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Enamel Structure of *Cuvieronius hyodon* (Proboscidea, Gomphotheriidae) with a Discussion on Enamel Evolution in Elephantoids

Marco P. Ferretti

Abstract Dental material of the South American elephantoid *Cuvieronius hyodon* from the Late Pleistocene of the Tarija Basin, Bolivia was sampled for a comprehensive analysis of the microstructure of the enamel. To examine variability at the dentition level, enamel samples of the upper incisor, second deciduous premolar, and molars were sectioned. The incisor and cheek teeth enamel is compared to that of other proboscideans in order to reveal phylogenetically and functional informative features useful to reconstruct the evolution of elephantoid enamel. Studies of the adaptations and evolution of proboscidean enamel have focused so far on molars. Nevertheless, given the possibility of an independent evolution of the enamel at different tooth positions, the variation of the enamel throughout the dentition needs to be taken into consideration when using enamel microstructural characters to infer proboscidean diversity and phylogeny. The results obtained from this study demonstrate the generality, among elephantoids, of the basic microstructural features of *Cuvieronius hyodon* enamel, allowing the characterization of the Elephantoid Enamel (EE). The differentiation between incisor and molar enamel seen in elephantoids is shown to represent a primitive elephantiform trait, as it also occurs in *Phiomia*. The three-layered enamel of the cheek teeth appears as the sole synapomorphy of the Elephantoidea, though the character might be homoplastic within the Proboscidea. Characters of the prisms cross-section might be used, on the other hand, to define less inclusive clades within the Elephantoidea.

Keywords Adaptation · Elephantiformes · Gomphotheriidae · Phylogeny · Schmelzmuster · South America · Tusk enamel

Introduction

Proboscideans possess a complex enamel microstructure, whose development and mechanical adaptation have attracted the attention of numerous investigators (Kawai 1955; Boyde 1964, 1969;

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Kozawa 1978; Kozawa et al. 1988; Bertrand 1988, 1989; Koenigswald 1988, 1997a; Pfretzschner 1992, 1994; Ferretti 2003a). Diversity of microstructure among proboscidean taxa offers, on the one hand, the possibility of discovering phylogenetically informative features and, on the other one, the opportunity of investigating the relationship between enamel microstructure and feeding adaptations (Remy 1976; Kozawa 1978, 1985; Bertrand 1988; Kamiya and Taruno 1988; Kamiya 1991; Koenigswald et al. 1993; Ferretti 2003b; Tabuce et al. 2007).

The diversification of the Proboscidea has been marked by a general trend toward larger body size and by extensive morphological changes of the craniodental system, considered to manifest in part a shift from a primitive browsing diet to a mixed or grass dominated one (Maglio 1973). Major evolutionary trends of the proboscidean dentition include the reduction of anterior teeth to a pair of enlarged incisors (tusks), the enlargement of the cheek teeth, the increase of relative tooth-crown height (hypsodonty), and the increase in the number of transversal crests forming the cheek teeth (Maglio 1973; Shoshani 1998). In order to cope with such different biomechanical situations, assuring an adequate resistance to fracture and abrasion, the enamel of proboscideans underwent important microstructural modification (Kozawa 1985; Bertrand 1988; Koenigswald et al. 1993; Pfretzschner 1994; Ferretti 2003a, b; Tabuce et al. 2007). Though the schmelzmuster of the incisors is known to differ from that of the molars (Kozawa 1983; Koenigswald 1988; Raubenheimer et al. 1995), most studies of the functional aspects and evolution of proboscidean enamel have been focused, so far, on molars. As a matter of fact, the antemolar dentition of proboscideans remains poorly sampled. Given the possibility of an independent evolution of the enamel at different tooth positions, the variation of the enamel microstructure throughout the dentition should be taken into consideration when using enamel microstructural characters to infer proboscidean diversity and phylogeny.

In this paper, the enamel of the upper incisor, second deciduous premolar, and molars (M1–M3) of the late Pleistocene South American gomphothere *Cuvieronius hyodon* is described in detail. Observations concern the nature of enamel outer surface, thickness, and microstructure. Thus, a comparison with other proboscideans, including a new description of the incisor enamel of *Phiomia serridens*, offers the opportunity to discuss the evolution of the enamel in the Proboscidea in general and in the Elephantoidea (sensu Tassy 1988), in particular.

Materials and methods

Primary study species

The species studied was *Cuvieronius hyodon*, a short-jawed gomphothere (Elephantoidea, Gomphotheriidae) representative of the so-called immigrant fauna that entered South America during the middle to late Pleistocene, at the acme of the Great American Faunal Interchange (Cione and Tonni 2001; Coltorti et al. 2007). It was widespread in the Central Andes during the late Pleistocene and was extinct by the end of the Last Glaciation (Hoffstetter 1950, 1986; Prado et al. 2005). The principal sample studied is from the late Pleistocene of the Tarija Basin, southern Bolivia. From this site several hundred remains of *C. hyodon* were collected, including tens of skulls and two complete skeletons (Nordenskiöld 1903; Boule and Thevenin 1920; Takai et al. 1982; Coltorti et al. 2007; Ferretti in press). Reconstructions of the palaeodiet of *C. hyodon*, based on stable isotope analysis, indicate that the species fed preferably on C3 leaves, although it had a significant fraction of more abrasive C4 phytolith grasses (MacFadden and Shockey 1997; Sanchez Chillon et al. 2004; Pellegrini 2005). This is

consistent with the relatively low-crowned, though not extremely brachydont cheek teeth possessed by *Cuvieronius*.

Cuvieronius hyodon possesses the derived elephantoid dental formula (1/0, 0/0, 0/0, 3/3) consisting of an upper incisor (Fig. 1a,b), modified as a continuously growing tusk and preceded by a deciduous tusk (tush), a vestigial lower deciduous incisor, three deciduous upper and lower premolars, and three upper and lower molars (Figs. 1c–g). The deciduous premolars are not vertically replaced by a second generation of premolars, a derived condition similar to, though independently acquired, from that of modern elephants (Shoshani 1996; Lambert and Shoshani 1998). A deciduous lower incisor was preserved in situ in one juvenile mandible, whereas some other mandibles presented a well-developed alveolus for the central incisor (Hoffstetter 1952; Ferretti unpublished data). In most of the examined juvenile mandibles, however, no traces of incisor alveoli were detected and, therefore, the tooth is considered as vestigial in *C. hyodon*. The studied material indicates that the lower deciduous incisor was lost
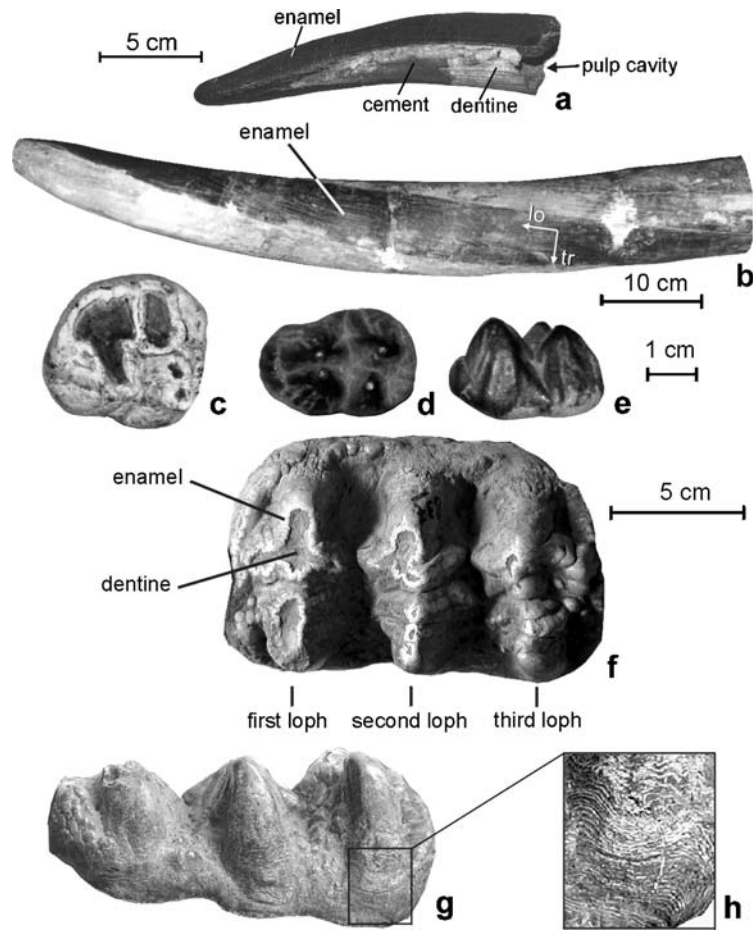
Fig. 1 Teeth of *Cuvieronius hyodon* from Tarija (Bolivia). **a** MNHN-TAR 799, juvenile tusk (*I2*), ventrolateral view. **b** NMR-M4562, left adult tusk (*I2*), lateral view. *Arrows* indicate the orientation of longitudinal (*lo*) and transversal (*tr*) enamel sections. **c** NMR 4429, right dP2, occlusal view. **d** MNHN-TAR 1234, left dp2, occlusal view. **e** Labial view. **f–h** MNHNLP 244, left M2. **f** Occlusal view. **g** Lingual view. **h** Close up of the outer enamel surface at the base of a cone to show perikymata. In all figures anterior is to the *left*.

