RHS) collected from Crevecoeur et al. (2010) (but see also Henry-Gambier, Maureille, & White, 2004).

To quantify the 3D enamel thickness, high-resolution micro-CT images of Cavallo D and of nine RHS deciduous lower central incisors (Northern Italian medieval specimens housed at the Department of Cultural Heritage, Ravenna, University of Bologna, Italy) were obtained with the Xalt micro-CT scanner (Panetta, Belcari, Del Guerra, Bartolomei, & Salvadori, 2012). All teeth were scanned at 50 kVp, 2 mm Al filtration, 960 views over 360°, 1.6 mAs/view. All the images were reconstructed using a modified Feldkamp algorithm (Feldkamp, Davis, & Kress, 1984), with an isotropic voxel size of 18.4 μm on a 512 × 512 × 1,200 volume dataset and then cropped to the tightest bounding box of each tooth. The

micro-CT images of the original sample were virtually segmented using Seg3Dv 2.1.4 (http://www.sci.utah.edu/cibc-software/seg3d.html). The segmented enamel cap and virtually filled dentin were converted to meshes using the Windged-Edge Mesh tool of the MeVisLab software (http://www.mevislab.de).

The digital models were then imported in Rapidform XOR2 (INUS Technology, Seoul, Korea). Following indications provided by Benazzi et al. (2014), the entire enamel cap was considered while the coronal dentine was separated from the root dentine using a spline curve digitized along the cervical line (Figure 1a). This curve was interpolated with a smooth surface to seal the bottom of the dentine core (Figure 1b).

