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Comparison of Enamel Microstructure of Ictidomys Tridecemlineatus Formed during Hibernation versus the Active Season

Amanda Cho
Andrews University, acho@andrews.edu

J. N. Andrews Honors Program
Andrews University

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Amanda Cho

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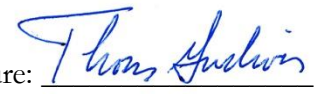
Advisor: Dr. Thomas Goodwin

Primary Advisor Signature: 

Department: Biology

Abstract

Ground squirrel incisors grow continuously, preserving a record of their most recent weeks of life. Previous research demonstrated that an abnormality in the surface of incisor enamel and dentin of thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) corresponded with hibernation. Using scanning electron microscopy, we compared internal microstructure of incisor enamel deposited during and outside of hibernation to determine if surface disruptions corresponded to differences in internal microstructure. For one specimen, hibernation enamel displayed irregularities in microstructure that were not present in non-hibernation enamel, but this difference was not observed in other specimens. Given these inconclusive results, further research is warranted.

Introduction

The incisors of rodents, including those of ground squirrels, grow continuously throughout their life; they grow from the base and are constantly being worn down from the tip. The growth of the incisor is incremental, with deposits formed daily at the base of the tooth (Schour & Steadman, 1935). As the tooth grows and is worn down, these daily increments contain a record of dental growth during the last couple of months of the animal's life (Goodwin, Michener, Gonzalez, & Rinaldi, 2005). This record of growth may shed insight into the last few weeks of life the animal's life.

A rodent's incisors are composed of enamel and dentin. The dentin is deposited starting from the base of the tooth in the form of cones inserting into each other from the center, pushing the tooth forwards. Enamel is deposited also starting from the base of the tooth. The enamel is laid down on the external surface of the tooth, overlapping the deposited dentin (Goodwin & Ryckman, 2006). The interface that is formed between the enamel and dentin is the enamel dentin junction (EDJ) (Schour & Steadman, 1935). Because of this pattern of growth, the enamel does not entirely cover the tooth, but only covers the external surface. While both the enamel and dentin are essential parts of the tooth, I will focus on structure of the enamel in this study.

Enamel is formed by first secreting a protein matrix that then becomes mineralized over time. This organic-rich protein matrix is converted over a few weeks to an almost entirely inorganic structure made up of apatite (calcium phosphate) crystals (Moradian-Oldak, 2012). The mineralization of the enamel begins as the enamel is being formed, thus mineralization begins at the base of the tooth and continues throughout its growth. Hence, the tip of the tooth will be more mineralized than the base of the tooth.

The internal microstructure of enamel displays hardened prisms made up of hydroxyapatite crystallites that are surrounded by an interprismatic matrix. The enamel prisms arise from the enamel dentin junction and extend towards the external surface of the tooth (Martin, 1994). The prisms extend from the enamel dentin junction through the portio interna (the internal layer of enamel) that contains Hunter-Schreger bands (HSB), or bands of similarly oriented prisms. Enamel microstructure then transitions into the portio externa (the outer layer of enamel) that displays radial enamel. In radial enamel the prisms are parallel to each other rather than decussating (Martin, 1999).

The internal microstructure of enamel has been classified based on variation in the organization of HSBs. These microstructural classifications include uniserial, multiserial, and pauciserial enamel. The HSBs in uniserial enamel are approximately one prism thick per band whereas the HSBs in multiserial enamel can range from two to six prisms in thickness; and HSBs in pauciserial enamel are around three prims in thickness (Martin, 1994). To summarize, rodent enamel microstructure therefore displays the characteristics of an enamel dentin junction, the portio interna and portio externa, and different orientations of the HSBs that vary by species.

Previous research done by Goodwin et al. (2005) showed that there is an outward disruption of enamel and dentin growth that is associated with hibernation in the lower incisors of ground squirrels. The hibernation mark has also been observed in prairie dogs (Goodwin & Ryckman, 2006). The hibernation mark can be characterized by a number of features such as an abnormally thickened enamel sleeve, a turbulent enamel surface structure or an irregular enamel dentin junction (Goodwin et al., 2005; Goodwin & Ryckman, 2006). However, prior studies only examined the surface of the incisors, and they did not investigate the internal microstructure

of dental tissues. Therefore, it is unknown how these external abnormalities might be reflected in the internal microstructure of the enamel.