at the time m1 came into wear and was not replaced by a permanent incisor. Following the hypotheses of homology with the therian dentition and the terminologies proposed by Raubenheimer et al. (1995), Tassy (1987, 1996a), and Luckett (1996), the teeth found in *C. hyodon* were designated as follows: the definitive upper incisor (tusk) is designated I2; upper and lower premolars are designated dP2 to dP4 and dp2 to dp4, respectively; upper and lower molars are designated M1 to M3 and m1 to m3, respectively.

The pattern of dental replacement in *C. hyodon* is similar to that of modern elephants: each cheek tooth (from dP2/dp2 to M3/m3) is replaced one after another in horizontal progression (horizontal tooth displacement) and no more than two functional teeth are usually present at any one time in each jaw quadrant.

Comparative material

Microscopic features of *C. hyodon* enamel were compared to the condition in other elephantoid taxa, which were selected to cover all the families presently recognized within the Elephantoidea (Table 1). To elucidate the origin of the elephantoid incisor and lateral teeth enamel, the comparison was extended to more basal proboscideans, including *Phiomia*, representing the sister taxon of the Elephantoidea (Tassy 1994, 1996b).

Comparative data are either based on direct observation of enamel samples (Table 1) or obtained from primary published descriptions. In particular, data on the following taxa were

Table 1 List of elephantoid enamel samples studied

Taxon	Specimen	Site	Age
Mammutidae			
Mammut americanum	KOE 446, M	New York, USA	L. Pleistocene
Gomphotheriidae			
Cuvieronius hyodon	MUT 5928, I2	Tarija, Bolivia	L. Pleistocene
Cuvieronius hyodon	MUT AG1a, I2	Tarija, Bolivia	L. Pleistocene
Cuvieronius hyodon	MNHN-TAR 799, I2	Tarija, Bolivia	L. Pleistocene
Cuvieronius hyodon	MUT 970, M1	Tarija, Bolivia	L. Pleistocene
Cuvieronius hyodon	MUT AG1b, M2	Tarija, Bolivia	L. Pleistocene
Cuvieronius hyodon	MUT 1197e, M3	Tarija, Bolivia	L. Pleistocene
Cuvieronius hyodon	MUT 266, dp2	Tarija, Bolivia	L. Pleistocene
Cuvieronius hyodon	MUT 5879, m2	Tarija, Bolivia	L. Pleistocene
Tetralophodon gomphotheres			
Anancus arvernensis	IGF 14296, M	Tuscany, Italy	L. Pliocene
Stegodontidae			
Stegodon sp.	KOE 439, M	Sangiran, Java	Pleistocene
Elephantidae			
Loxodonta africana	KOE 164, M	Zoo specimen	Recent
Mammuthus meridionalis	IGF 145, dp3	Upper Valdarno, Italy	E. Pleistocene
Mammuthus meridionalis	IGF 151, dp4	Upper Valdarno, Italy	E. Pleistocene
Mammuthus meridionalis	IGF 44, m2	Upper Valdarno, Italy	E. Pleistocene
Mammuthus meridionalis	IGF 13730, M3	Upper Valdarno, Italy	E. Pleistocene
Mammuthus primigenius	IGF 2289, m1	Tiber Valley, Italy	L. Pleistocene
Elephas maximus	IGF-E1, M3	Asia	Recent

taken from the papers indicated within parentheses: *Phosphatherium* (Tabuce et al. 2007), *Numidotherium* (Bertrand 1988, 1989; Tabuce et al. 2007), *Barytherium grave* (Bertrand 1988, 1989), *Moeritherium* (Bertrand 1988, 1989; Pfretzschner 1994), *Deinotherium* (Bertrand 1988, 1989; Pfretzschner 1994), *Palaeomastodon* (Kozawa 1978; Bertrand 1988, 1989; Pfretzschner 1994), *Phiomia* (Bertrand 1988, 1989; Pfretzschner 1994), Elephantoidea indet. (Koenigswald 1988), *Gomphotherium* (Fox 2000), *Loxodonta* (incisor enamel; Kozawa 1983; Raubenheimer et al. 1995).

Micrographs of thin sections of the incisor enamel of *Phiomia* "*wintoni*" (=*serridens*) from the Oligocene of El Fayum (Egypt), prepared by A.E. Anderson and included in the second volume of Osborn's monograph on the Proboscidea (1942: plate 27, fig. 6; pl. 28, figs. 1 and 2) were reinterpreted for the purpose of the present comparative analysis. As remarked by G. G. Simpson in the appendix to Osborn's monograph (1942: 1607–1608), the analysis of these sections was only preliminary undertaken by A.E. Anderson, who provided short notes "[....] useful as a guide to the study of his photographs." Considering that no other analysis of *Phiomia* incisor enamel has been produced up to now, Anderson's study, which has been largely unnoticed in subsequent literature on proboscidean enamel microstructure, remains the sole source of information on the incisor enamel microstructure in this taxon.
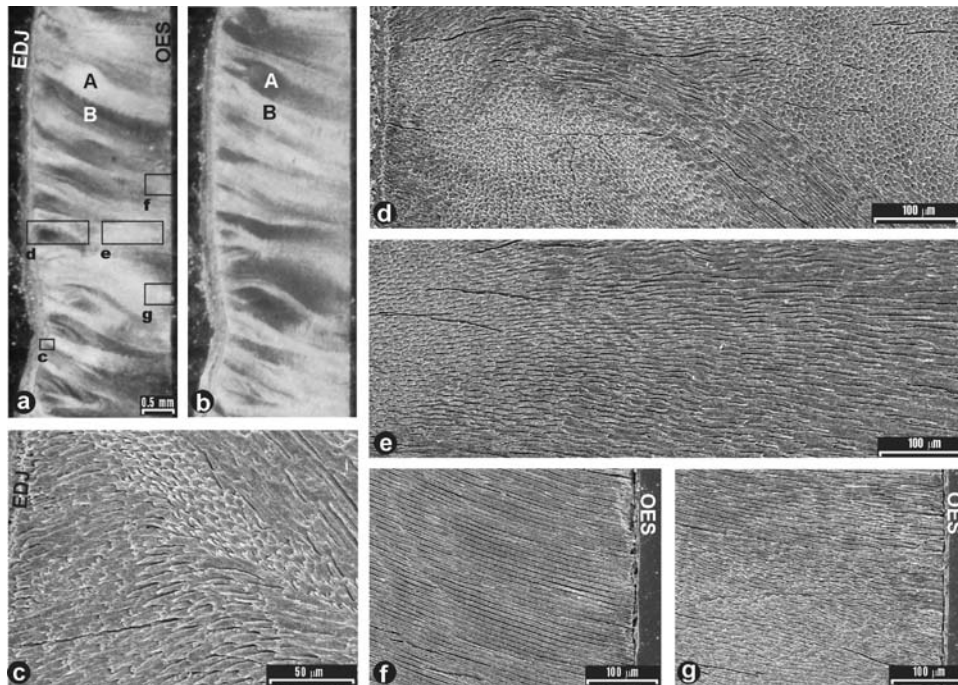
Fig. 2 Transversal sections of *Cuvieronius hyodon* tusk enamel (MUT 5928). **a–b** Reflected light images of the same section illuminated from opposing sides. Depending on the direction of the light relative to prism orientation, single bundles of prisms (*A*, *B*) appear as either light or dark bands. *Boxes* in **a** indicate the enamel regions magnified in **c–g**. **c–g** SEM micrographs of the enamel section depicted in **a**. **c**, **d** Most of the enamel consists of 3DE. **e** Toward the OES, the decussation weakens and **f** eventually prisms become parallel to each other forming a thin RE layer. At some places, close the OES, slightly decussated prisms are observed **g**.

