Structure and evolution of mammoth molar enamel

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This work investigates the structure of Eurasian Plio–Pleistocene *Mammuthus* enamel, with attention to diagenesis and individual variability. A focal point of this study was to determine whether morphological trends in *Mammuthus* molars were accompanied by correlated enamel microstructure changes. In the examined four taxa the enamel of the cheek teeth consists of three layers delimited by two major discontinuities in enamel prism direction. Noticeably, the enamel capping the occlusal end of the unworn molar plates retains a less derived two-layered structure, similar to that found in the basal proboscidean *Moeritherium*. In *Mammuthus meridionalis* the third deciduous premolar is differentiated from all other teeth in having more strongly decussating Hunter-Schreger bands in the middle layer, as a possible reinforcement of the very thin enamel. Evidence from this analysis shows that, in the transition from late Middle Pliocene *M. rumanus* to Late Pleistocene *M. primigenius*, the middle enamel layer, which is made up of prisms at an angle to the occlusal surface, providing greater resistance against wear, increased its relative thickness. This is consistent with the hypothesis that *Mammuthus* adapted to a more abrasive diet. Comparison with other proboscidean taxa indicates that the schmelzmuster (enamel pattern) found in *Mammuthus* is a synapomorphy of the Elephantoidea.

Key words: Mammalia, Proboscidea, *Mammuthus*, enamel microstructure, evolution, systematics.

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Introduction

Enamel microanatomy has become an important feature in the taxonomy and phylogeny of several mammal groups (Korvenkontio 1934; Kawai 1955; Boyde 1965, 1969, 1971; Koenigswald 1980; Carlson and Krause 1982; Gantt 1983; Sahni 1985; Koenigswald et al. 1993; Martin 1993, 1994; Pfretzschner 1993; Koenigswald and Sander 1997a; Stefen 1997; Kalthoff 2000). Enamel mechanical properties, especially resistance to wear and fracture propagation, were also demonstrated in recent studies (Rensberger and Koenigswald 1980; Fortelius 1985; Pfretzschner 1988, 1994; Rensberger 1992, 1995a, b, 1997; Srivastava et al. 1999), providing an important complement to morpho-functional analysis of teeth for interpreting the feeding habits of fossil vertebrates.

Proboscidean enamel has been the subject of several studies that have highlighted the group's complex enamel structure and discussed some functional adaptations (Remy 1976; Kozawa 1978, 1985; Okuda et al. 1984; Bertrand 1987, 1988; Sakae et al. 1991; Pfretzschner 1992, 1994; Kamiya 1993; Ferretti 2003).

Previous studies found five principal enamel types occurring in the various proboscidean families (Kozawa 1978; Bertrand 1987, 1988; Pfretzschner 1994). Four of them are common among placental mammals (Koenigswald 1997). A fifth, very complex enamel type, is restricted to the Proboscidea.

The present paper is a contribution to knowledge of enamel microstructure of mammoths. Four mammoth species of the Eurasian *Mammuthus* lineage were investigated, focusing on individual variability of the structure of the molar enamel, with attention to possible alteration due to diagenesis.

Mammuthus is a monophyletic genus; with respect to *Elephas* and *Loxodonta*, it is distinguished on both morphological and molecular evidence (Maglio 1973; Shoshani et al. 1985; Tassy and Darlu 1986; Lister 1996; Thomas et al. 2000). The three elephant genera *Loxodonta*, *Elephas*, and *Mammuthus* diverged as early as the Early Pliocene, around 5 Ma, or somewhat earlier (Maglio 1973; Beden 1987; Kalb and Mebrate 1993). Thus *Mammuthus* dispersed outside of Africa to Eurasia in the Middle Pliocene. The last *Mammuthus* representative, the woolly mammoth *M. primigenius* (Blumenbach, 1799), became extinct about 3.7 thousand years ago (Lister and Bahn 2000). During the approximately 3 million years covered by the four *Mammuthus* species examined here, mammoth molars underwent a morphological change characterized by increment of crown height, multiplication of the number of plates forming the tooth, and thinning of the enamel, as a probable adaptation to a progressively predominant grass diet. These modifications could have also affected enamel microstructure and its verification is one of the aims of this paper.

Terminology and abbreviations

For description of enamel microstructure the terminology of Carlson (1995), and Koenigswald and Sander (1997) was followed.

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Table 1. List of specimens analyzed in this study.

Taxon	Specimen	Locality	Age
Mammuthus rumanus	IGF 1932[1], M3	Montopoli, Italy	late Middle Pliocene
Mammuthus meridionalis	IGF 12787, M1	Upper Valdarno, Italy	Early Pleistocene
Mammuthus meridionalis	IGF 13730a, M2	Upper Valdarno, Italy	Early Pleistocene
Mammuthus meridionalis	IGF 44, M2	Upper Valdarno, Italy	Early Pleistocene
Mammuthus meridionalis	IGF 13730b, M3	Upper Valdarno, Italy	Early Pleistocene
Mammuthus meridionalis	IGF 1147, M3	Upper Valdarno, Italy	Early Pleistocene
Mammuthus meridionalis	IGF 145, dp3	Upper Valdarno, Italy	Early Pleistocene
Mammuthus meridionalis	IGF 151, dp4	Upper Valdarno, Italy	Early Pleistocene
Mammuthus meridionalis	IGF 69, m3	Upper Valdarno, Italy	Early Pleistocene
Mammuthus meridionalis	IGF P-61, M3	Farneta, Italy	late Early Pleistocene
Mammuthus meridionalis	IGF-PM1, m3	Pietrafitta, Italy	late Early Pleistocene
Mammuthus trogontherii	IQW 3046, M3	Süssenborn, Germany	early Middle Pleistocene
Mammuthus primigenius	IGF 1086b, M3	Arezzo, Italy	late Middle Pleistocene
Mammuthus primigenius	KOE 389 M3	Sickenhofen, Germany	Late Pleistocene
Mammuthus primigenius	IGF 2289, m1	Tiber Valley, Italy	Late Pleistocene
Elephas maximus	IGF-E1, m3	India	Recent
Loxodonta africana	KOE 164, M?	zoo specimen	Recent
Loxodonta africana	IGF-L1, M2	zoo specimen	Recent

[1]Specimen previously attributed to *Archidiskodon gromovi* (Aleskeeva and Garutt, 1965) by Azzaroli (1977).

Enamel microstructure terminology and abbreviations.—Enamel cement junction (ECJ): the boundary plane between enamel and crown cement; enamel dentine junction (EDJ): the boundary plane between dentine and enamel. During tooth formation, amelogenesis starts at the EDJ; enamel prism: bundles of apatite cristallites extended from the EDJ close to the outer enamel surface; Hunter-Schreger bands (HSB): a specific mode of prism decussation, with prisms decussating in layers. Prisms within one HS band are oriented parallel to one another, and at an angle to prisms in adjacent bands; interprismatic matrix (IPM): the apatite cristallites between prisms; outer enamel surface (OES): the outer limit of enamel (in elephant molars, the OES is usually covered by cement); prism decussation: crossing over of prisms or groups of prisms, due to lateral bending of prisms along their way from the EDJ to the OES; prism sheath: boundary plane produced by an abrupt change in apatite cristallite orientation, delimiting a single enamel prism.

Abbreviations of collections and institutions.—KOE, enamel collection of the Institut für Paläontologie, Rheinische Fridrich-Wilhelms-Universität Bonn, Germany; IGF, Museo di Storia Naturale – Sezione di Paleontologia e Geologia, Università degli Studi di Firenze, Italy; IQW, Forschungsinstitut und Naturmuseum Senckenberg Forschungsstation für Quartärpalaeontologie, Weimar, Germany; MAA, Museo Archeologico, Arezzo, Italy.

Other abbreviations.—M, upper molar; m, lower molar; DP, deciduous upper premolar; dp, deciduous lower premolar.

