Original Article

Vertical Prism Decussation of *Teleoceras* (Rhinocerotidae) Molar

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Abstract

Most mammals possess microstructural decussation in the tooth enamel. The Hunter-Schreger bands (HSB) are the important microstructure created by differently directed enamel prisms, as observed under a reflective light microscope. The prism decussation of some rhinocerotoids reveals that HSB run vertically along the axis of the tooth. The boundary between the bands is definite in the tangential section. *Teleoceras* is an extinct rhinocerotoid that was commonly found in the Miocene area of North America. Our observations revealed vertical prism decussation in the inner two-thirds to four-fifths of the *Teleoceras* molar enamel. The prisms strongly twisted on each axis, and the path of each prism undulated at the border of the bands. Their relative relations revealed inclinational changes and displacement to the adjacent zone of the HSB. Reconstructed three-dimensional images were used for complementation of microscopic observations.

Introduction

The shape of enamel prisms and Hunter-Schreger bands (HSB) varies among the mammalian taxa. The enamel structure of the cheek teeth (i.e., molars and premolars) was distinguished among the orders of mammals by Kawai (1), and additional studies indicated that there are five types of HSBs (2, 3). Previous studies reported that rhinocerotoid HSB of cheek teeth run vertically (3–9), whereas horizontal HSB is the most prevalent feature in mammals. Molars of *Teleoceras* (10–14) were used as experimental material. *Teleoceras* lived in North America during the late Miocene and Pliocene (approximately 23–3 million years ago). Their proportions were similar to those of hippopotamus, i.e., over 1.5 ton in weight, and had a single small nasal horn. The microstructure of the molar enamel was observed using scanning electron microscopy (SEM) supplemented by three-dimensional images based on serial SEM images.

Materials and Methods

Materials

Fragments of *Teleoceras* molars from the Thomas Burke Memorial Washington State Museum in Seattle (USA) (UWBM no.61586) were examined. Additionally, a molar of *Teleoceras* (a private collection, SKPC #0014) from the Bone Valley Formation in Florida was observed. Some fragments were embedded in polyethylene resin and sectioned tangentially, vertically, and horizontally. After polishing and slight etching with 0.05 N HCl, the specimens were sputter-coated with Au-Pd.

Scanning electron microscopy analysis

The specimens were then analyzed under a reflected light microscope (SZ; Olympus Corp., Tokyo, Japan), and the enamel microstructure was examined using SEM (S2700 and S3400N; Hitachi Ltd., Tokyo, Japan). To elucidate the three-dimensional course and profile of the enamel prisms, the sections were consecutively ground down in 10-μm steps tangentially to the dentinoenamel junction up to a depth of 50 μm for SEM.

Three-dimensional reconstructions

The identification of the successive images of prisms was simplified by creating holes as location marks on the enamel surface using an Er: YAG laser (Erwin Advel; Morita Corporation, Tokyo, Japan). These holes remained in a

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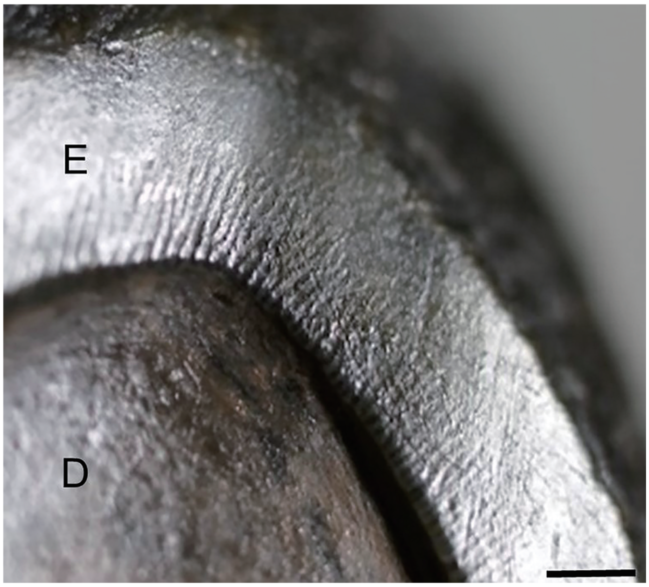