Enamel sample preparation and levels of complexity

The Tarija *Cuvieronius hyodon* tooth collection kept at the Museo Nacional Paleontologico–Arquelogico of Tarija, was sampled to include incisors (tusk) and both deciduous and permanent cheek teeth (Table 1). Additional observation on tusks and molars were made on the Tarija samples stored at the Museo de Paleontologia of La Paz, the Museum national d'Histoire Naturelle of Paris, and the Swedish Museum of Natural History of Stockholm.

Given the possibility of infradental variability, samples from various regions of the tooth were prepared. Enamel samples were prepared for observation at the SEM according to the procedure described in Ferretti (2003a). This includes embedding in epoxy resin, sawing according to various planes of sections (see below), polishing with abrasive powder, etching with 2N HCl for 2–3 s, and sputter-coating with gold-palladium. In order to obtain information on the three-dimensional structure of the enamel, the samples were sectioned according to definite planes. Incisor enamel samples were sectioned along (1) longitudinal (normal to the outer enamel surface and parallel to the long axis of the enamel band; Fig. 1b), (2) transversal (normal to the long axis of the enamel band; Fig. 1b), and (3) tangential (parallel to the outer enamel surface) planes. Cheek teeth samples were sectioned according to (1) vertical (parallel to the mesio-distal axis of the tooth), (2) horizontal, and (3) tangential (parallel to the outer enamel surface) planes. To document both the extension and the pattern of the decussated area throughout the entire enamel section, coated samples were also observed under the reflected light microscope. Photographs of the enamel sections under various orientation of the incident light aided in better defining the boundary between bundles and/or bands of differently oriented prisms. Description of the enamel microstructure was done at hierarchically increasing levels of complexity (see Koenigswald and Clemens 1992). At the lowest level here considered, the size, cross-section, and packing arrangement of enamel prisms (prism pattern) were described. These mirror the size, morphology and orientation of Tomes' processes on the distal side of the ameloblasts with respect to the mineralizing front, during amelogenesis (Boyde 1964, 1967, 1969). To document prism pattern variability through the enamel layer, tangential sections at different depths from the outer enamel surface were prepared. Then, in comparing prism pattern differences between taxa, equivalent planes of section and enamel zones where used, as several works have shown that enamel prisms do not maintain their same cross-sectional shape throughout their path from the EDJ to the OES (Shellis 1984; Grine et al. 1987; Radlanski et al. 2001).

At the next level, "enamel types" have been described, referring to the orientation of enamel prisms in discrete portions of the enamel (Koenigswald and Sander 1997). The distribution of the various enamel types within the tooth was then used to determine the schmelzmuster of each tooth category (Koenigswald and Sander 1997). Finally, by combining the information from the various teeth examined (incisor, deciduous premolars, molars), variation of the enamel microstructure throughout *C. hyodon* dentition was determined. Nomenclature of enamel microstructure features follows Boyde (1964) and Koenigswald and Sander (1997). Classification of the Proboscidea is according to Tassy (1996b) and Gheerbrant et al. (2005).

Enamel and tooth measurements

For the purposes of the present study, three linear measurements were collected from the *Cuvieronius* tooth sample kept at the Museo Nacional Paleontologico–Arquelogico of Tarija, Bolivia: (1) maximum crown width (W) of deciduous premolars and molars, measured perpendicular to the tooth mesiodistal axis; (2) incisor and cheek tooth enamel thickness (ET); and (3) transversal diameter of the incisor enamel band, measured at the base of the tusk.

Incisor enamel thickness was measured perpendicular to the outer enamel surface, at places were natural fractures exposed the entire enamel layer. Up to ten enamel thickness measurements, taken perpendicular to the outer enamel surface, were collected on the occlusal surface of each cheek tooth. To compare enamel thickness across the *Cuvieronius* dentition, a relative enamel thickness (RET) was calculated using the following formula:

$$RET = (ET/W)100.$$

One linear measurement, prism diameter, was used to estimate the average prism size. Measurements were collected from SEM micrographs. Because the size of prism cross-sections can be significantly effected by the angle at which prisms are sectioned (Dumont 1995), special care was taken in order to use only nearly orthogonal cross-sections.

Dental Abbreviations.

dP	deciduous upper premolar
dp	deciduous lower premolar
I	upper incisor
i	lower incisor
M	upper molar
m	lower molar

Enamel Microstructure Abbreviations.

3DE	3D enamel (enamel type with irregularly decussated—i.e., intercrossed—prisms)
EDJ	enamel-dentine junction
ET	enamel thickness
HSB	Hunter–Schreger bands (enamel with prisms decussated in layers)
IPM	Interprismatic matrix
OES	outer enamel surface
PLEX	prismless external layer.
RE	radial enamel (enamel type with parallel prisms)

Institutional Abbreviations.

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IGF	Museo di Storia Naturale (Sezione di Geologia e Paleontologia), University of Firenze
KOE	enamel collection of the Institut für Paläontologie, Freidrich-Wilhelms-Universität, Bonn
MUT	Museo Nacional Paleontologico–Arquelogico, Tarija, Bolivia
MNHN	Museum national d'Histoire Naturelle, Paris, France
MPLP	Museo de Paleontologia, La Paz, Bolivia.
NMR	Swedish Museum of Natural History, Stockholm

Results

Enamel of *Cuvieronius hyodon*

Upper incisor (tusk)

In unworn juvenile tusks (e.g., MNHN-TAR 799; Fig. 1a) the enamel forms a complete cap around the tip. The enamel cap continues laterally to form a band that extends to the basal end of the incisor. On the ventromedial side, a much shorter band-like projection is present (Fig. 1a). The outer enamel surface is originally rather rough and at places a thin cement cover