Material and methods

Material

Fossil teeth of *Mammuthus meridionalis* (Nesti, 1825) from Upper Valdarno (Italy; early Early Pleistocene; Azzaroli 1977) were sectioned for enamel microstructure analysis under the SEM and the light microscope. Five of the six cheek teeth constituting mammoth primary and adult dentition, were sampled. These teeth are here referred to as deciduous third (DP3) and fourth (DP4) premolar; first (M1), second (M2), and third (M3) molar (lower case characters are used for corresponding lower teeth). Further comparative material was prepared and examined in order to cover the successive Plio–Pleistocene Eurasian mammoth species and the two extant elephant species (Table 1). The comparative material includes specimens of: *M. rumanus* (Stefanescu, 1924), from Montopoli (Italy; late Middle Pliocene), representing the earliest occurrence of *Mammuthus* in Western Europe, (Azzaroli 1977; Lister and Sher, 2001; Lister and van Essen 2003); *M. meridionalis* from Pietrafitta and Farneta (Italy; late Early Pleistocene; Ferretti 1999); *M. trogontherii* (Pohlig, 1885) from Süssenborn (Germany; early Middle Pleistocene; Günther 1969); *M. primigenius* from various European late Middle Pleistocene to Late Pleistocene localities; *Elephas maximus* Linnaeus, 1758 (Recent); *Loxodonta africana* (Blumenbach, 1797) (Recent). The four *Mammuthus* taxa investigated in this work are morphologically coherent units with a defined boundary in time and space (Fig. 1), distin-
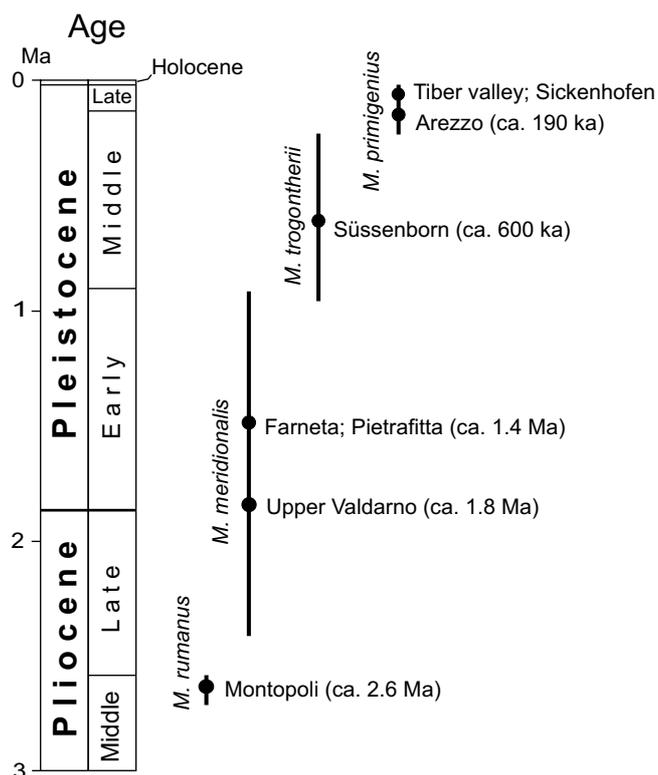
Fig. 1. Chronological distribution of *Mammuthus* species in Europe and ages of fossil samples considered in this study.

guished from each other by morphological differences that are quite stable across fossil samples (Lister 1996; Lister and Sher 2001). Current evidence makes it increasingly likely that they are not just successive samples from a reproductive continuum (i.e., chrono-species), but, actually, that each younger taxon is the product of a cladogenetic event (allopatric speciation) of its corresponding ancestral species (Lister and Sher 2001; Ferretti 1999). This is here interpreted as evidence in support of their status as "real" species.

Sample preparation

From each molar or molar fragment, small portions of dental tissue were removed and their position with respect to the complete tooth recorded. Enamel samples were then embedded in epoxy resin and sawn according to defined planes of sections (see below) with a circular diamond saw for mineralogical sampling. Sections were polished with wet aluminum oxide powder from coarser to finer (grit number 400, 600, and 1000) and then etched with 2N HCl for 2–5 seconds, in order to render visible prisms. After this step specimens underwent different preparation according to the method of analysis (see below). Occlusal enamel fragments were examined without preparation.

Thin sections.—Polished enamel surfaces were glued on slides using a transparent two-component epoxy glue. Specimens were then ground to a thickness of about 10 micrometres, followed by polishing and etching with HCl 2N for

2–3 seconds. Finally, thin sections were covered with a coverslip fixed with a photo-hardening glue.

SEM.—Samples for SEM analysis were glued on aluminum stubs using a conductive carbon paint and then coated with gold-palladium alloy or with graphite. The latter was used to prepare specimens for microprobe microanalysis.

R**eflected light microscopy**.—Coated enamel sections were examined under the reflected light microscope using the optic-fiber effect of prisms (Koenigswald and Pfretzschner 1987). This method allowed a rapid documentation of relatively large enamel portions.

Planes of section

In order to achieve a three-dimensional reconstruction of the enamel structure, sections of enamel were examined in various planes. Plane orientation is relative to the tooth vertical axis (occlusal-basal axis; Fig. 2), to the EDJ or to the OES. For thin sections sagittal (parallel to the tooth occlusal-basal and mesio-distal axes) and horizontal (perpendicular to the occlusal-basal axis) planes were prepared. In addition to these, tangential (parallel to the OES) and oblique (relative to the EDJ) sections were prepared for SEM and reflected light analysis.
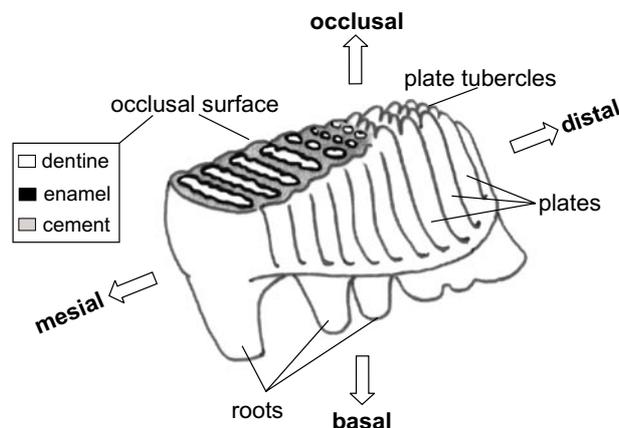
Fig. 2. Schematic representation of a mammoth lower molar. Anatomical features, distribution of dental hard-tissues on the occlusal surface, and principal directions are shown.

Measurement of enamel thickness

In order to quantify differences among taxa in relative thickness of the layers forming the molar enamel (see below), mean percentage thickness of each layer relative to the whole enamel thickness, has been calculated. Measurements of enamel layer thickness were made using a micrometric cursor connected to a light microscope. Measurements were taken along a line perpendicular to the EDJ. As enamel thickness may vary locally along the enamel section, a minimum of three measurements was taken at different sites on each section (sagittal), and a mean value then calculated for each

specimen. Further measurements were then added and the mean newly calculated, until the addition of the last measurement did not modify significantly (P < 0.05) the mean from the previous score.

Enamel types and schmelzmuster

Enamel types are defined on the basis of the direction of the prisms relative to the EDJ (Koenigswald and Clemens 1992). Inclination of prisms was measured as the angle between prism and the normal to the EDJ, according to Korvenkontio (1934).

The term schmelzmuster, introduced by Koenigswald (1980), refers to the spatial distribution of the various enamel types within a single tooth. The structural organization of enamel (schmelzmuster) was here considered independently from the thickness of the various enamel types (differential enamel thickness).

Results

The following description summarizes the morphological characters of the enamel common to all the considered *Mammuthus meridionalis* molars.

Crystallite orientation

Apatite crystallites, the basic constituent of enamel prisms, are parallel to each other and to the prism long axis. Crystallites in the interprismatic matrix are also oriented parallel to the neighboring prisms.