In this study, I used scanning electron microscopy (SEM) to document the internal microstructure of incisor enamel of thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), a species known to hibernate and form a hibernation mark (Kisser, 2009). The focus of my study was to compare microstructure of enamel within the hibernation mark with enamel formed during the active season to see if there were internal differences that correlated with surface abnormalities of the tooth during hibernation.

The incisors examined in this study were from squirrels sacrificed in spring 2009 during a study of how surface features of incisors are related to body-temperature variation during hibernation (Kisser, 2009). In that study, body temperature of squirrels was tracked overwinter by attaching a temperature sensor on a collar to free-ranging squirrels captured in fall; and the specimens were then recaptured in spring, sacrificed, and their incisors were extracted. Because the timing of entry into and exit from hibernation, as well as details of body-temperature variation during hibernation, were known from the body-temperature record, it was possible to fairly precisely associate hibernation with the growth record and hibernation mark of each incisor (Kisser, 2009; Kisser & Goodwin, 2012).

Methodology

Documentation of the enamel microstructure of the incisors was done using the scanning electron microscope (SEM). The procedure for preparing the tooth for viewing in the SEM was modified from Martin and Wahlert (1999) and adapted to our lab. To prepare the incisors for viewing in the SEM, first the incisors were examined and their hibernation marks indicated with

a wax pencil. Each incisor was then glued down with a drop of glue on the base and the tip of the tooth on the edge of a silicon mold longitudinally with the medial (internal) side of the tooth facing outwards. Once the glue was dried, the tooth was set in a block of clear polyester resin (Castin' Craft Clear Polyester Casting Resin). The resin was made based on the manufacture's guidelines using a ratio of six drops of catalyst for every ounce of resin and mixed for 60 sec. Then the resin was poured into the silicon mold to set the tooth and was allowed to harden for at least 48 hours. The embedded incisor was then sanded down on the medial surface using finer and finer grits of sandpaper until the internal enamel was exposed in longitudinal section.

Once the internal enamel was exposed and there were no obvious scratches from the sand-paper, I cut away the section of the block of resin extending from the lateral surface of the incisor. This was done to create a smaller block that could be worked with readily in the SEM.

The location of the hibernation mark on the exposed medial surface of the tooth was indicated on the resin adjacent to the tooth by using a small pin to scratch a small marker. Next, to improve contrast of the prisms for viewing with the SEM, each tooth was etched with 2N HCl for 30 sec and immediately afterwards, the tooth was rinsed for 45 sec with deionized water to neutralize the reaction. After etching the enamel, the embedded tooth was set on a SEM stub and sputter coated with a thin layer of gold for 30 sec. This helped to improve the conductance of the specimen and to reduce the charging of the tooth during examination under SEM. The tooth was then ready to be viewed in the SEM. This preparation was done for four specimens.

Images on the SEM (Zeiss EVO LS10) were taken at magnifications ranging from 310X to 520X. The following parameters were varied to achieve the best image: brightness, contrast, spot size, stigmation, scan speed and noise reduction. Saturation was set at 2.580A, the IProbe at 201 pA and the EHT at 10 kV.

Results

Viewed in longitudinal section, the four incisors of thirteen-lined ground squirrels all displayed uniserial enamel microstructure with alternating singular bands of decussating enamel prisms; vertically oriented rows of prisms arising from the enamel dentin junction alternated with rows of prisms that project toward the viewer (Fig. 1). Enamel displayed a clear enamel dentin junction; a clear distinction between the portio externa and portio interna of the enamel; and defined enamel prisms surrounded by an interprismatic matrix. The portio externa showed radial enamel with parallel enamel prisms with no decussation while the portio interna displayed uniserial HSBs (Fig. 1).

In all incisors, enamel prisms were well defined along the ~two-thirds of the tooth toward the tip of the tooth, with portio interna and portio externa clearly visible (Fig. 2A, 3A). In contrast, SEM imagery from the base of the tooth invariably showed no definition in the prisms, making them undistinguishable from the surrounding resin (Fig. 2C). A transition in prism definition occurred ~one-third from the base of the tooth, with prism definition lost first near the external enamel surface; prisms remained clearly defined near the enamel dentin junction after the portio externa and upper half of the portio interna had no definition (Figs. 2B, 3B).