can be detected. Whereas the enamel cap is progressively worn down, the longitudinal enamel band usually persists throughout the animal's life, as enamel continues to be formed at the base of the tusk (Fig. 1b). The prolonged abrasion and polishing to which the tusk is subjected during lifetime produces the extremely smooth OES that characterizes the tusk of adults. Among tusks with fully formed apical wear facets (belonging to mature individuals), the width of the enamel band, measured at the basal end of the tusk, ranges from 23 to 70 mm, with an average of 48 mm (N=17). The ET of the band is 2.3 mm (N=8), on average. In transverse section (Figs. 2a–b), the EDJ appears undulated, with relieved points corresponding to the longitudinal ridges observable on the outer surface of the tusk dentine, where the enamel has been spalled off. The OES is straight. In longitudinal section (Figs. 3a–b), the EDJ is straight. Enamel prisms, as observed at the SEM, have a keyhole cross-section (pattern 3 of Boyde 1964; Fig. 4a), with a mean transverse diameter of 7 μm, in most of the enamel thickness. In contrast, at the EDJ and near the OES, the prism cross-section is circular (pattern 1), ranging
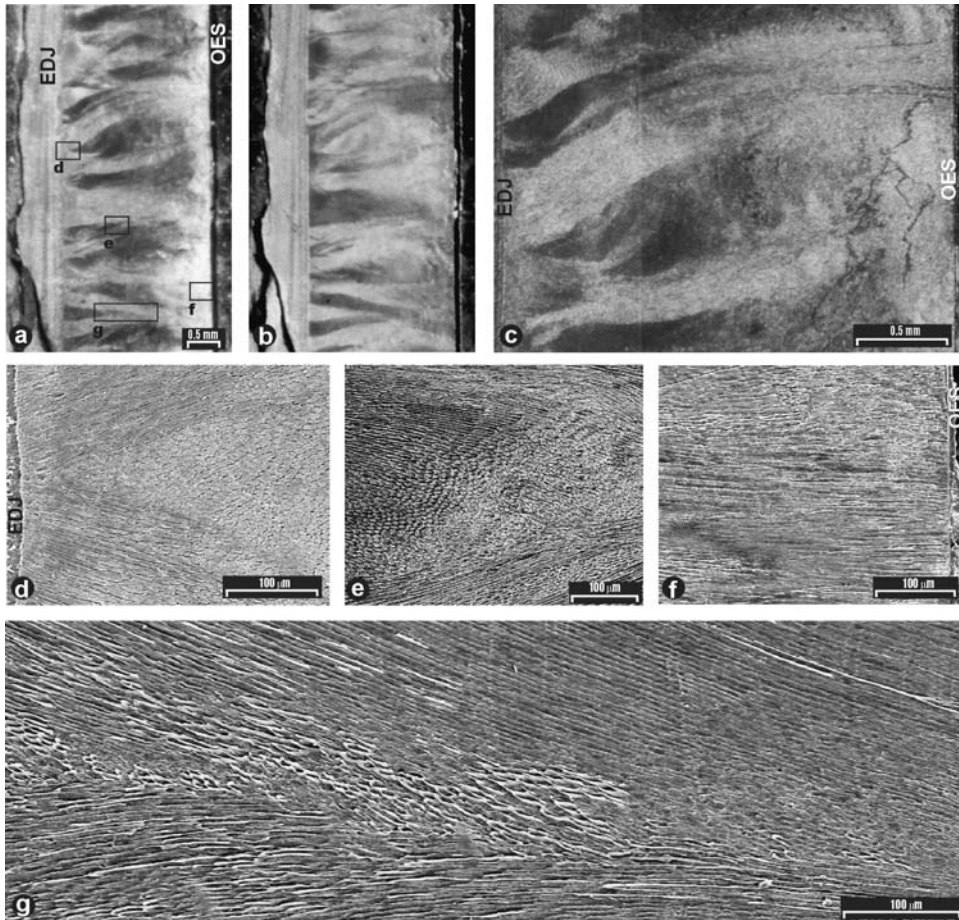
Fig. 3 Longitudinal sections of *Cuvieronius hyodon* tusk enamel (MUT AG1). **a–b** Reflected light images illuminated from opposing sides, highlighting the optic-fiber effect of prisms. **c** Close up of **a**, showing the irregular attitude of the prism bundles (*light and dark bands*). **d–g** SEM micrographs of the enamel section depicted in **a**. The irregularly decussated prism bundles in 3D enamel (**d, e**) tend to converge towards the OES (**g**). Incremental lines are clearly visible in the outermost portion of enamel (**g**). **f** External RE layer.

Fig. 4 SEM images of oblique/
tangential sections of *Cuvieronius
hyodon* tusk enamel (MUT AG1).
a Pattern 3 (*keyhole*) prisms in
the inner portion of the enamel
thickness. **b** Transition from pat-
tern 1 (*round*) to 3 prisms near
the OES. **c** Incisor outermost
enamel layer. A thin PLEX is
present at the OES. Beneath the
PLEX, small prisms with a prism
seam (s) are observable.
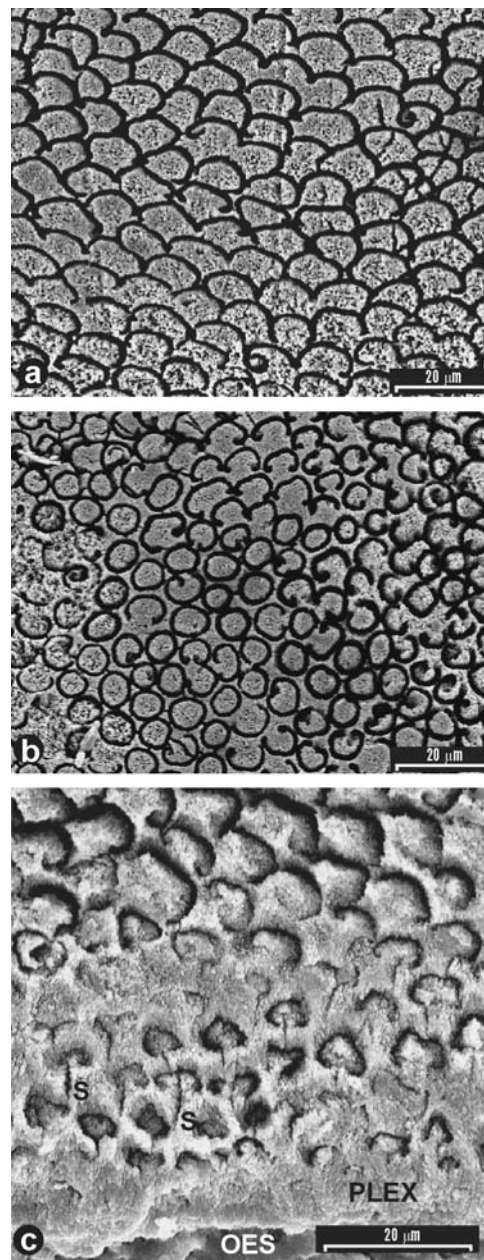
from 5 to 6 μm in diameter (Fig. 4b). In oblique-tangential section, at locations where the
original outermost enamel layer was preserved from wear, a 8 to 10 μm thick PLEX is
present. Noteworthy, next to the PLEX, three to four rows of small prisms characterized by a
central linear discontinuity that bisects the prism without reaching the prism sheath are present
(Fig. 4c). This structure seems to correspond to the prism "seam" described in many Mesozoic
mammals and in a number of extant placental and marsupial taxa (e.g., chiropterans,

dermopterans, humans, and *Tarsipes*; Lester and Hand 1987; Lester 1989; Lester and Koenigswald 1989; Wood and Stern 1997). The majority of the incisor enamel is decussated. Examination of transverse, longitudinal, and tangential enamel sections reveals that the decussating prisms are arranged into bundles (i.e., they do not form HSB), interwoven in the three dimensions (Figs. 2c,d and 3c,d). This irregular type of prism decussation, occurring also in the cheek teeth (see below), has been termed 3D enamel (3DE) by Pfretzschner (1992) and is typical of proboscideans. In the outer half of the incisor enamel, the prism bundles change direction and start to converge (Figs. 2e and 3e,g), gradually reducing the angle of decussation, so that prisms eventually became parallel to each other, forming a thin layer of RE (Figs. 2f,g and 3f).