Prisms cross section

In their course from the EDJ to the OES, prisms vary both in cross section and absolute size. The predominant prism type is the key-hole pattern (type 3 of Boyde 1965). In the inner enamel prisms are arcade-shaped with a diameter of about 5–6 microns (Fig. 3A$_1$). More outwardly, prisms widen slightly and develop two lateral expansion (lobes) producing the so called "ginko-leaf" morphology (Kozawa 1978), typical of proboscidean enamel (Fig. 3A$_2$). In the outer enamel, prisms more markedly augment their diameter, while their cross section becomes more simple, losing the ginko-leaf outline (Fig. 3A$_3$, C). Frequent in this zone is the apparent fusion between contiguous prisms. This unique feature would have important implications for the understanding of enamel formation. However, the possibility that either diagenesis (but see below) or the sample preparation technique could have caused the disappearance of part of the (originally present) prism sheath, cannot be ignored. Ongoing analyses are specifically addressing this point.

Restricted to a very thin zone near the OES, a basically different prism type is found, characterized by small, sub-circular and irregularly spaced prisms, separated by abundant interprismatic matrix (IPM). Prism sheaths are open basally or, in some places, they completely surround the prisms (Fig. 3B$_1$, B$_2$), a pattern that can be considered intermediate between type 1 and 3 of Boyde (1965).

Enamel types

3-D enamel.—The three-dimensional pattern of this enamel type is rather irregular, with no recognizable structural "units". Prisms depart from the EDJ parallel to each other and immediately join to form bundles of varying thickness that interweave in all the three directions (Fig. 4A, B$_1$). On the average bundles are 15–20 prisms thick. However, the number of prisms along each bundle is not constant, as single prisms may leave one bundle to join another after changing direction. The thickest and predominant bundles are those rising to the occlusal surface at 45°–50° (Fig. 4B$_1$). These bundles usually maintain their orientation throughout the entire thickness of the 3-D enamel zone and pass, without deviating, into successive enamel zones characterized by different enamel types. A second set of bundles is directed basally at about 50° (Fig. 4B$_1$). These latter bundles usually maintain their orientation for short distances, always limited within the 3-D enamel zone. A third orientation assumed by the prisms bundles is sub-horizontal and also in this case prisms maintain this attitude only within the 3-D enamel zone (Fig. 4B$_1$). In sagittal section, regions of about 100 micrometres height, where all prisms are parallel and are either occlusally or, less frequently, basally oriented, are found. The bundles are here particularly extended vertically but yet lack a lateral continuity (they do not form layers). In thin section, in fact, it is clearly observable, by slowly changing the focus, and thus the depth of the observed plane, that adjacent bundles (in a bucco-lingual direction) possess different orientations. Prisms associated with 3-D enamel posses a key-hole cross section.

Hunter-Schreger bands.—The frequency of successive HSB and the sinuosity of each band vary on their way to the outside. In sagittal sections HSB are initially densely packed and markedly undulated. HSB frequency then quickly decreases while the course of the bands become rather straight or only slightly undulated, rising toward the occlusal surface (Fig. 4B$_2$). In tangential section the bands are always wavy. Borders between bands may be either sharp or more or less fuzzy. Seen at the SEM each band corresponds to a layer of equally oriented prisms, at an angle with those within adjacent layers (horizontal decussation; Fig. 4C). Prisms within bands are occlusally oriented, rising at about 60°. Individual HSB shows varying thickness as variable is the angle of decussation which is, generally speaking, low. Prisms cross section displays mostly the ginko-leaf morphology.