In one tooth (AU 7984), the junction between the portio interna and portio externa from the region of the hibernation mark was irregular and somewhat difficult to define (Fig. 4A), whereas in active season enamel, the junction between the portio externa and portio interna was well-defined and fairly linear (Fig. 4B–C). However, on another tooth (AU7982) with a documented hibernation mark, this boundary between the portio externa and portio interna was similarly linear and well-defined in both the hibernation mark enamel (Fig. 5B, C) and the active

season enamel (Fig. 5A). Unfortunately, we were unable to visualize enamel of the hibernation mark in two specimens because the hibernation mark occurred near the base of the tooth, and therefore in the segment of enamel with poorly defined prisms (as shown in Fig. 2C).

Discussion

In this study, I characterized the enamel microstructure of thirteen-lined ground squirrels and demonstrated that the enamel displayed uniserial enamel microstructure of the HSB in the portio interna and radial enamel in the portio externa (Fig. 1). These findings are consistent with the types of enamel microstructure that have been demonstrated in some other rodents (Martin, 1999). Thirteen-lined ground squirrel enamel was also shown to exhibit the characteristic features of rodent enamel microstructure including enamel prisms, an interprismatic matrix, an enamel-dentin junction, and a portio interna and portio externa (Fig. 1).

The transition in definition of the prisms that were visualized from the tip of the tooth, where the prisms were well-defined, to the base of the tooth, where the prisms were undistinguishable, probably reflected differences in the mineralization of the tooth. The protein matrix of the enamel is mineralized during the incisor's growth starting from the base of the tooth, causing the tip of the tooth to be more mineralized than the still forming base (Moradian-Oldak, 2012). In the methodology of preparing the incisor for viewing in the SEM, acid was used to etch the enamel and give the prisms more definition. However, if the enamel is not well mineralized then the acid would be less able to etch it, and as a consequence the prisms will lack definition. Therefore, the enamel microstructure was not visible in the basal, poorly mineralized segment of each tooth.

While I did document a difference in enamel microstructure between hibernation enamel versus active season enamel microstructure in one of the incisors (AU7984), I was unable to detect this difference in other teeth. This was partially due to the hibernation mark being present in the basal one-third of the incisors for two of the specimens that we imaged, a region that was poorly mineralized and thus did not display visible microstructure. The other specimen with visible hibernation enamel microstructure (AU7982) had a longitudinally extended hibernation mark that spanned the transition from well-defined (toward the tooth tip) to poorly-defined prisms (toward the tooth base). Although I did not observe microstructural abnormalities in the portion of the hibernation mark with well-defined prisms, it is possible that such an abnormality would have been present at the base of the hibernation mark: on the surface of incisors, irregularities in the enamel are often present at the basal end of the enamel sleeve and hibernation mark (Goodwin et al., 2005). Thus, these results suggest that abnormalities in enamel microstructure (notably an irregular and undefined junction between the portio interna and portio externa) may be associated with hibernation. While the results are not definitive, they do warrant further research.

In summary I have shown that thirteen-lined ground squirrel enamel microstructure displays uniserial enamel microstructure and contains common features characteristic of rodent enamel microstructure. I was also able to record that incisors consistently displayed a transition in prism definition from the tip of the tooth (more definition) to the base of the tooth (no definition) probably due to the process of mineralization of the tooth. Lastly, I found that there is a potential difference in the enamel microstructure of thirteen-lined grounds squirrels during hibernation versus the active season that requires further research.