Fig. 1. Radial belted structure on the occlusal surface of the *Teleoceras* molar enamel. The enamel surface is slightly inclined outward from the center.
Scale bar=1 mm. E, enamel; D, dentin

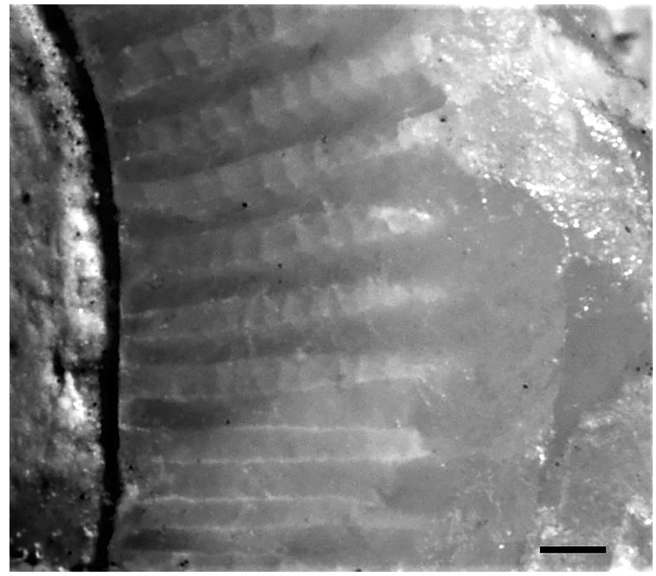

Fig. 2. The occlusal surface of the molar. Radial HSB from the dentinoenamel junction could be observed. Scale bar=200 μm.

constant position throughout the series of sections. The location marks enabled the registration of successive tracings by adjusting the function of the Photoshop Element (Adobe Systems Incorporated, San Jose, CA, USA) using serial SEM images. Separate tracings of each successive outline of 10 adjacent prisms were then made on layers of the Photoshop Element, and the outlines of corresponding prisms were identified, numbered, traced, and digitized via a graphics tablet (Intuos 3; Wacom, Saitama, Japan). Three-dimensional reconstructions were produced with the "OZ" image rendering software (Rise Corporation, Sendai, Japan). In these reconstructions, each prism was shown in a different color and could be viewed from any perspective.

Results

Observation of the *Teleoceras* molars using reflected light microscope revealed that the radial ridges came up to the inner two-thirds to four-fifths of the occlusal enamel (Figs. 1, 2).

In the polishing surface, some bands bifurcated and discontinued on their course on the tangential section observed using reflected light microscope (Fig. 3). The central crack on the specimen was made at the final stage of polishing, and the laser opened the holes for location marks. In SEM image of the tangential sections, the arrangement of the prisms belonging to each band seemed to be in the opposite direction, oblique-cut surface. One or two lines of prisms, which appeared as a cross-cut surface, formed the border between the bands. The boundary prisms were compressed in round and more irregular shapes than the