Radial enamel.—This is formed by parallel prisms, directed radially away from the EDJ. In *M. meridionalis* three different subtypes of radial enamel are found. The first subtype (A) is characterized by prisms rising toward the occlusal plane
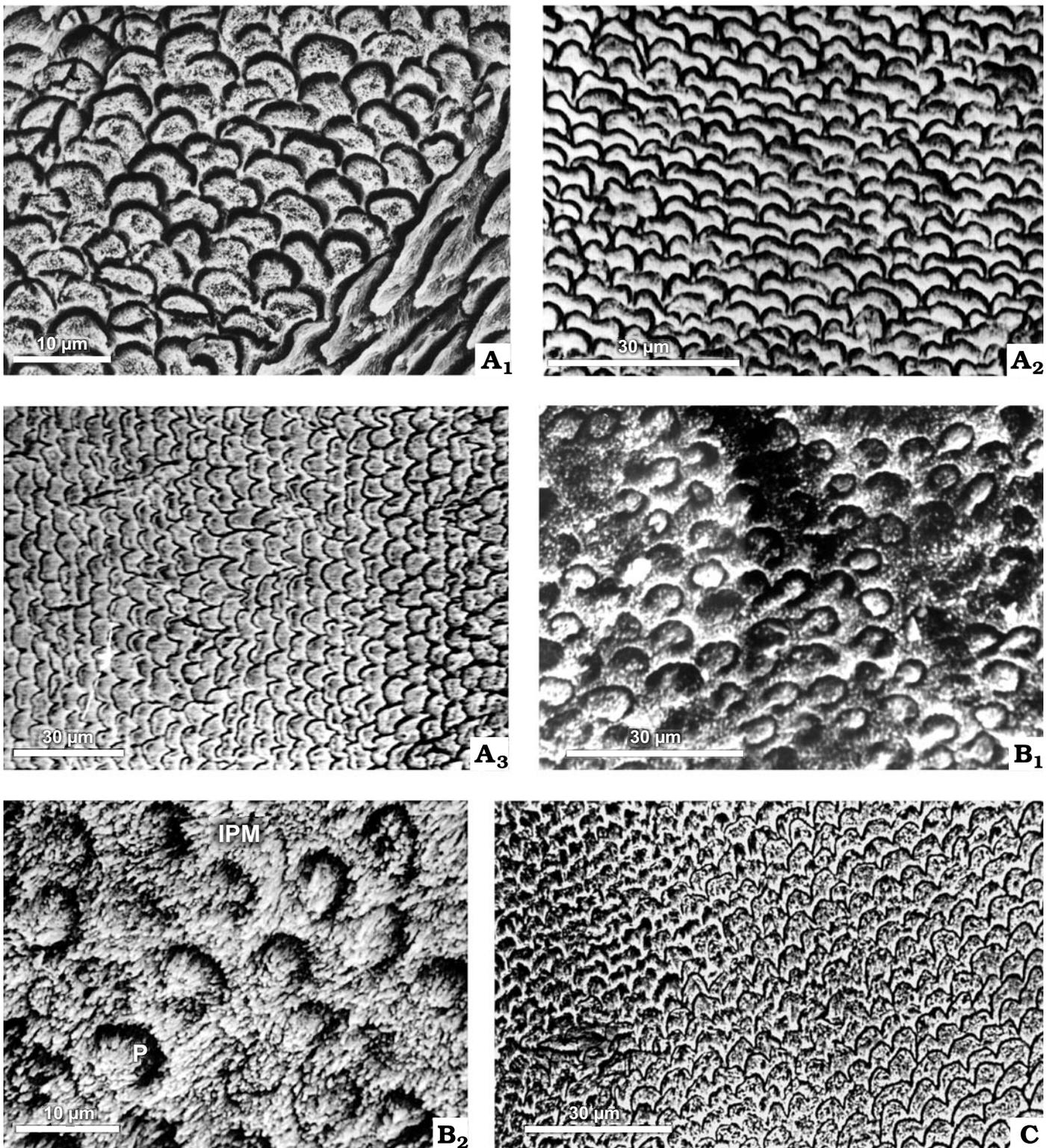
Fig. 3. Variability of the enamel prism cross-sections in the molars of Early Pleistocene southern mammoth, *Mammuthus meridionalis* (Nesti); Upper Valdarno, Italy. **A**. IGF 13730a; M2. A_1. Sagittal section of inner enamel layer with prisms showing a keyhole cross-section. A_2. Horizontal section of middle enamel layer; prism cross-sections show a ginko-leaf pattern. A_3. Horizontal section of outer enamel layer with prisms showing arch-shaped cross-sections and frequent fusion with adjacent prisms. **B**. IGF 13730b; M3. B_1. Tangential section of enamel outer layer, near the ECJ, showing prisms with square cross-sections, surrounded by abundant IPM. B_2. Orientation as in D, but at higher magnification, showing the thick interprismatic matrix (IPM) between the prisms (P). IPM cristallites are parallel to the prisms. **C**. IGF 1147; M3; oblique section through enamel middle and outer layers showing how prism cross-sections vary from the EDJ (top, left) to the OES (bottom, right).
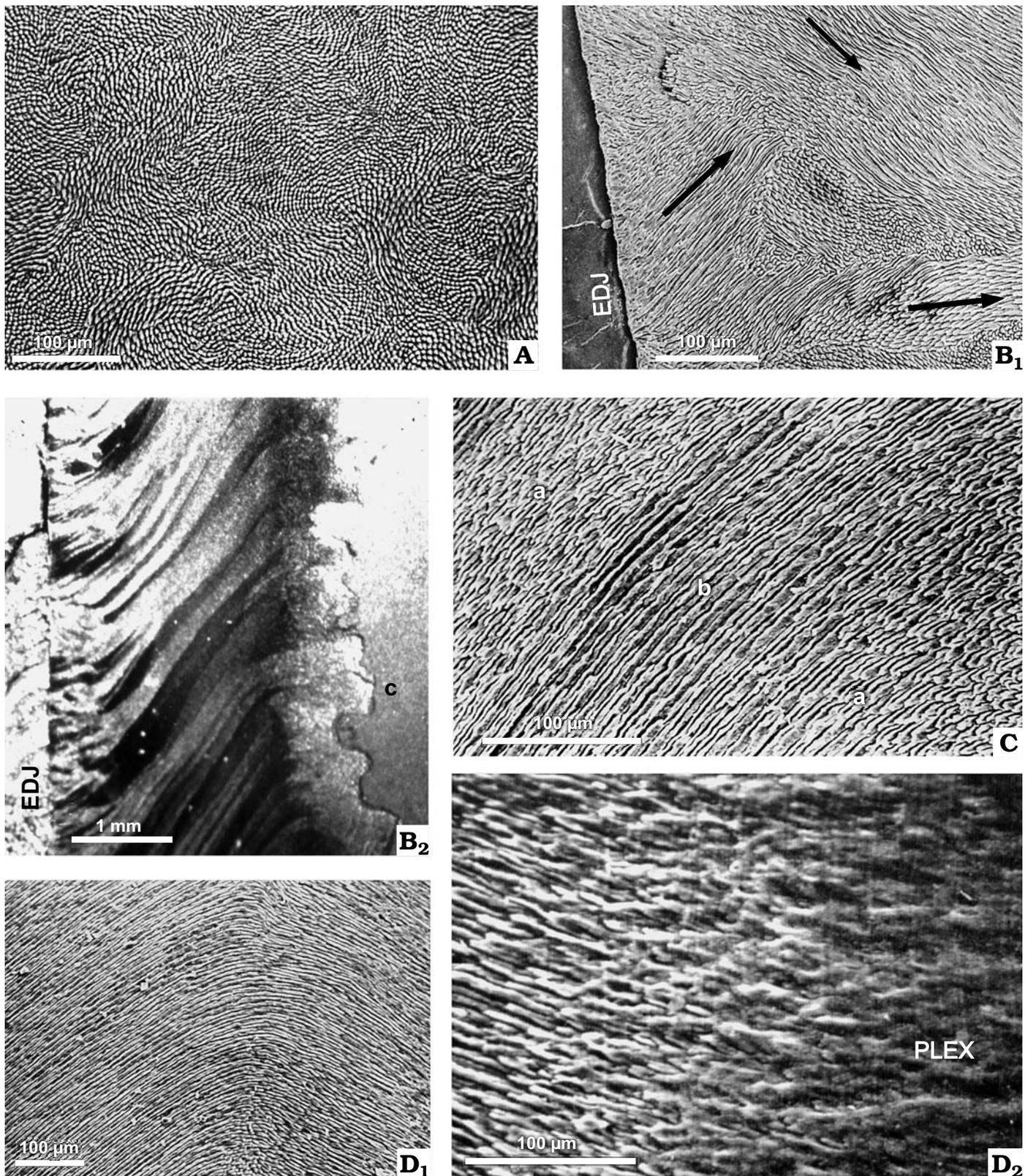
Fig. 4. Enamel types in the molars of Early Pleistocene southern mammoth, *Mammuthus meridionalis* (Nesti); Upper Valdarno, Italy. **A**. IGF 13730a; M2, tangential section of enamel at the EDJ, viewed toward the OES; occlusal surface at top; almost all prisms are sectioned perpendicularly to their long axis and are irregularly packed. **B**. IGF 13730b; M3. B_1. Sagittal section of inner enamel layer made up of 3-D enamel; arrows show the directions of three prism bundles surrounding a central triangular area with prisms directed perpendicularly to the figure plane; occlusal surface at top. B_2. Reflected light micrograph of the same sample as in B_1; occlusal surface at top; the optic-fiber effect of prisms evidences the complex structure of the inner enamel layer, along the EDJ, and the wavy HSB in the middle layer. The OES, in contact with the cement (c), is particularly irregular. **C**. Sagittal section of enamel middle layer com- →

with an angle of about 60° (Fig. 4D$_1$). Prism outline varies from the ginko-leaf pattern to the key-hole one.

In the second subtype (B), prisms are sub-parallel to the occlusal surface and normal to the enamel outer surface (Fig. 4D$_1$), possessing an irregular key-hole transverse section (Fig. 3A$_3$). The transverse diameter of the prisms forming radial enamel subtype B is greater than in the previous enamel types and the apparent fusion (see paragraph on Prism cross section) between contiguous prisms is frequent (Fig. 3A$_3$, C). IPM is absent. The third radial enamel subtype (C) is also characterized by prisms parallel to the masticatory surface, but prism cross section narrows and becomes sub-circular. The prismatic sheath may either completely surround the prisms or be open basally (Fig. 3B$_1$, B$_2$).

Prismless enamel.—This type lacks a prismatic organization and is composed by a matrix of apatite crystallites parallel to each other (Fig. 4D$_2$). However, average crystallite orientation is locally variable (see below).

Distribution and variability of enamel types

Schmelzmuster.—The schmelzmuster of *M. meridionalis* molars is three-layered (Fig. 5). The innermost layer, in contact with the dentine, is formed by 3-D enamel. It represents about 15–20 per cent of the entire enamel thickness. The middle layer makes up almost 50–60 per cent of the total enamel thickness and has two zones: in an innermost zone the enamel is formed by HSB; the outermost zone consists instead of radial enamel. The transition between these two enamel types is rather gradual. Passing from the zone with HSB to that with radial enamel, prism decussation weakens and eventually prisms become parallel to each other. On the contrary the boundary between the middle and the third, outermost layer, is marked by a sudden flattening of the inclination of the prisms, which become parallel to the occlusal plane (Fig. 4D$_1$). The outermost layer can be divided, however, into two rather distinct zones: (1) an innermost one characterized by prisms showing a key-hole cross-section and without IPM (radial enamel subtype B), and (2) an outer zone exhibiting prism with a rounded cross-section and abundant IPM (radial enamel subtype C). The outermost layers constitutes, on the average, about 21 per cent of the enamel thickness. In almost all the samples a thin layer of prismless enamel occurs near the OES. This last enamel type will be discussed further below.