Lower second deciduous premolar (dp2)

The OES is smooth, with poorly developed external ornamentations (Figs. 1c–e). The dp2 possesses the thinnest enamel among all the lower lateral teeth of *Cuvieronius*, with a mean ET of 1.4 mm ($N=8$), corresponding to a RET value of 5. In horizontal section, the EDJ and OES are undulated. In vertical section, they both are straight (Fig. 5a). In the studied specimen (MUT 266), close to the EDJ, there is a very thin layer with a poorly defined prismatic structure (Figs. 5e–g), possibly corresponding to the layer with pattern 1 prisms observed at
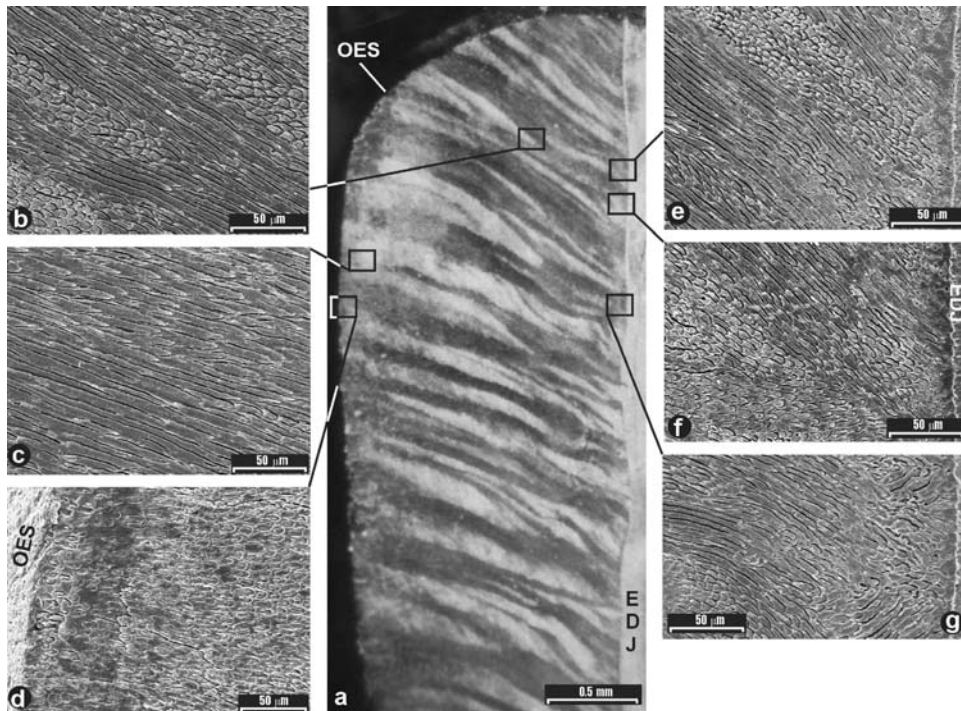
Fig. 5 Vertical sections of *Cuvieronius hyodon* dp2 enamel (MUT 266). **a** Reflected light image showing well-defined HSB through most of the enamel thickness. Natural occlusal surface is visible at the top. **b–g** Close ups (SEM micrographs) of portions of the enamel section shown in Fig. 5a. **b** Middle enamel zone with bifurcating HSB. **c** Outer enamel zone with nearly parallel prisms. **d** The enamel close to OES consists of RE with pattern 1 prisms and thick IPM. **e** HSB forming the innermost enamel layer at the top of the only slightly worn tooth crown. **f, g** 3DE is present close to the EDJ in the rest of the crown.

this location in the enamel of all other teeth of *Cuvieronius*. Prisms with a rounded cross-section surrounded by abundant IPM are, on the other hand, clearly observable near the OES (Fig. 5d), where they form a layer of circa 200 μm. The remainder of the enamel of the dp2 is dominated by pattern 3 prisms. The transversal diameter of the prisms varies from 5 to 6 μm. The majority of the enamel thickness (>90%) is decussated (Fig. 5a). The enamel at the top of the tooth cones (cusps) is formed by HSB and a very thin outer layer of RE (Fig. 5e). In the rest of the tooth crown, a thin layer of 3DE (representing 5–10% of the whole enamel thickness) is present near the EDJ (Figs. 5f–g), in combination with HSB and RE. In vertical section, the HSB are of variable thickness, nearly straight, and occlusally directed (Fig. 5a). The mean occlusal angle of prisms decreases moving to the OES. HSB are well defined. Frequent are both the anastomosis between two bands and the bifurcation of individual HSB (Fig. 5b). At the SEM, it is possible to observe that prisms of adjacent bands are strongly decussated (Figs. 5b,e). Approaching the OES the prisms became parallel to each other, forming the thin RE layer, without changing their occlusal orientation (Fig. 5c).

Molars (M1–M3)

The outer enamel surface of the molars is corrugated and presents small ridges and tubercles. At the base of the tooth crown, perikymata (microscopic wavelike grooves on the enamel outer surface, corresponding to Retzius lines reaching the OES) are frequently observable (Figs. 1f–h). As is typical for elephantoids, ET increases from M1/m1 to M3/m3, i.e., with tooth size. The average RET value for each molar category is about 7, indicating that ET scales isometrically with molar size. As expected, the molars possess a relatively thicker enamel than the deciduous premolars, whose average RET values, in all checked teeth (dP2–dP4; $N=18$), are about 5. All of the studied molars of *C. hyodon* (Table 1) show similar enamel microstructural features, which are summarized below.

At the prism level, the variation of the prism pattern moving from the EDJ to the OES (i.e., 1-3-1) is similar to that observed in the I2 and dp2. Pattern 3 prisms vary in diameter from 5 to 7 μm (Fig. 6d). No prism seam has been observed in the enamel of molars. Prisms decussation in the molar enamel is significantly less extended than in the dp2, representing about 70% of the total enamel thickness. Three enamel layers can be easily distinguished at mid-crown, on the basis of prism orientation: an inner layer with 3DE, representing from 10 to 15% of the total enamel thickness (Fig. 6a), a middle layer formed by HSB and RE with occlusally oriented prisms, and an outer layer composed by RE with prisms nearly perpendicular to the OES (Fig. 6b). As in the dp2, 3DE is absent in the enamel capping the cones of the molars. The borders between adjacent HSB are less defined than in the dp2, due to the weaker angle of decussation between prisms. Incremental lines, linked to rhythmic changes in enamel secretion, are clearly observable in the outer enamel of molars (Fig. 6c). Comparisons of enamel samples taken from different regions of the same tooth at mid-crown, failed to reveal any fundamental structural differences.

Upper incisor enamel of Phiomia serridens

The enlarged upper incisor of *Phiomia* is considered to be homologous to the upper tusk of elephantoids (i.e., I2; Gheerbrant et al. 2005: 325). It possesses a large lateral enamel band that persists throughout the life of the animal (Andrews 1906). In the thin tranversal sections prepared by Anderson (Osborn 1942: plate 28, figs. 1 and 2) the incisor enamel of *Phiomia serridens* appears almost completely decussated (Figs. 7a–c), except for a thin zone near the OES, where prisms are nearly parallel to each other and normal to the OES (Fig. 7c). The
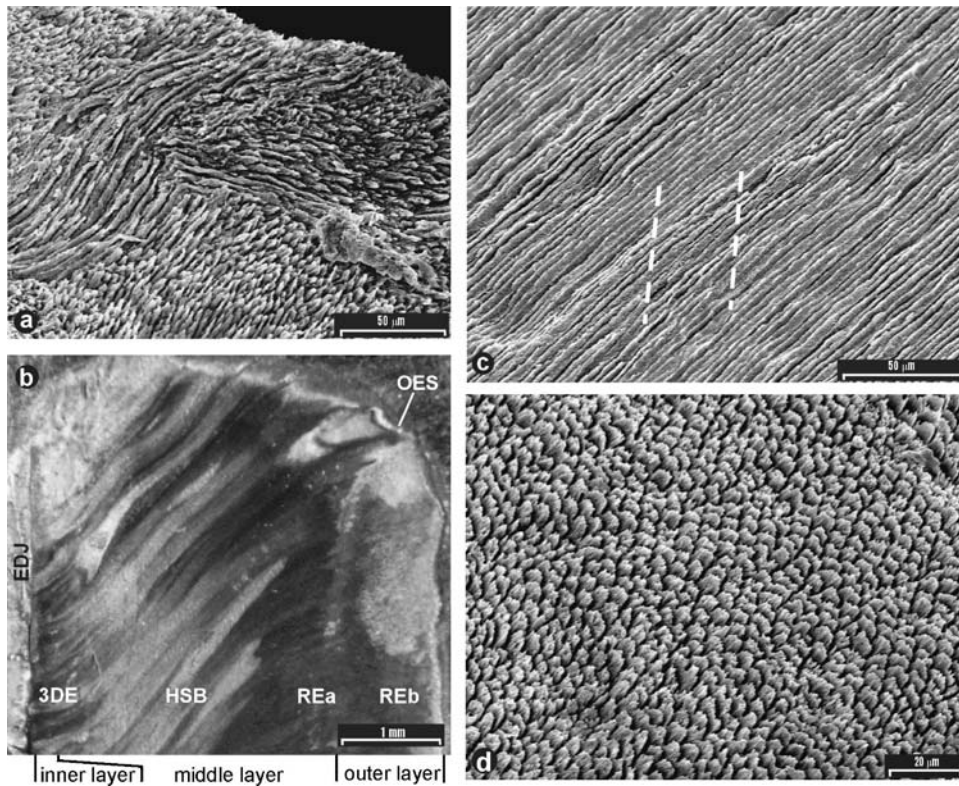
Fig. 6 a–c Vertical and **d** tangential sections of *Cuvieronius hyodon* M2 enamel (MUT AG1b). **a** SEM micrograph of a natural vertical fracture plane showing the irregularly decussated bundles of 3DE. **b** Reflected light image showing the three-layered schmelzmuster of the molar. The outer zone of the middle layer consists of radial enamel (REa) with occlusally oriented prisms; in the outer layer, prisms are normal to the OES (REb). The occlusal surface is to the top. **c** Outer zone of middle enamel layer with RE. Several incremental lines, whose direction is highlighted by the *dotted lines*, are visible. **d** Slightly oblique cross-section of pattern 3 prisms, in the middle enamel layer of the molar.