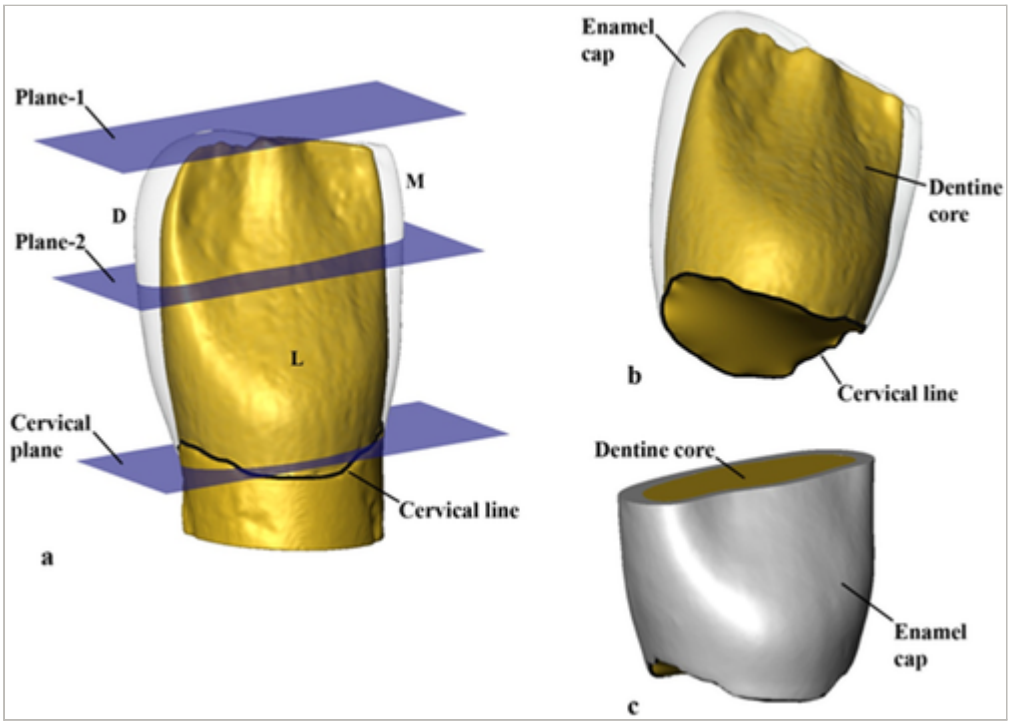

Figure 1

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(a) A spline curve (black) was digitized on the cervical line of the digital model of the recent *Homo sapiens* (RHS) lower left deciduous first incisor (the enamel is transparent to better show the underlying dentine); D = distal; L = lingual; M = mesial. (b) For 3D enamel thickness analysis, the coronal dentine was separated from the root dentine based on the cervical line, which was then interpolated by a smooth surface to seal the bottom of the dentine core. (c) A large portion of the crown was removed using plane-2, which is both parallel to the cervical plane and apically displaced 2 mm from plane-1.

We measured the volume of the enamel cap (mm^3); the volume of the dentine core (which includes the volume of the coronal pulp - mm^3); the enamel-dentine junction (EDJ) surface (the interface between the enamel cap and the dentine core - mm^2). These measurements were used for the computation of both the 3D average enamel thickness (3D AET = volume of enamel divided by the EDJ surface; index in millimeters) and the 3D relative enamel thickness index (3D RET = the average enamel thickness index divided by the cubic root of dentine volume; scale free index).

Additional comparative data for the 3D enamel thickness for three Neandertals (Abri Suard, Roc-de-Marsal 1, Spy VI) and the UPHS specimen La Madeleine 4 were sourced from Bayle (2008) and Bayle, Braga, Mazurier, & Macchiarelli (2009). Note that the method used by the authors for these four teeth to

compute the values of the components of 3D enamel thickness follows the protocol provided by Olejniczak et al. (2008), which has been conceived for molar teeth but not for incisors and cuts the crown from the root differently from what done for the rest of the sample (for more details see Benazzi et al., 2014).

As Cavallo D is rather worn, while the wear stage of the RHS sample ranges between 2 and 4 (Molnar, 1971; see Table 2 for information about wear stages), the latter was digitally worn down simulating a wear stage comparable to Cavallo D to evaluate whether an increase of tooth wear might affect the taxonomical attribution (as tooth wear affects more the enamel volume than the dentine volume, an increase of wear causes a decrease of the AET and RET indices). Owing to the lack of protocols to simulate artificial wear in incisors, and because Ungar, Fennell, Gordon, & Trinkaus (1997) did not find any clear pattern of beveling with tooth wear in permanent lower central incisors, we defined an objective procedure that involves the following steps (Figure 1a): the best-fit plane through the points of the cervical line was computed (cervical plane), and the most incisal plane that is parallel to the cervical plane was then identified (plane-1); a further plane (plane-2) was then computed at a distance of 2 or 3 mm from plane-1, depending on both the wear stage of each RHS specimen and the crown height; plane-2 was used to remove a large portion of the original RHS incisors (Figure 1c), so that the remaining crown has an average mid-lingual height from the cervix of 2.76 ± 0.32 mm (ranging from 2.1 to 3.2 mm), which is much shorter than the crown height measured in Cavallo D (see below).

Table 1. Dimensions (in mm) of the tooth Cavallo D compared with a sample of Neandertals, Upper Paleolithic *Homo sapiens* (UPHS) and recent *Homo sapiens* (RHS)a di1s. n = sample size, SD = standard deviation.

	MD (mm)			LL(mm)		
	n	**Mean ± SD**	**Z-score**	**n**	**Mean ± SD**	**Z-score**
Cavallo D		4.87			4.77	
Neandertals	17	4.94 ± 0.33	−0.21	17	4.66 ± 0.25	0.44

UPHS	13	4.37 ± 0.22	2.27	12	3.96 ± 0.28	2.89
RHS	54	3.97 ± 0.36	2.50	58	3.69 ± 0.34	3.18

- *n* = sample size, SD = standard deviation, MD = mesio-distal diameter; LL = labio-lingual diameter.
- [a] Comparative data from Henry-Gambier et al. (2004) and Crevecoeur et al. (2010).