Intraspecific variability.—Analysis of an unworn molar plate (IGF 13730b) showed that the schmelzmuster of *M. meridionalis* varies along the height of the crown. The enamel capping the plate tubercles does not have the inner 3-D enamel layer and consists of only two layers. The innermost zone of the inner
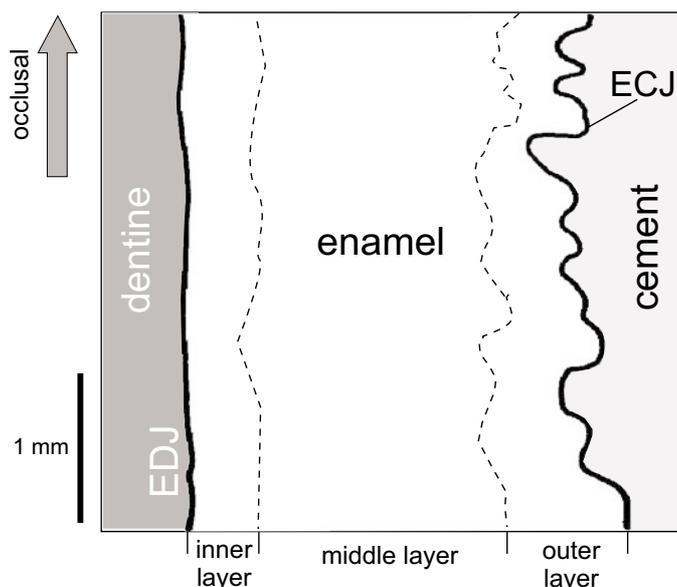
Fig. 5. Schematic representation of a sagittal section of upper molar enamel of *M. meridionalis*, based on Fig. 4B$_2$. Boundaries between inner, middle, and outer enamel layers are shown by vertical dotted lines.

layer is composed, instead, by thin HSB (Fig. 6A$_1$, A$_2$), disposed in concentric layers around the tubercles' vertical axis. More outwardly the HSB give place to a zone of radial enamel (subtype A). A simultaneous bend of the prisms from a steeply rising direction to a nearly horizontal one, produces a thick outer layer formed by radial enamel subtype B. Further down, about 20 mm from the tubercle tips, the enamel displays the three-layered schmelzmuster, as described in the previous section. The same vertical variation is found in *Loxodonta africana* (specimen KOE 164) and has been described in *Elephas recki brumpti* Beden, 1980 by Bertrand (1987). The entire sample of *M. meridionalis* teeth, both uppers and lowers, examined in this study, adheres to this schmelzmuster. The only noticeable difference was found in the dp3 (specimen IGF 145), that shows a stronger decussation of the HSB (Fig. 6B). On the other hand, the dp4 (specimen IGF 157) is characterized by HSB with a lower angle of decussation, comparable to that of the adult molars (M1, M2, and M3).

Specimen IGF 13730a is a molar fragment formed by the posterior enamel crest of a plate and the anterior one of the succeeding one. No differences in thickness and microstructure between the two enamel crests were detected.

Growth structures

In thin section the Striae of Retzius are clearly visible. They are nearly parallel to the EDJ (Fig. 6C). Each stria represent

posed by weekly decussating horizontal prisms layers (HSB): a, b label bands with prisms running, respectively, occlusally and labially (intersecting the figure plane), and occlusally and parallel to the figure plane; EDJ at left, occlusal surface at top. **D**. IGF 69; m3. D$_1$. Sagittal section of enamel showing the boundary between middle (left) and outer (right) enamel layers, marked by the simultaneous bending of prisms (at center); EDJ at left, occlusal surface at top. D$_2$. Sagittal section of the same, outer enamel layer, near the OES; EDJ at left, occlusal surface at top; between radial enamel subtype B (left) and PLEX (right) there is a narrow transitional zone made up by radial enamel with thick IPM (subtype C; at center).
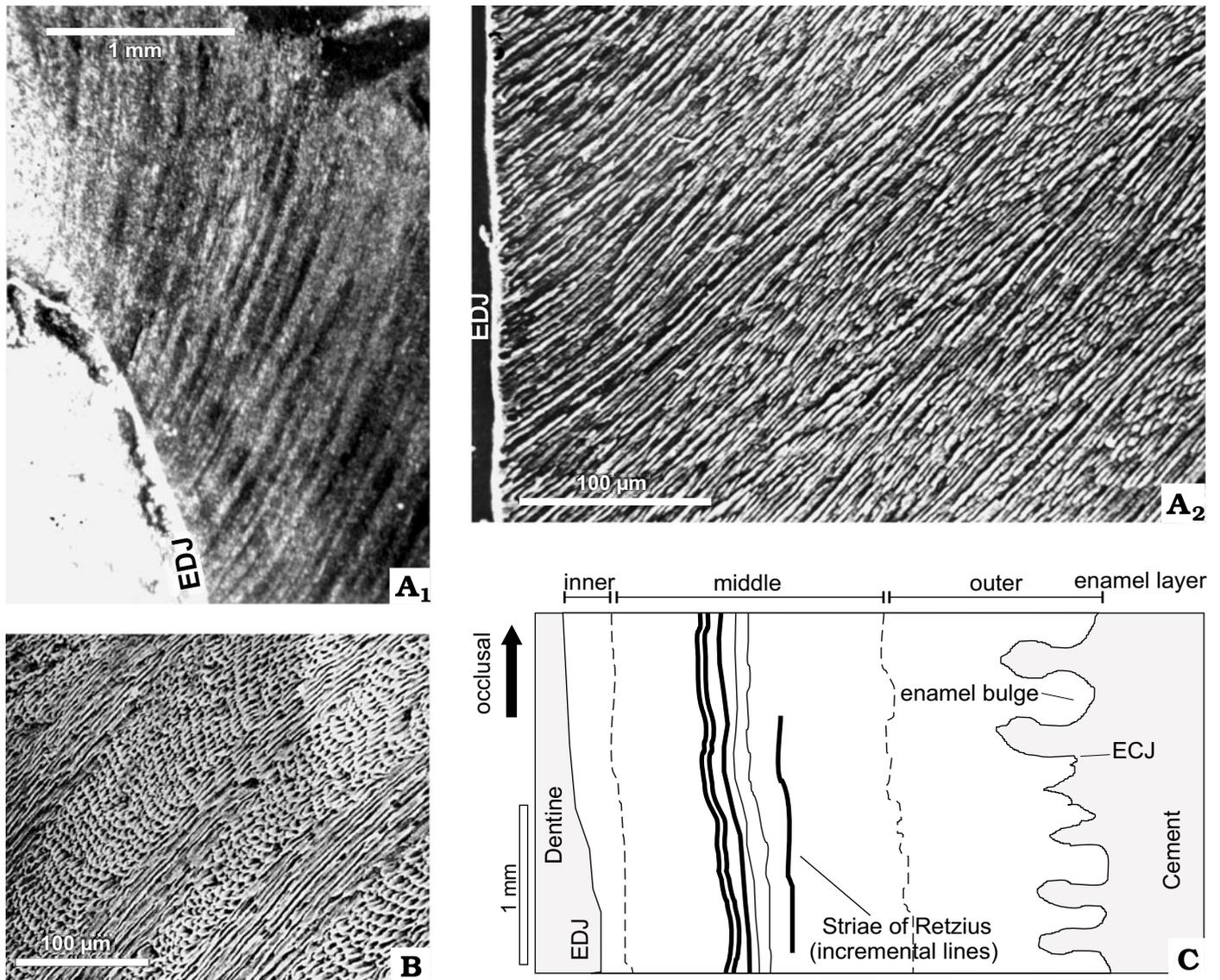
Fig. 6. Enamel types and growth structures in the molars of early Pleistocene southern mammoth. **A**, **B**. *Mammuthus meridionalis* (Nesti); Upper Valdarno, Italy. **A**. IGF 13730b; M3. A$_1$. Reflected light micrograph of a sagittal section of a molar plate tubercle, showing thin HSB in the innermost portion of enamel; occlusal end at top. A$_2$. SEM micrograph of the same sample as in A$_1$, showing the thin, straight and only slightly decussating HSB. **B**. IGF 145; dp3, sagittal section of the middle enamel layer made up by strongly decussating HSB; EDJ at left, occlusal surface at top. **C**. Schematic representation of a sagittal section of upper molar enamel of *M. meridionalis*, based on a thin section prepared from specimen IGF 13730b. The drawing outlines a set of incremental lines (striae of Retzius) in the center of the middle enamel layer. Morphological details of the OES are showed.

the position of the enamel growth front in correspondence of periodical interruptions or slowing down of the mineralization process. The contrast between light and dark bands seems due to a different degree of mineralization of the enamel (see Carlson 1995 for a review). The incremental lines seen in *M. meridionalis* enamel are characterized by variable thickness. They met the OES at a very low angle.