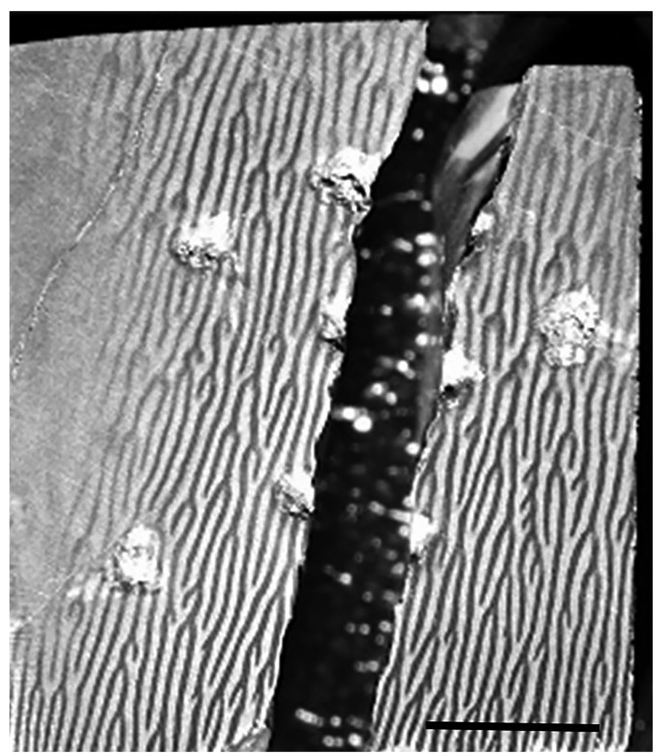

Fig. 3. Tangential surfaces of the molar under a reflective light microscope. Scale bar=4 mm.

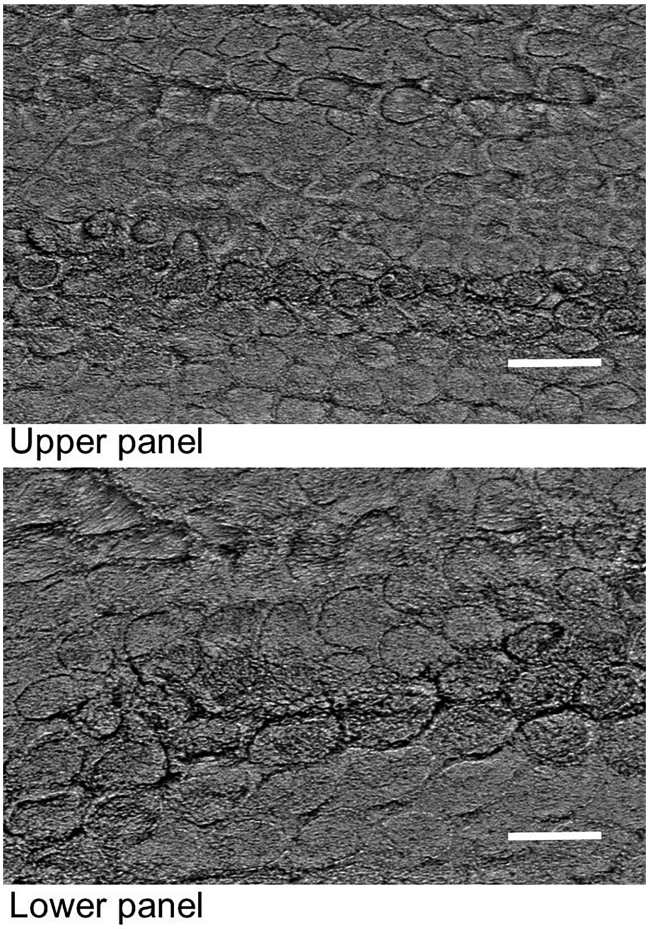

Fig. 4. Scanning electron microscopic observation of a tangential section (backscatter mode). Upper panel: One or two lines of prisms compose the boundary between the bands. Scale bar=10 μm. Lower panel: Note the shape of the prisms of the boundary between the bands. Scale bar =5 μm.

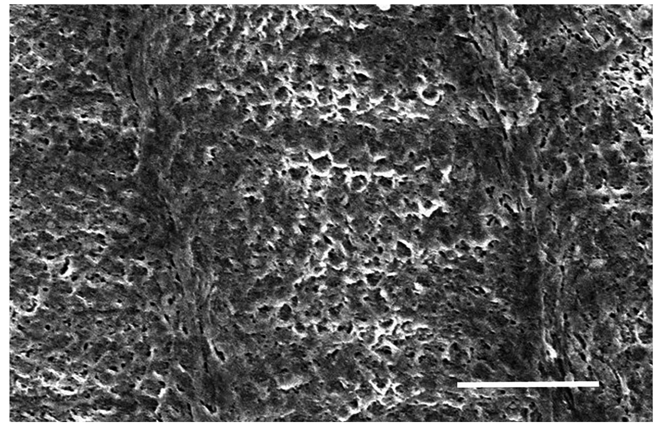

Fig. 5. Scanning electron microscopy observation of a horizontal section. The boundary is composed of the longitudinal cutting surfaces of prisms. The enamel surface direction is toward the top. Scale bar=25 μm.