Table 2. Values of the components of three-dimensional (3D) enamel thickness of Cavallo D (dri1), Neandertals, UPHS, and RHS di1s

	Wear stage[a]	Enamel volume (mm^3)	Coronal dentine + pulp volume (mm^3)	EDJ surface (mm^2)	3D AET (mm)	3D RET (scale-free)	*Z*-score for RET index
Cavallo D	5	10.86	42.62	53.57	0.20	5.80	
Neandertals[b](3)	1–3	12.89 ± 3.07 (9.91–16.05)	40.05 ± 0.76 (39.18–40.53)	57.40 ± 5.85 (50.65–61.06)	0.22 ± 0.04 (0.20–0.26)	6.52 ± 1.08 (5.70–7.74)	−0.67

La Madeleine 4 UPHS[c]	3–4	10.16	18.94	31.66	0.32	12.04	
RHS (9)[d]	2–4	10.02 ± 2.53 (5.55–14.45)	22.27 ± 2.46 (18.22–25.60)	39.56 ± 3.72 (33.15–43.37)	0.25 ± 0.05 (0.17–0.33)	8.89 ± 1.51 (6.36–11.83)	−2.05
RHS (9)[e]	5	4.78 ± 1.13 (3.12–6.19)	16.35 ± 2.63 (12.21–19.93)	22.98 ± 3.31 (16.89–27.43)	0.21 ± 0.03 (0.15–0.26)	8.15 ± 1.12 (6.11–10.28)	−2.10

- For the samples, (*n*): mean ± SD (range).
- [a] Based on Molnar (1971).
- [b] ForAbriSuard S14 and Spy VI, see Bayle (2008); for Roc de Marsal, see Bayle et al. (2009).
- [c] Bayle (2008).
- [d] Original data.
- [e] Digital removal of tooth crown up to wear stage 5.

Standardized scores (*Z*-scores) of Cavallo D crown diameters and RET index were computed to establish to which group means the value of Cavallo D was closest to.

2.3 AMS radiocarbon dating

A minimum absolute age for the Cavallo D tooth has been obtained by dating a sample of mixed charcoals collected from layer FII, treated following the so-called ABA (Acid-Base-Acid) procedure, whose purpose is the removal of the possible natural contaminations such as carbonates and humic substances. According to laboratory quality check practice, after this treatment, the dried cleaned sample was split into two fractions, separately combusted and then graphitized (Fedi et al., 2013), to obtain two graphite pellets to be inserted into the accelerator source (lab codes Fi0822 and Fi0824). Accelerator Mass Spectrometry measurements were performed on the dedicated beam line of the 3 MV tandem accelerator of INFN-LABEC, Florence (Fedi, Cartocci, Manetti, Taccetti, & Mandò, 2007). Measured $^{14}C/^{12}C$ isotopic ratios were corrected for isotopic fractionation ($^{13}C/^{12}C$ ratios were also measured on the accelerator

beam line) and for background counts (by measuring blank samples, i.e., nominally without ^{14}C), and eventually normalized to the $^{14}C/^{12}C$ isotopic ratios measured in a set of samples prepared from a standard reference material, namely NIST Oxalic Acid II.

3 Results

3.1 Morphological description

The overall dimensions and appearance of the tooth point to the human deciduous incisors. It is not MD elongated to be a di_1 and its preserved LL crown diameter (4.76 mm) is lower than the minimum value recorded in a sample of Neandertals di_2 ($n = 18$) and close to the mean of the samples of Neandertals di_1 ($n = 12$) and di_2 ($n = 22$) (Bailey & Hublin, 2006; Chech, Vandermeersch, Arensburg, & Tillier, 2003; Crevecoeur et al., 2010; Ménard, 1984; Pap, Tillier, Arensburg, & Chech, 1996; Tillier, 1983; Trinkaus, Ranov, & Lauklin, 2000; Wolpoff, 1979). As regards the lower deciduous incisors, in lingual view (Figure 2) Cavallo D shows M and D parallel and nearly rectilinear crown margins, as seen in the di_1, while the di_2 have a convex D margin. Regarding the side, in incisal view, Cavallo D has convex lingual and labial surfaces, the former is symmetrical, the latter (ASUDAS grade 4) has its most anterior point closer to one side (mesial) than to the other one (distal), thus suggesting that Cavallo D (Figure 2) is a lower first deciduous right incisor.