Prisms themselves present along their longitudinal aspect periodic micro-variations of crystallite orientation that give origin to a series of varicosities, analogous to that described in human enamel (Schroeder 1992). These structures are also related to the accretionary rate, but of shorter period, possibly daily (Boyde 1976), with respect to the striae of Retzius.

Prismless enamel and the enamel-cement junction

The OES, covered by cement, is extremely wrinkled. (Figs. 4B$_2$, 6C). This roughness is determined by (1) vertical undulations (parallel to the plate vertical axis), affecting, at variable depth the entire enamel band, and (2) small rounded protuberances, affecting only the outer portion with prismless enamel. Under the light microscope these protuberances are visible, in sagittal section, as marked, sometimes drop-like, convexity of the ECJ. As a result, the ECJ appears extremely indented (Fig. 6C). As a consequence there is a deep interlocking of the enamel outer surface with the cement layer. Observed under

high resolution polarized light, the enamel can be seen to bulge along the ECJ, and it is possible to note that crystallites are not parallel, as in the rest of the prismless enamel zone, but radially oriented, around the bulge's symmetry axes. Moreover, incremental lines, visible in some cases, evidence an arch-shaped growing front for the bulges. These observations seem to suggest that the occurrence of such an external enamel zone, which lacks a prismatic organization, is essential to the development of the rough OES.

Enamel occlusal surface

The wear-induced occlusal surface (secondary surface) of elephant (subfamily Elephantinae, sensu Maglio 1973) molars is not perpendicular to the tooth vertical axis, but rather mesially sloping in both upper and lower molars (actually, in lower molars this is the case when they are at an intermediate stage o wear). The enamel band of each plate is sectioned on the occlusal plane and gives origin to two transverse crests, distally inclined in upper molars, and normal to distally inclined in the lower ones (Fig. 7). In the molars of this sample, the enamel crests have a round sagittal section due to polishing from food items and repeated contact with opposing enamel crests during occlusion. The prevalent direction of the power stroke in the mastication process is revealed by the dentine and cement profile, as observable in sagittal sections (cf. Koenigswald et al. 1994). In the lower molars of the sample, dentine and cement between enamel crests are more excavated in a distal direction. The reverse occurs in the upper molar (Fig. 7). It follows that in *Mammuthus*, as it is generally accepted for recent elephants, mastication is mostly unidirectional. In particular the power stroke direction is horizontal and forward. This determines the leading and trailing edges (Greaves 1973; Costa and Greaves 1981), that correspond, respectively, to the plate mesial and distal enamel crests in lower molars and to the distal and mesial ones in upper molars (Fig. 7). As already reported above, the leading and trailing crests have the same schmelzmuster in *M. meridionalis*, and do not differentiate in thickness. However, because of the symmetry between the leading and trailing enamel crests with respect to the plate's dentine core, the enamel prisms of the two crests
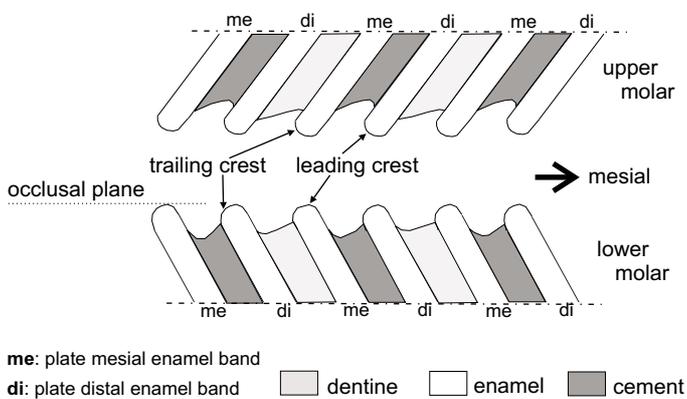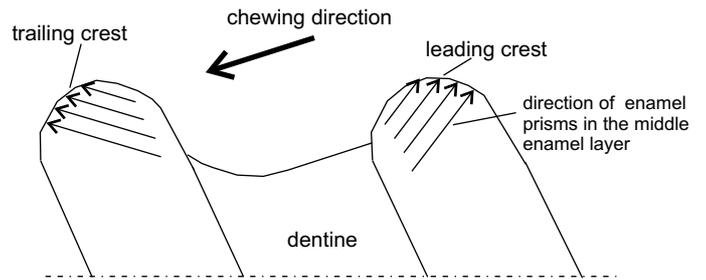
Fig. 8. Schematic representation of the sagittal section of the leading and trailing enamel crests of a lower molar plate, showing the convex outline of the enamel crests, the direction of enamel prisms (arrows) in the middle enamel layer, and the chewing direction. The latter is determined by the relative movement of the opposite teeth during mastication.
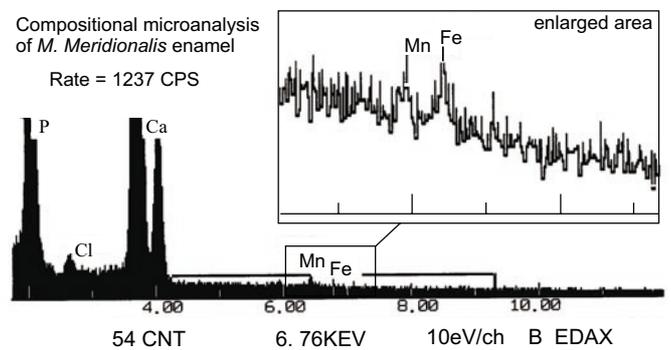
Fig. 9. Chemical compositional microanalysis of *M. meridionalis* enamel (IGF 13730b). The analysis of the outer enamel layer revealed, beside the presence of calcium (Ca) and phosphate (P), the occurrence of iron (Fe) and manganese (Mn), interpreted as diagenetic secondary elements. The chlorine (Cl) signal is due to the use of HCl to etch the enamel section. CPS, counts per second.

are directed in opposite directions. In particular, in the central and most elevated portion of the leading crest, corresponding to the enamel middle layer, prisms meet the surface at an angle and are oriented opposite to the chewing direction. In the trailing crest prisms are also occlusally directed, but point toward the chewing direction (Fig. 8).

Diagenetic alterations of the enamel

The high stability of apatite makes enamel particularly resistant to diagenetic processes. This is testified by the SEM micrographs which show that microstructural features of the fossil samples are perfectly preserved. This is further proven by comparison with recent material of *E. maximus* and *L. africana*. For these reasons all the features of *M. meridionalis* enamel so far described are here considered as "primary". Nevertheless, enamel of *M. meridionalis* presents often, in correspondence to the outermost layer, a brownish coloration, very evident in thin section, where it contrasts with the transparent inner layers. In polarized light this layer assumes the typical colors of iron (Fe) minerals. A compositional micro-analysis with a microprobe confirmed the

Fig. 7. Schematic representation of the sagittal section of opposite *Mammuthus* molars, showing details of the occlusal surface.

Table 2. Enamel thickness differentiation in the upper M3 of four successive Eurasian *Mammuthus* species.

Taxon	Sample	Relative enamel thickness (%)						Enamel thickness (mm)		
			Inner layer		Middle layer		Outer layer			
		N	mean	min–max	mean	min–max	mean	min–max	N	mean	min–max
M. rumanus	Montopoli	1	15	–	54	–	31	–	2	3.5	3.2–3.9
M. meridionalis	Upper Valdarno and Farneta	3	17	13–21	62	55–67	21	18–24	43	3.2	2.6–3.9
M. trogontherii	Süssenborn	1	13	–	66	–	21	–	46	2.3	1.9–3.0
M. primigenius	Sickenhofen and Arezzo	2	9	6–10	75	71–79	16	13–23	3*	1.9	1.5–2.3

*material from Arezzo, Italy (IGF).

occurrence of Fe and manganese (Mn) within the outer enamel layer in two sample of *M. meridionalis* (Fig. 9). On the other hand, no traces of Fe have been found in the middle and inner layer of the same samples or at any location in the enamel samples of *E. maximus* or *L. africana*.