decussation is irregular, and the degree of complexity matches that of the 3DE of *Cuvieronius hyodon*. It must be emphasized, however, that lack of further views of this specimen prevents a definitive interpretation of the mode of prism decussation in *Phiomia* incisor enamel. In transverse section, the EDJ presents the same characteristic undulation observed in *C. hyodon*. The OES is straight. The microstructure of the incisor enamel of *P. serridens* differs from the two-layered enamel of the molars, formed by HSB and a thick outer layer of RE (Bertrand 1988, 1989; Pfretzschner 1994).

Discussion

Comparative proboscidean enamel microstructure

In this section, the variability of the enamel microstructure of the upper tusk and of the molars among proboscideans is discussed in the different levels of complexity. The systematic distribution of key enamel microstructural traits is summarized in Fig. 8.

Fig. 7 Thin transversal section of *Phiomia serridens* upper tusk enamel observed with **a** crossed nicols and (**b**) under plane light. **c** Enlarged view of the enamel area within the circle in Figure 7b. The schmelzmuster is characterised by 3DE and a thin outer layer of RE. (Modified after Osborn 1942).
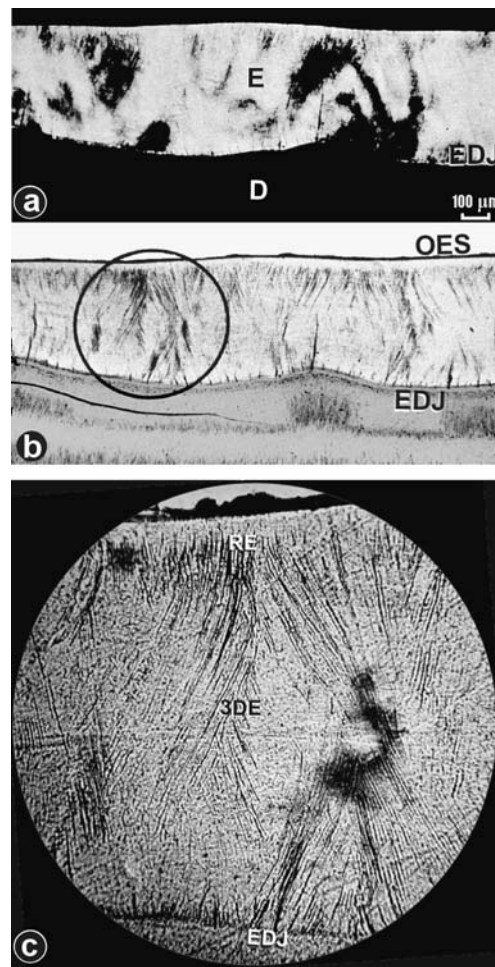
Prism pattern

The pattern of variation of the prism cross-section, moving from the EDJ to the OES, observed in the teeth of *Cuvieronius hyodon*, is shared by all examined proboscideans. Similar changes are known, actually, in other mammalian taxa, including man (Boyde 1964, 1989; Shellis 1984; Radlanski et al. 2001), pointing to a common therian developmental pattern. A noticeable trait of *Cuvieronius hyodon* incisor enamel is the occurrence of prisms with a well-defined prism seam close to the OES. This structure, recognized as a feature of the mammalian plesiomorphic prismatic enamel (Lester and Koenigswald 1989), was lost in most Cenozoic mammalian groups (Wood and Stern 1997; Wood and Rougier 2005). It has not been reported thus far in Proboscidea or in any other tethythere. Considering the paucity of data on proboscidean incisor enamel, it is possible that prism seams might have previously gone unnoticed in other proboscideans, rather than representing a unique feature of *Cuvieronius hyodon* incisor enamel.

   In the teeth of all examined taxa, the enamel is dominated by pattern 3 prisms. These are relatively large prisms, ranging from 5 to 7 µm in diameter (the common range is from 2.5 to

	Schmelzmuster		Prism	
	Incisors	Lateral teeth	cross-section	Source
Phosphatherium	?	HSB-RE	keyhole	Tabuce et al., (2007)
N. koholense	3DE	3DE	keyhole	Bertrand (1988); Tabuce et al., (2007)
Barytherium	?	3DE	keyhole	Bertrand (1988)
Moeritherium	?	HSB-RE	keyhole	Bertrand (1988)
Prodeinotherium	?	3DE	keyhole	Bertrand (1988)
Deinotherium	?	3DE	keyhole	Bertrand (1988); Pfretzschner (1994)
Palaeomastodon	?	HSB-RE	keyhole	Kozawa (1978); Bertrand (1988)
Phiomia	3DE-RE	(3DE)-HSB-RE	keyhole	Pfretzschner (1994); this study
Mammut	?	3DE-HSB-RE	keyhole	This study
Gomphotherium	3DE-RE	3DE-HSB-RE	fan-shaped	Fox (2000); Bertrand (1988)
Cuvieronius	3DE-RE	3DE-HSB-RE	fan-shaped	This study
Anancus	?	3DE-HSB-RE	fan-shaped	This study
Stegodon	?	3DE-HSB-RE	Ginkgo-leaf	This study
Elephas	3DE-RE	3DE-HSB-RE	Ginkgo-leaf	Kozawa (1978, 1983); this study

Fig. 8 Distribution of enamel microstructural characters (schmelzmuster and morphology of pattern 3 prism cross-section in the middle enamel layer) among selected proboscidean taxa. Phylogenetic relationships are based on Tassy (1996b) and Gheerbrant et al. (2005). Numbers refer to nodes discussed in the text.

6.5 μm, on average, among eutherians; Wood and Rougier 2005), irrespective of tooth position and dimension. Differences between taxa occur, however, in the morphology of the prism cross-section in molars. Prisms with a typical keyhole pattern, characterized by a nearly rounded prism head (Fig. 9a), are the most widely distributed form and dominate the enamel of *Numidotherium*, *Moeritherium*, *Deinotherium*, *Phiomia*, and *Mammut*, among the considered taxa. In *Cuvieronius*, *Gomphotherium*, and *Anancus* prisms showing a slightly transversally enlarged, fan-shaped head (Fig. 9b) are present in the middle enamel layer with HSB and RE. Derived Stegodontidae (*Stegodon*) and Elephantidae (*Loxodonta*, *Elephas*, and *Mammuthus*) show, at this location, prisms with a Gingko-leaf pattern (Kozawa 1978),
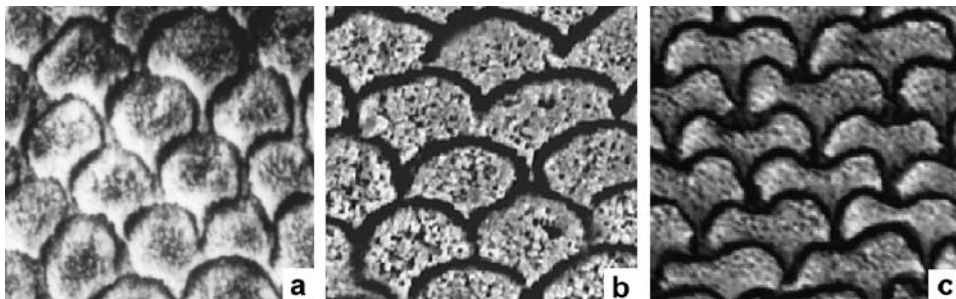
Fig. 9 SEM micrographs of tangential sections of molar middle enamel layer showing the different morphologies of pattern 3 prisms occurring in proboscideans. **a** Typical keyhole pattern (*Mammut americanum*, Mammutidae). **b** Fan-shaped pattern (*Cuvieronius hyodon*, Gomphotheriidae*)*. **c** Ginkgo-leaf pattern (*Mammuthus primigenius*, Elephantidae). The height of each box corresponds to about 25 μm.