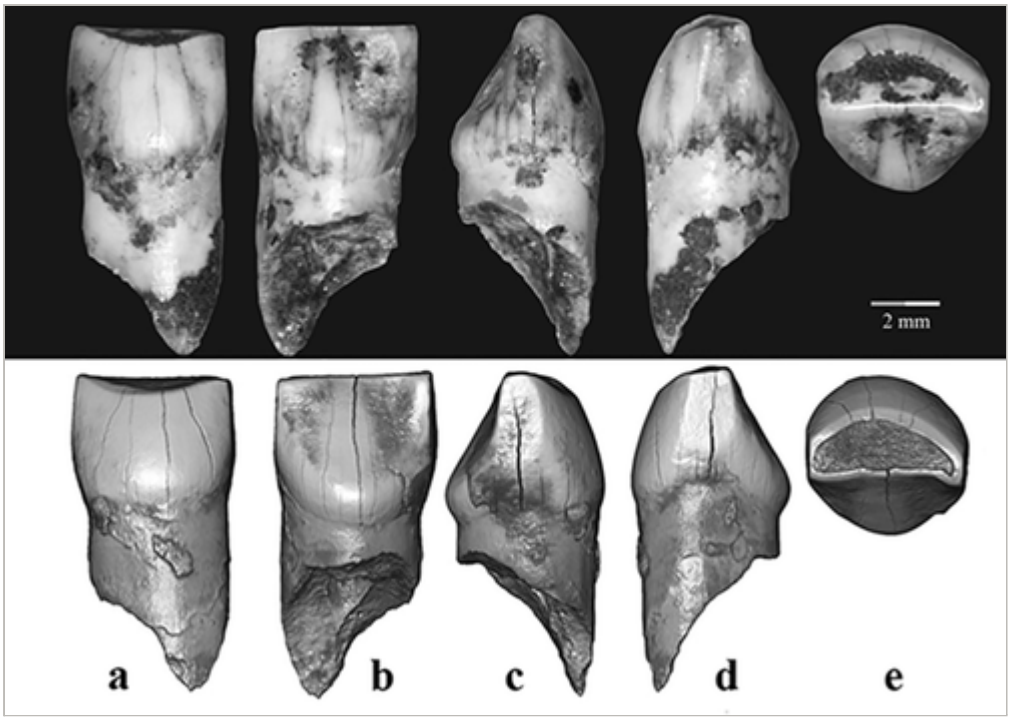

Figure 2

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Cavallo D, the original tooth(upper) and the digital reconstruction (lower). (a) labial view; (b) lingual view; (c) distal view; (d) mesial view; (e) incisal view.

Cavallo D has a worn crown (Stage 5, after Molnar, 1971) and resorbed root, which is more elongated on the labial side (5.69 mm) than on the lingual side (1.11 mm). Several fissures run superior-inferiorly from the incisal edge to the cervix on the entire crown.

The incisal wear facet has a convex labial and a straight lingual border. Its maximum LL diameter is in correspondence with the most anterior point of the labial crown margin, and is sloping from lingual to labial and from distal to mesial. The beveling angle is 101° (Ungar et al., 1997). In labial view, the mesial and distal sides are nearly straight and bear large interproximal wear facets. The lingual side shows a faint cervical eminence, surmounted by fainter median ridge separating two shallow fossae, the distal one larger than the mesial one. There is no trace of a mesial marginal ridge, but a weakly expressed marginal ridge is present along the distal margin (ASUDAS grade 1).