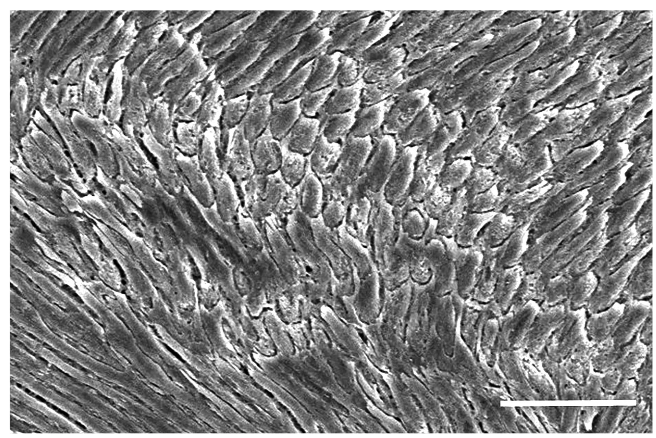

Fig. 6. Changes in the direction of prisms observed in the longitudinal section under a scanning electron microscope. Scale bar=25 μm.

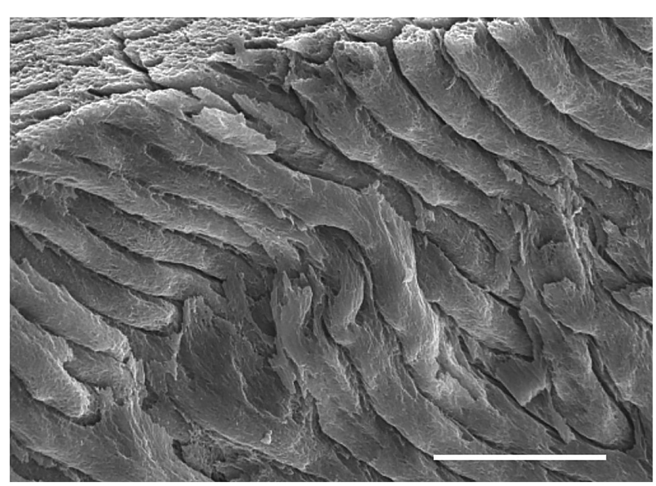

Fig. 7. Changes in the prism direction and distortions observed in this broken longitudinal section. Scale bar=15 μm.

non-boundary prisms (Figs. 4, 5).

In the horizontal sections, prism direction was slightly different between the bands, and the boundaries between the bands appeared as longitudinal cutting surfaces of prisms, each of which had a different width (Fig. 6). In the longitudinal section, some prisms did not demonstrate flexure in portions of the band, whereas changes in direction occurred abruptly in a particular part (Fig. 7).

In SEM images of a longitudinal section of a natural fracture, the enamel prisms were intricately intertwined to twist each other (Fig. 8). Each of prisms twisted itself and, as a result, its positional relationship changed with the adjacent prisms (Fig. 8).

The three-dimensional visualization of the group of the prisms was viewed from the upper-left oblique, and each