Enamel differentiation in *Mammuthus*

Both the studied molars of late Middle Pliocene *M. rumanus* from Montopoli and late Early Pleistocene *M. meridionalis* from Farneta and Pietrafitta show the same schmelzmuster as the specimens from Upper Valdarno (early Early Pleistocene). *M. trogontherii* and *M. primigenius* are also characterized by the same schmelzmuster of *M. meridionalis* and *M. rumanus*, even though they possess thinner enamel. On the other hand the molars of the four mammoth species examined differ in the relative thickness of the three enamel layers.

Table 2 reports the mean percentage thickness (see Meth-

ods) of the three enamel layers in the M3 of *M. rumanus*, *M. meridionalis*, *M. trogontherii*, and *M. primigenius*. Given the small number of specimens for each species, data should be evaluated with caution, however the results are consistent and allow some functional interpretations (see Discussion). The inner layer constitutes, in the first three species mentioned above, approximately 15 per cent of the whole enamel thickness. In *M. primigenius* the inner enamel is thinner than in the three older species, representing from 6 to 10 per cent of the whole thickness. The middle layer is, on the contrary, significantly ($P < 0.05$) thinner in *M. meridionalis* (62 per cent) and thicker in *M. primigenius* (75 per cent). Accordingly the outer enamel layer become relatively thinner passing from the oldest (*M. rumanus*; 31 per cent) to the youngest (*M. primigenius*; 16 per cent) species (Fig. 10).

Discussion and conclusions

Structure and function

This analysis reveals that molars of Eurasian *Mammuthus* species are characterized by a schmelzmuster composed by four main enamel types, with three radial enamel subtypes. Two major discontinuities delimit three layers: in an innermost layer the enamel is formed by a complex enamel type, termed 3D enamel by Pfretzschner (1994); the middle layer has occlusally rising prisms, organized into HSB in an innermost zone and into radial enamel, with occlusally rising prisms, in an outermost one; the outermost layer is built up by radial enamel with nearly horizontally oriented prisms, and a thin outer zone of a prismless enamel, in contact with the cement cover. This latter, "primitive" enamel type, which in mammals is frequently formed when ameloblast activity is ending (Boyde 1964; Schroeder 1992), and constitutes the so called prismless external layer (PLEX; Martin 1992), is found both in *M. meridionalis* and in the two extant species *E. maximus* and *L. africana*. PLEX seems thus to represent a constant component of the schmelzmuster of elephants too. It was also observed in the Eocene proboscidean *Numidotherium koholense* Mahboubi et al., 1986 (Numidotheriidae) from the Eocene of Algeria (Bertrand 1987). It is here suggested that PLEX, forming the extremely rough OES, increases adhesion of the crown cement.

While HSB, radial, and prismless enamel are common among placental mammals, 3-D enamel is restricted to the
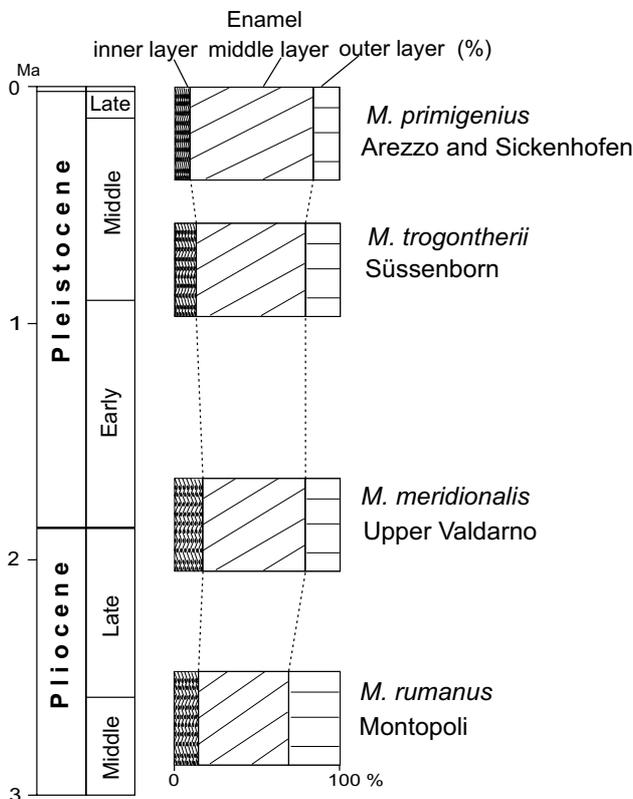
Fig. 10. Enamel thickness differentiation in four successive European mammoth samples. Descriptive statistics are given in Table 2.

Proboscidea. It can be defined as an irregular enamel, following the definition by Koenigswald and Sander (1997c) and was called 3-D enamel by Pfretzschner (1994) because it contains prism bundles running in all the three directions of space. 3-D enamel is found adjacent to the EDJ, where intensity of shear forces produced during occlusion are higher and shear forces are oriented in the three directions of space (Pfretzschner 1992, 1994). The complex structure of 3-D enamel seems thus a mechanical adaptation to withstand this stress pattern. Other mammals with hypsodont molars (e.g., *Equus caballus*, *Phacochoerus aethiopicus*, *Bos taurus*) also developed specialized enamel types near the EDJ, that, though structurally different, are functionally similar to 3-D enamel (Pfretzschner 1992, 1994). 3-D enamel is not present in the enamel capping the plate tubercles, where a two-layered enamel occurs. HSB are instead encountered in an innermost zone. This simplified schmelzmuster has been found also in *Elephas recki* and *Loxodonta africana* at the same location, which indicates this is a pattern typical for Elephantinae. A similar two-layered schmelzmuster characterizes the enamel of the Eocene proboscidean *Moeritherium lyonsi* Andrews, 1901 (Moeritheriidae) (Bertrand 1987; Ferretti unpublished data) and is therefore believed to be the plesiomorphic condition for Proboscidea. The occlusal end of an unworn elephant plate thus exhibits plesiomorphic features in both its gross morphology (i.e., subdivision in tubercles) and microstructure, relative to the rest of the plate. From this it also follows that increment of crown height, a trend characterizing elephant evolution, involves extension of the ontogenetic phase when the central portion of the tooth crown is formed. The same pattern has been demonstrated for hypsodont representatives of the Arvicolidae (Koenigswald 1993; Koenigswald and Kolfschoten 1996).

The predominant prism type in *Mammuthus* enamel is the type 3, or key-hole pattern, while a thin zone of an irregular type, intermediate between type 1 and 3, is confined to the outermost layer. Prism cross-sections, thus, varies from an open prism sheath to a closed prism sheath, along their course from the EDJ to the OES. Bertrand (1987, 1988) reports the occurrence of type 1 enamel close to the EDJ in two Eocene species from Pakistan of the basal proboscidean family Anthracobunidae, in *N. koholense*, and in a molar of an unidentified species of *Palaeomastodon* Andrews, 1901 from the Oligocene of Egypt. However, all the specimens examined in the present study have type 3 prisms at the EDJ.

Within each plate there is no differentiation of the enamel between "leading" and "trailing" enamel crest, even though the prevalently protinal (i.e., forwardly directed; Koenigswald et al., 1994) mastication of elephantids (Elephantidae, sensu Maglio 1973) should in theory produce different mechanical stress on the mesial and the distal enamel band of a plate (cf. Koenigswald 1980; Pfretzschner 1994). However, if enamel microstructure actually reflects stress direction and intensity, one would presume that in the molars of the investigated *Mammuthus* species these should be the same on both enamel crests (cf. Rensberger 1995b). Enamel differentiation

between leading and trailing crests is observed usually in species with very thin enamel (e.g., rodents), a characteristic that strongly constrains enamel microstructure (cf. Koenigswald and Sander 1997b). It can be argued that the relatively thick enamel of elephants (from 1 to 5 mm) guarantees an adequate robustness to both enamel crests, even though they may be subject to different tensile stresses.