The root resorption due to the emerging permanent incisor suggests that the tooth might have been lost ante-mortem through dental development, a stage which corresponds to an age of 6-year old in recent modern children (AlQahtani et al., 2010), but could be significantly lower in Neandertals (Ramirez Rozzi & Bermudez de Castro, 2004; Smith, Toussaint, Reid, Olejniczak, & Hublin, 2007).

3.2 Morphometric analysis

The tooth crown has a MD diameter of 4.87 mm (minimum estimation due to wear) and a LL diameter of 4.77 mm, with a mid-labial height from the cervix of 3.6 mm, and a mid-lingual height of 4.58 mm. At the cervix, MD is 4.31 mm and LL is 4.22 mm. Comparative data for crown diameters are reported in Table 1. Cavallo D has a large crown size, and the computed *Z*-score is much closer to the Neandertal mean than to Upper Paleolithic and recent *H. sapiens*.

Similar results were obtained considering the 3D enamel thickness (Table 2). The large dentine volume and EDJ surface of the specimen contribute to convey a low RET index, which falls inside the Neandertal di_1s variability, but is outside the range currently known for *H. sapiens* (UPHS and RHS). Results do not change when the RHS sample is artificially worn down (see method above), as the computed *Z*-score is still closer to Neandertals than RHS.

3.2.1 AMS radiocarbon dating

As the two measured pellets resulted to be statistically consistent between each other and being obtained from the same original sample, the best estimation of the radiocarbon age of the Cavallo layer FII was calculated as their weighted average (Table 3). The conventional radiocarbon age is 40,600 ± 1,500 years BP and was calibrated using OxCal 4.2 (Bronk Ramsey, 2009) and IntCal13 (Reimer et al., 2013) calibration curve (Table 3). According to calibration, the layer FII is thus dated to the period 45,600–42,900 cal BP, at 68% level of probability. The absolute date for FII is to be considered as a *terminus ante quem* for the FIIIb layer (where indeed Cavallo D has been found). Considering the already dated samples of the uppermost layers in the cave (Benazzi et al., 2011), we cannot directly compare the conventional radiocarbon ages, since the present date refers to a specimen of terrestrial origin, while those referred to marine organisms. However, following the Bayesian inference, we can add this date as the bottom of the already dated stratigraphical sequence, just preceding the EIII Uluzzian layer, obtaining a consistent chronology of the site. Our result suggests a short chronological gap between the Final Mousterian (layer F) and the Early Uluzzian (layer E) and indeed the layers F was found to be partially eroded in its upper part.

Table 3. Radiocarbon result obtained from INFN-LABEC, Florence, calibrated using OxCal 4.2 (Bronk Ramsey, 2009) and IntaCal13 (Reimer et al., 2013) calibration curve

Lab code	**^{14}C age (years BP)**	**Sample**	**^{14}C age (years BP)**	**Calibrated age (cal BP, 68%)**	**Calibrated age (cal BP, 95%)**
Fi0822	39,300 ± 1,900	Cavallolaye r FII	40,600 ± 1,500	45,600–42,900	47,900–42,100

Fi0824	42,000 ± 2,400

4 Discussion and Conclusions

The interest of Cavallo D is highlighted by the discovery, in the same site, of Uluzzian associated anatomical modern human teeth, Cavallo B and C (Benazzi et al., 2011). The stratigraphy of the site clearly shows that Cavallo D predates Cavallo B and C, but the former could be only slightly earlier than the latter based on absolute ^{14}C dating. Indeed, the new ^{14}C dating obtained for layer FII is extremely important, because it constrains the bottom of the Uluzzian deposit and confirm previous Bayesian model that dates the oldest Uluzzian to 45,000–43,000 cal BP (Benazzi et al., 2011).