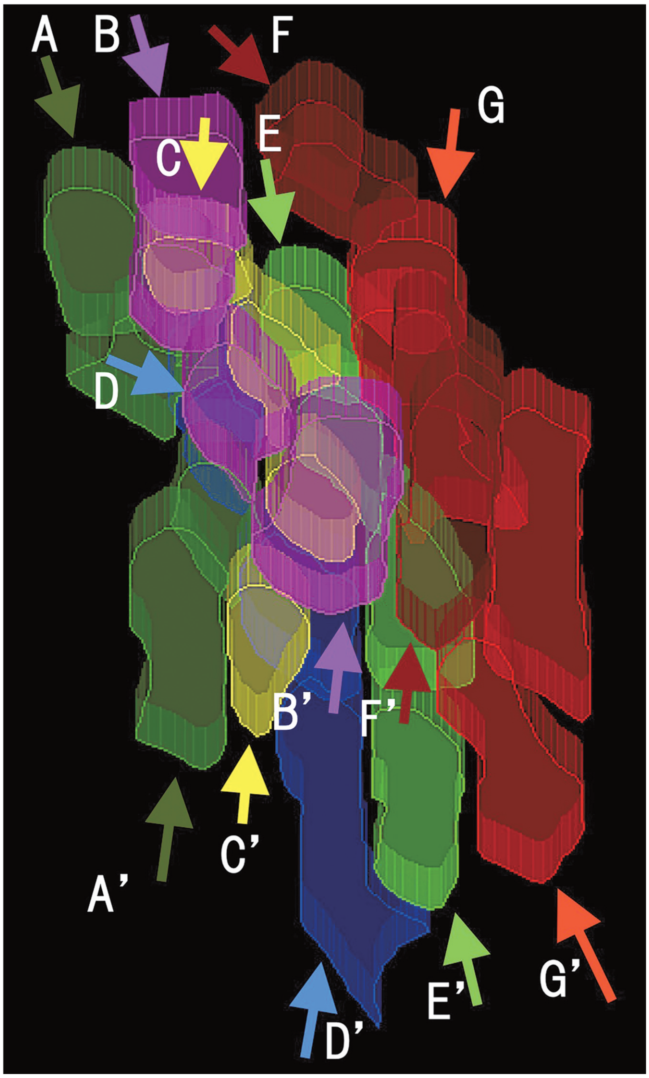

Fig. 8. Three-dimensional visualization of the group of the prisms viewed from the upper left oblique. Each prism appeared in a different color with the same letter(A-A' et al.). The A-G shows the surface and A'-G' shows the bottom of a serial cutting plane, respectively.

prism appeared in a different color for the same letter(A-A' et al.; Fig. 9). Prisms A-G and A'-G' stood in the same plane. Inclinations and orientation changed the shape of prisms as the surface was ground off in the stages of depths from A-G to A'-G'. This reconstruction revealed a displacement of position and shape in the areas of different depths.

Discussion

Teleoceras was a hippopotamus-like rhinocerotoid that lived during the Miocene epoch in North America(10–14). *Teleoceros* was the most prosperous genus of rhinoceroids; therefore, it was considered as a material for researching the typical decussation pattern.

As previously described, for rhinocerotoids cheek teeth (3–9) the molar of *Teleoceras* showed vertical decussation of prism along the tooth axis, which makes a radial ridge in the occlusal enamel. The different reflectivity of the bands, observed in the sections viewed using incident light is presumed to be due to the different directions of the prisms in each band.

The rows of the prisms belonging to each band appeared to run in the opposite direction in the tangential sections, and one or two lines of prisms formed the boundary between the bands. The cutting feature in a tangential section of the boundary prisms was either round or oval, but it was disintegrating the shape in place and more irregular and deformed than in the non-boundary prisms. The prisms seemed to be pressured and twisted from the circumference. The enamel prisms of the fracture surface showed twists on each axis and bending, and it was recognized that the positional relationship between the prisms changes. Concerning previous researches(9, 15–21), polishing serial sections were observed using SEM to restore the three-dimensional image of the enamel prisms. The obtained image of the group of prisms showed that the prisms changed their vicinity or position and bent, twisted, and/or crossed with each other. Each prism changed its shape and was distorted along the path. The prism of the fracture surfaces corresponded to the three-dimensional image(Figs. 7, 8), indicating changes in the respective directions and locality relations of prisms along their rows. Such features were frequent in the border of the bands, and the prisms that did not show flexure were observed in the middle portion of the band. These observations; therefore, suggest the presence of no clear boundaries between the bands of HSB due to changes in the direction of prisms near the border and the entry of the prism to the adjacent band. It could thus be inferred that the prisms in the boundary area became torsional or disordered during amelogenesis. Unlike almost all other mammals, rhinocerotoids had acquired the ability of ameloblast to form vertical decussation in their phylogeny. Thus, significant radial ridges of the occlusal surface (Fig. 1) resulting from vertical decussation attributed to the effective function of chewing fine food to the rhinocerotoids lineage.

Conflicts of interest

The authors have no potential conflicts of interest.

Acknowledgments

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