All the studied cheek teeth of *M. meridionalis* present the same schmelzmuster. Only the dp3 is different in having stronger decussating HSB. As higher angles of decussation reinforce enamel to withstand fracture (Koenigswald and Pfretzschner 1987; Pfretzschner 1988) this could represent an adaptation to strengthen the relatively thinner enamel of the dp3 (about 1 mm).

Evolution

A characteristic of the dental evolution of Eurasian *Mammuthus* is the progressive reduction of enamel thickness (Lister 1996; Maglio 1973; Aguirre 1969). From the present study it is concluded that a thinner enamel evolved in late *Mammuthus* species through a progressive shortening of the period of formation of the enamel band during tooth ontogenesis (see Martin 1985), and not by losing one or more enamel zones, as is the case in some rodent lineages (e.g., *Microtus* and *Arvicola*; Koenigswald 1980). The schmelzmuster maintained, in fact, the same structural characteristics along the Eurasian mammoth line. On the other hand, evidence presented in this study indicates that, along with the enamel thinning trend, the relative thickness of the three layers constituting the mammoths' enamel underwent a differential modification. In particular the relative thickness of the outermost layer, where enamel prisms are parallel to the occlusal surface and thus offer a lesser resistance against wear (Rensberger and Koenigswald 1980), decreased in the transition from *M. rumanus* to *M. primigenius* (Fig. 10). In contrast, the middle layer, which is made up by prisms set at an angle with the occlusal surface (an attitude that makes enamel more resistant to wear) increased its relative thickness. The enamel differentiation observed in the Eurasian mammoth line could represent an adaptation to keep the rate of wear to a minimum as the enamel became thinner and/or the diet more abrasive. To corroborate this hypothesis, however, additional data are needed. It would also be of great interest to investigate whether the American *Mammuthus* lineage (*M. "hayi"* – *M. imperator* – *M. columbi*), that also evolved thin-enameled hypsodont molars (Madden 1981), underwent similar microstructural changes.

Diagenesis

The presence of iron demonstrated in many *M. meridionalis* enamel samples and its absence in those from extant species, would suggest a secondary, i.e., diagenetic, origin of the iron, an hypothesis that is supported here. This hypothesis, however, does not easily explain why the iron-bearing layer ex-

actly matches with the enamel outer layer and no traces of iron has been revealed in the middle layer. The outer layer is made up mostly by a radial enamel with prisms wider than in the remainder of the enamel. If there is a relationship between iron enrichment and enamel structure, it must be supposed that the radial enamel forming the outermost layer is somehow more permeable to Fe ions than the other enamel types occurring in *M. meridionalis*.

Kamiya (1981), in a study on the alteration of the enamel in *E.* (*Palaeoloxodon*) *naumanni* Makiyama, 1924, considers the occurrence of prisms with badly defined outlines and of areas with prismless enamel near the OES, as modification of the apatite crystals due to diagenetic processes. From the figures and description of Kamiya, the supposed altered areas in *E.* (*P.*) *naumanni* enamel show similarities, respectively, with the radial enamel with IPM (subtype C) and to the PLEX found in *M. meridionalis*. The numerous observations made in the present analysis and comparisons with enamel of the extant species, however, strongly support the opinion that in *M. meridionalis*, these are primary features of the enamel.

Enamel structure and proboscidean systematics

Mammuthus species possess the same schmelzmuster in all their cheek teeth. The analysis revealed some minor differences among specimens, including the complexity of 3-D enamel, and the thickness of HSB, but at present, due to the small sample size, these cannot be distinguished from individual variability. It is important to distinguish the portion of the tooth crown being examined, since the apicies of the tubercles display a simpler structure, as opposed to that of the rest of the molar plate. The other elephant taxa examined (*E. maximus* and *L. africana*) exhibit the same microstructural pattern, further supporting the conclusion that in the cheek teeth of Elephantinae no structural differentiation exists. On the other hand the observed thickness differentiation in the Eurasian mammoth lineage would indicate that differential relative thickness of the enamel layers could represent a useful diagnostic character for intrageneric systematics. Preliminary comparison, as part of an ongoing work, with representatives of the principal proboscidean clades (Fig. 11) suggests that the occurrence, in the central portion of the tooth crown, of 3-D enamel and a schmelzmuster composed by six enamel types and subtypes (3-D enamel-HSB-radial enamel subtypes A, B, and C-prismless enamel) are synapomorphies of the Elephantoidea (sensu Tassy 1990). This complex schmelzmuster developed from a more simple one, like that found in the Eo–Oligocene *Moeritherium lyonsi* (Bertrand 1987, 1988; Pfretzschner 1994). The early diverging families Numidotheriidae, Barytheriidae, and Deinotheriidae developed, possibly from a moerithere-like pattern, a specialized enamel made up almost entirely by 3-D enamel (Remy 1976; Bertrand 1987; Koenigswald et al. 1993; Pfretzschner 1994). Representatives of the Early Oligocene genus *Palaeomastodon*, the sister taxon of all Neogene elephantoids (Tassy 1990), display an intermediate condition between
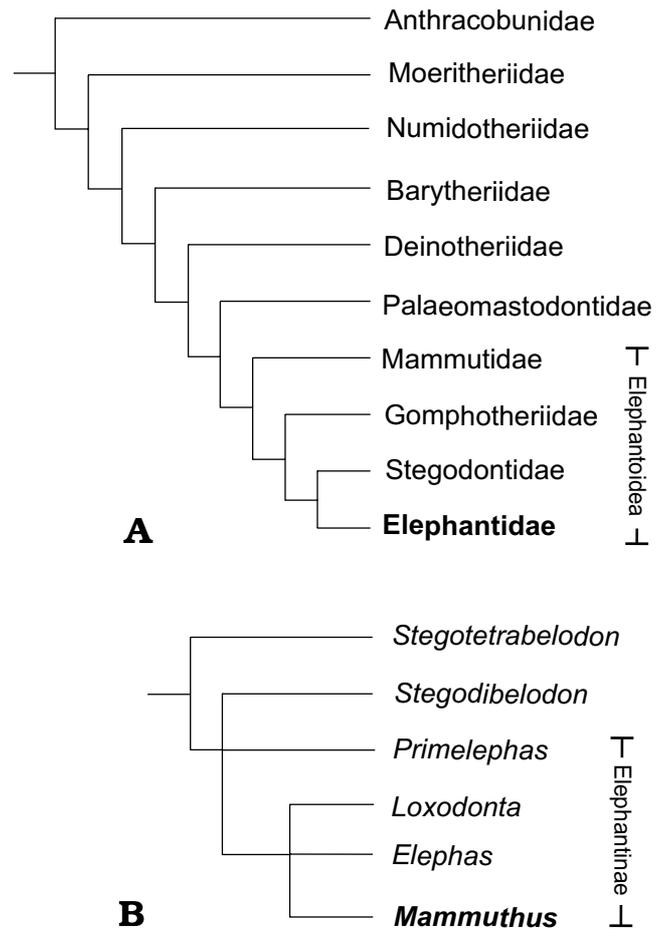
Fig. 11. **A**. Phylogeny of the Proboscidea (based on Tassy 1990). **B**. Cladogram of the Elephantidae, depicting the phylogenetic relationships of *Mammuthus* to the other elephant taxa (based on Tassy 1990 and Thomas et al. 2000).

those of the latter group and *M. lyonsi* (Bertrand 1997; Pfretzschner 1994). From these results it appears that enamel structure in proboscideans may represent an important tool for ingroup systematics at the species and family level.

Acknowledgments

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