characterized by a wide and flat prism head possessing a "dorsal" central depression (Fig. 9c). Tabuce et al. (2007) report the occurrence of prisms with a flattened cross-sectional outline, described as so-called "Gingko-tree-leaf" pattern, along with prisms showing typical rounded key-hole outlines, in the early Eocene *Phosphatherium escuilliei*, the oldest and most primitive known bona fide proboscidean (Gheerbrant et al. 1998, 2005). The flattened prism outline described in *Phosphatherium* (Tabuce et al. 2007: fig. 1c), however, differs from the typical Gingko-leaf pattern found in elephantids (Fig. 9c), in lacking a clear bilobed shape and a sagittal depression, suggesting the two structures are not homologues. It should be noted, however, that sectioning obliquity would produce asymmetric and transversally expanded prism outlines, like those observable in Tabuce et al.'s fig. 1c. In any case, based on the description and figures provided by Tabuce et al. (2007: figs. 1b–d and 2a–b), it appears that the key-hole outlines predominate the enamel of *Phosphatherium*.

The distribution of the various prism types among proboscideans (Fig. 8) indicates that the round-headed keyhole morphology is primitive for elephantiforms, whereas the fan-shaped and Gingko-leaf patterns are derived. Further investigation needs to address the phylogenetic significance of these two derived patterns within elephantoids.

Enamel types

Three basic organizational types of prismatic enamel are recognized in proboscideans: radial enamel (RE), Hunter-Schreger Bands (HSB), and 3D enamel (3DE). Each enamel type possesses specific biomechanical properties linked to tooth function (Koenigswald and Pfretzschner 1991). Prisms decussation (as in HSB and 3DE) enhances resistance to crack propagation in the enamel of teeth subjected to high occlusal stresses, whereas occlusally rising prisms in RE are more resistant to abrasive wear (Rensberger and Koenigswald 1980; Pfretzschner 1988; Rensberger 2000). The structural variability of single enamel types across the proboscidean taxa that have been considered is generally limited. The morphology of HSB shows, actually, a certain degree of interspecific variability, mainly in the angle of decussation and the thickness and the waviness of the band in vertical section. In *Moeritherium* molars, at mid-crown, HSB are relatively thin, of nearly constant thickness and straight (Bertrand 1988; Pfretzschner 1994). In contrast, the middle enamel layer of elephantoid molars show more complex HSB, characterized by an undulated path, irregular thickness and frequent bifurcations of the bands.

Molar schmelzmuster

A three-layered (3DE-HSB-RE) schmelzmuster, similar to that of the molar of *C. hyodon*, is found in all the elephantoids here considered (*Mammut*, *Gomphotherium*, *Anancus*, *Stegodon*, *Loxodonta*, *Mammuthus*, and *Elephas*). Note that in elephantoids the 3DE is absent from the enamel capping the occlusal end of the tooth, where a combination of HSB and RE is found. *Phiomia* differs from elephantoids in the absence of a distinct 3DE layer close to the EDJ. In this taxon, Pfretzschner (1994) reported the occurrence of irregular and poorly defined HSB at places along the EDJ, interpreted as a rudimentary type of 3DE. In contrast, only HSB are present in the inner enamel of *Palaeomastodon* and *Moeritherium* (Bertrand 1988; Pfretzschner 1994). Deinotheres and "barytherioids" (*Numidotherium koholense* and *Barytherium grave*) markedly differ from the pattern of *Moeritherium* and the Elephantiformes (i.e., *Palaeomastodon*, *Phiomia* plus Elephantoidea) in having an almost completely decussated enamel consisting of 3DE (Bertrand 1988, 1989). Different from elephantoids, 3DE extends to the entire height of the crown in both deinotheres and barytherioids (Bertrand 1988, 1989). Tabuce

et al. (2007) found a three-layered schmelzmuster, with 3DE, HSB, and RE, in the Eocene "*Numidotherium*" *savagei*, unlike that described in *Numidotherium koholense*, suggesting the two species are not closely related. The authors stress the similarity between the schmelzmuster of "*N.*" *savagei* and that of elephantoids. Indeed, both consist of the same enamel types. However, in "*N.*" *savagei* the 3DE, forming the inner enamel layer, represents 45% of the entire enamel thickness, whereas in elephantoids the inner 3DE layer never exceeds 20%. This suggests the possibility that the schmelzmuster of "*N.*" *savagei* is not homologous with that of elephantoids. Additional work, in particular on the vertical variation of the schmelzmuster and the details of the HSB morphology in "*N.*" *savagei* molars, is needed to solve this problem.

A two-layered schmelzmuster, consisting of HSB and RE, characterizes the molar enamel of *Phosphatherium escuilliei* (Tabuce et al. 2007).

In all studied taxa, enamel decussation (either as HSB, 3DE or a combination of both enamel types) starts close to the EDJ and occupies at least half of the whole enamel thickness. In those schmelzmusters where both 3DE and HSB occur (e.g., elephantoids), the former enamel type is always closer to the EDJ and directly overlained by HSB. If present, RE forms the outermost layer, extending close to the OES.

The relationship of function to schmelzmuster in proboscideans is still partly unclear. The impact of crown height on enamel microstructure has been explored by Pfretzschner (1992, 1994) who interpreted the evolution of 3DE in the inner enamel of the elephantoid molar as a biomechanical adaptation to hypsodonty. Indeed, Pfretzschner (1994) stressed that, though the molars of primitive elephantoids (e.g., *Gomphotherium*) are regarded as brachyodont, the functional units of the elephantoid tooth, i.e., the cones forming the loph(id)s, could be considered biomechanically analogous to the hypsodont teeth of ungulates. Bertrand (1988) suggested that the difference in schmelzmuster between derived lophodont forms (*N. koholense*, *Barytherium*, and deinotheres) and bunolophodont proboscideans (*Moeritherium* and elephantiforms) is functionally related to tooth shape and thus to diet. Indeed, the sharing by barytherioids and deinotheres of a similar schmelzmuster implies a remarkable convergence that supports the adaptive nature of this structure. More work is needed, however, to fully corroborate this hypothesis.

Dentition level

At present, incisor enamel is known only in *Numidotherium koholense* (Bertrand 1988; Tabuce et al. 2007), *Phiomia* (Anderson in Osborn 1942; this study), Gomphotheriidae (Koenigswald 1988; Fox 2000; this study), and Elephantidae (Nogami 1981; Kozawa 1983; Raubenheimer et al. 1995). Koenigswald (1988) described the enamel of a proboscidean tusk from Esselborn (Dinotheriensande; late Miocene) referred to *Tetralophodon longirostris*. The tusk is indicated as a lower one (Koenigswald 1988: caption of Fig. 6). This seems very unlikely, as no Old World Neogene elephantoid possesses an enamel band on the lower incisor. On the other hand, in *T. longirostris* the upper tusk also lacks an enamel band. The specimen described by Koenigswald (1988) should therefore pertain to a different and presently unidentified elephantoid.