Bailey (2006) emphasized that the crown morphology of lower permanent incisors is not particularly useful in sorting out taxonomy among Middle-Late Pleistocene hominins, and it is reasonable to assume that the same limits can be extended to the lower deciduous incisors as well. However, Bailey also suggested that Neandertal lower permanent incisors do appear to be larger than those of modern humans (relative to the posterior teeth), a condition that has been also observed by other authors for lower deciduous incisors, at least when

comparing Neandertals with UPHS (e.g., Crevecoeur et al., 2010; Trinkaus et al., 2000).

Indeed, the LL crown diameter of Cavallo D points to Neandertal affinities: it is very close to the Neandertal mean and larger than the UPHS mean (Table 1). Both the morphology and the enamel thickness, point to the same direction. As far as the morphology is concerned, Cavallo D is similar to the Neandertal remains of, for example, Combe Grenal 31 and Spy VI (Figure 3).

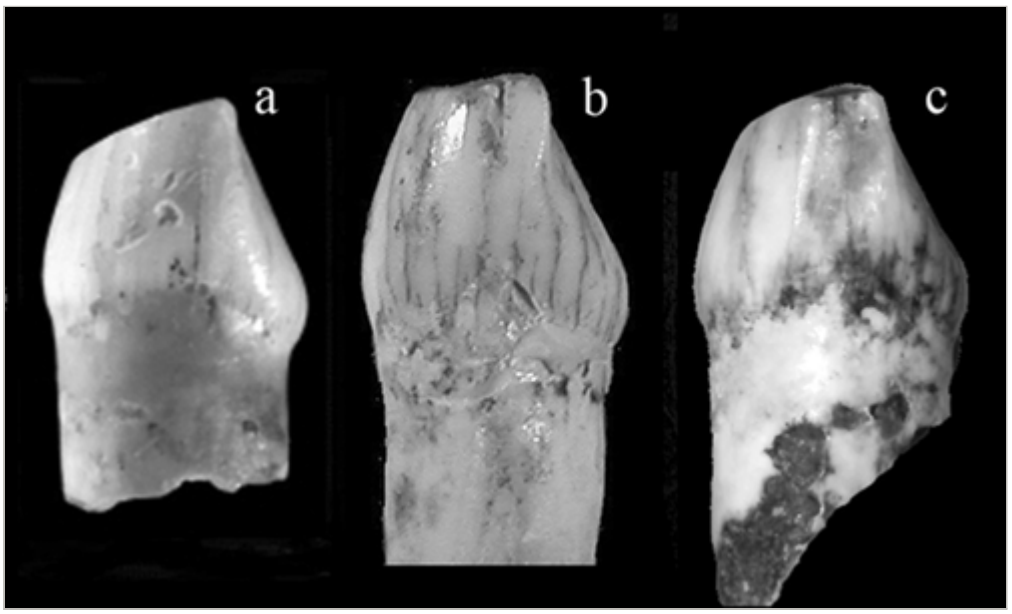

Figure 3

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Mesial view of: (a) Combe Grenal 31, Maureille et al. (2010); (b) Spy VI, Crevecoeur et al. (2010); (c) Cavallo D.

The quantification of the 3D enamel thickness generally requires an unworn or slightly worn crown, but as tooth wear is almost ubiquitous in the dental fossil record it greatly reduces the sample size for analysis. Following Benazzi et al. (2014), we digitally wore down the comparative sample until it reached the same degree of wear of the specimen under study. Actually, we exaggerated the artificial wear, reducing the RHS crown height of 2–3 mm, depending on the original tooth dimension and wear stage: the mean value for the RET index decreased of less than one point, but it remained still higher than the RET value for Cavallo D, further supporting the attribution of Cavallo D to Neandertal.

Owing to the limits of metric approaches due to the ubiquitous worn condition of the fossil dental sample, we believe that future contributions should develop robust methods for a reliable quantification of the 3D enamel morphology of worn teeth (Benazzi et al., 2012, 2015).