All investigated elephantiforms show a differentiation between the enamel of the enlarged upper incisor and that of the lateral teeth (not checked in *Palaeomastodon*). Different from previous descriptions, the elephantiform incisor schmelzmuster is shown to be formed by 3DE and a thin outer layer of RE. In *N. koholense*, however, both the incisors and lateral teeth show a similar derived schmelzmuster, dominated by 3DE (Bertrand 1988, 1989; Tabuce et al. 2007). Enamel differentiation between anterior and lateral teeth has been observed in numerous mammals with enlarged front teeth (either incisors or canines), and has been related to the different biomechanical requirement of the two types of teeth (Koenigswald 1988).

The present analysis revealed a differentiation of the schmelzmuster between the anterior deciduous premolars and the molars of elephantoids. In *C. hyodon*, the dp2 has HSB extending close to the OES, whereas they are confined to the middle enamel zone in the molars. The HSB of the dp2 are also more strongly decussated than those of molars. In elephants (Elephantidae), the dp3 has a similarly highly decussated enamel as in the dp2 of *C. hyodon* (Ferretti 2003a), indicating this is a common feature of the enamel of the elephantoid anterior deciduous premolars. Considering that deciduous premolars have both absolutely and relatively thinner enamel than molars, it is possible that strong decussation in elephantoid cheek teeth has an inverse relationship with enamel thickness.

Evolution of the elephantoid enamel

By comparing the distribution of the enamel characters here discussed with recent phylogenetic hypotheses of the Proboscidea based on dental and skeletal gross morphology (Tassy 1996b; Gheerbrant et al. 2005), it is possible to reconstruct the evolutionary path of some significant microstructural characters of the proboscidean enamel with special attention to the emergence of the elephantoid pattern.

Molar enamel

An enamel formed by HSB covered by RE as displayed by *Palaeomastodon* is observed in the molars of *Moeritherium* and *Phosphatherium*, though in this latter taxon the outer RE is much thinner (Tabuce et al. 2007). Deinotheres (checked in *Prodeinotherium* and *Deinotherium*), regarded as the sister-group of Elephantiformes (Gheerbrant et al. 2005; Fig. 8), possess an all-decussated enamel composed of 3DE. The simplest explanation for the distribution of these traits is that primitive elephantiforms retained the plesiomorphic condition seen in *Moeritherium* and *Phosphatherium*, whereas deinotheres evolved from this primitive enamel a schmelzmuster convergent to that of barytherioids, possibly in relation to the secondary acquisition of a lophodont dentition (see Bertrand 1988). The unique combination of a lophodont dentition and a three-layered schmelzmuster in "*N.*" *savagei* (Tabuce et al. 2007) seems to challenge, at least in part, this hypothesis. The contribution of "*N.*" *savagei* to the understanding of the evolution of elephantoid molar enamel is still unclear, as the place of this poorly known taxon among the Proboscidea has not been definitively settled. Nevertheless, all available data indicate that the elephantoid molar schmelzmuster derived from a structural predecessor similar to that of *Palaeomastodon*, with the introduction of a thin 3DE layer close to the EDJ.

3DE represents the most characteristic structural traits of proboscidean enamel. This enamel type occurs, indeed, in many proboscideans, but not in the most primitive one (Tabuce et al. 2007). This raises the question whether 3DE represents a synapomorphy within the Proboscidea or evolved several times in parallel. The distribution of 3DE on the proboscidean cladogram depicted in Fig. 8, indicates that the character is homoplastic. At least three clades evolved independently an irregular enamel in the molars from a primitive pattern comprising HSB as the sole decussated enamel type: in barytherioids (*B. grave* and *N. koholense*; node 3), in Deinotheriidae (node 4), and in the Elephantoidea (node 6). The alternative hypothesis, that 3DE represents a synapomorphy at node 2 (Fig. 8), implying two reversals to the primitive condition in *Moeritherium* and in basal elephantiforms, though more parsimonious, would be more difficult to accept from a structural and developmental basis. Indeed, cases of parallel evolution of similar enamel types have been shown to be rather diffuse within Mammalia and have been hypothesized to be related to the limited potential of the ameloblasts to produce

different structures (Koenigswald 1997b). Nevertheless, considering the recurrent origins of 3DE in several proboscidean groups and its absence in non-proboscidean tethytheres (Koenigswald 1997a), it is tempting to regard the potentiality to develop an irregular type of prism decussation as an "underlying synapomorphy" of the Proboscidea (see Saether 1979 and Koenigswald 1997b).

Incisor enamel

The lack of data on the upper incisor enamel of *Phosphatherium*, *Daouitherium*, *Barytherium*, and *Moeritherium* allows, at present, only some speculations on the evolution of incisor enamel in elephantiforms in particular and in proboscideans in general to be made. An attractive hypothesis is to consider the differentiation of the upper incisor enamel microstructure as an apomorphy of elephantiforms, linked to the transformation of the second upper incisor into an ever-growing tusk. Yet, the occurrence in the incisors of *Numidotherium koholense* of an enamel formed by 3DE (Bertrand 1988; Tabuce et al. 2007) suggests the possibility that this is a more inclusive basal feature. Pending knowledge of the condition in *Phosphatherium*, it is not possible to assess whether an incisor schmelzmuster built up almost exclusively of 3DE (with or without a thin outer RE layer) represents the ancestral condition of the Proboscidea or is an apomorphy of the clade including barytherioids, *Moeritherium*, deinotheres, and elephantiforms (node 2, Fig. 8). Also, the possibility that this character is homoplastic among proboscideans cannot be ruled out, at present.

Conclusions

The analysis of the Tarija *Cuvieronius hyodon* sample, which includes the first description of the enamel of a proboscidean dp2, permitted the determination of the variation of the enamel microstructure at the dentition level. The results obtained from the comparative study demonstrate the generality, among elephantoids, of the basic microstructural features of *Cuvieronius hyodon* enamel, allowing a general characterization of the basal Elephantoid Enamel (EE).

Of the dental categories forming the complete primitive elephantoid dentition, only the second generation premolars (P2–P4), not present in *C. hyodon*, remain unsampled.

The basic features of the EE can be summarized as follows:

(a) The bulk of the enamel is formed by pattern 3 prisms. Prism cross-section may be either keyhole shaped (primitive), fan-shaped (derived) or Ginkgo-leaf shaped (restricted to the Stegodontidae and Elephantidae among elephantoids). A prism seam may be a further feature of the elephantoid incisor outer enamel prisms, though additional work is needed to definitively prove this point.

(b) The schmelzmuster of the upper tusk differs significantly from that of the cheek teeth, consisting of 3DE and a thin outer zone of RE.

(c) In both deciduous premolars and molars, there is a vertical differentiation of the enamel. The enamel cap at the apex of the tooth cones is formed of HSB and RE, whereas in the rest of the crown a three-layered schmelzmuster with 3DE overlained by HSB and RE is present.

(d) No enamel differentiation between mesial, distal, labial, and lingual regions of the tooth crown exists in the cheek teeth.

(e) The second and third deciduous premolars (dp3 enamel checked in elephantids; Ferretti 2003a) differ from more distal cheek teeth in the greater proportion of decussated enamel

(>90% of the total enamel thickness) and in having HSB with a stronger prism decussation.

Of the various features of the EE, the three-layered enamel of the cheek teeth appears as the sole synapomorphy of the Elephantoidea (not checked in *Hemimastodon*, the most primitive member of the superfamily; Tassy 1996b). As discussed above, the differentiation of the incisor enamel represents a primitive elephantiform trait, as it also occurs in *Phiomia*. Actually, observations of *Numidotherium koholense* incisor enamel (Bertrand 1988; Tabuce et al. 2007) indicate the possibility that a schmelzmuster dominated by 3DE is a more basal proboscidean character. Characters of the prisms cross-section might be used, on the other hand, to define less inclusive clades within the Elephantoidea.

Many aspects related to the function and phylogeny of proboscidean enamel remain unresolved. Of great help would be the acquisition of data on the incisor enamel of basal proboscideans and of *Moeritherium*, as well as a more detailed knowledge of enamel variation at the dentition level in non-elephantoid proboscideans. The unavoidable "sacrifice" of some rare fossils, necessary in the analysis of enamel, would be rewarded by a significant step forward in our understanding of the evolution of this remarkable vertebrate hard tissue.

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