Acknowledgements

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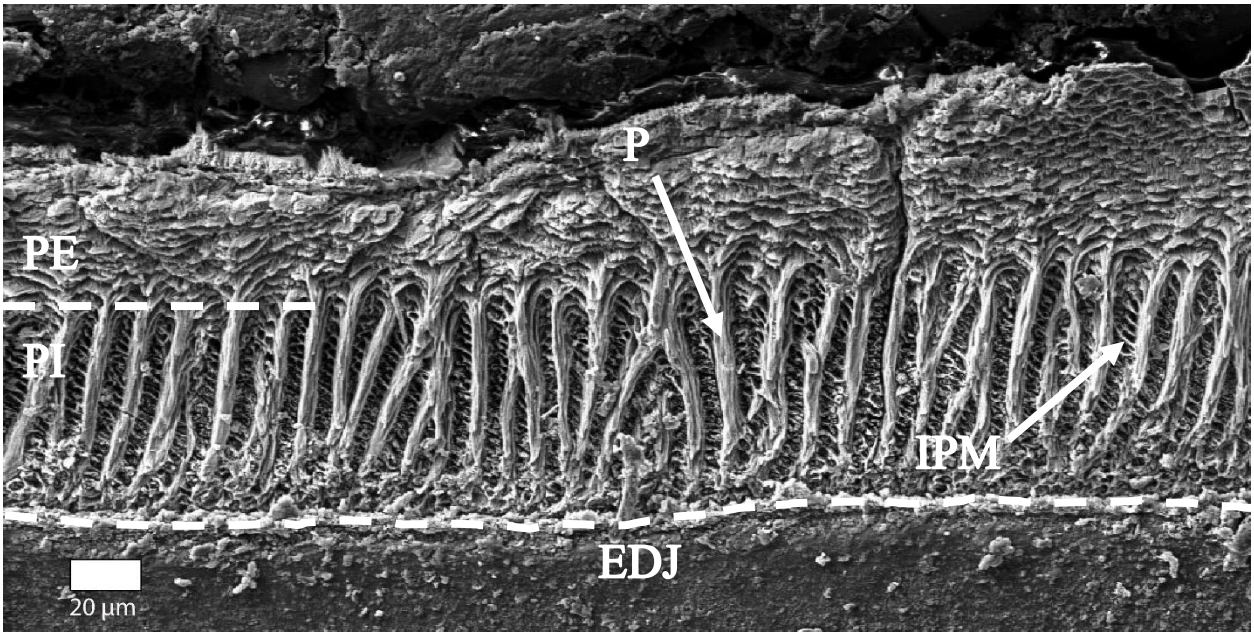

Figure 1. Features of thirteen-lined ground squirrel enamel. Features evident in enamel microstructure of *Ictidomys tridecemlineatus*. This incisor displayed uniserial enamel microstructure (alternating single rows of prisms). Abbreviations: EDJ = enamel dentin junction, PI = portio interna, PE = portio externa, P = prism, IPM = interprismatic matrix. (Specimen AU7982)

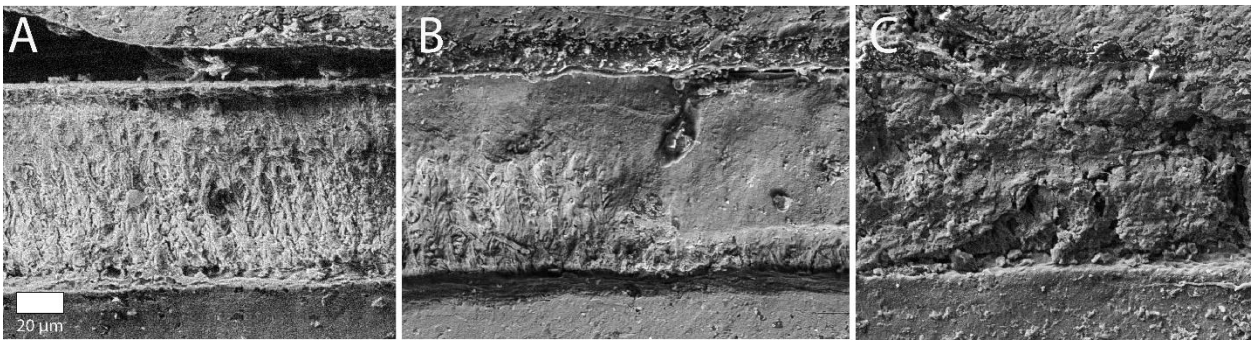

Figure 2. Transition in definition of enamel prisms. Series depicting transition in prism definition from the tip of the tooth (A) to the base of the tooth (C). The tip of the tooth is on the right and the base of the tooth is on the left. (A) Enamel microstructure at the tip of the tooth, shows clear definition of prisms. (B) Enamel microstructure near the base of the tooth, shows transition in the definition of prisms; on the lower left near the EDJ there is clear definition of prisms that begins to fade as you precede towards the right which is more towards the base of the tooth. (C) Enamel microstructure at the base of the tooth where there is no definition of prisms. (Specimen AU7976)

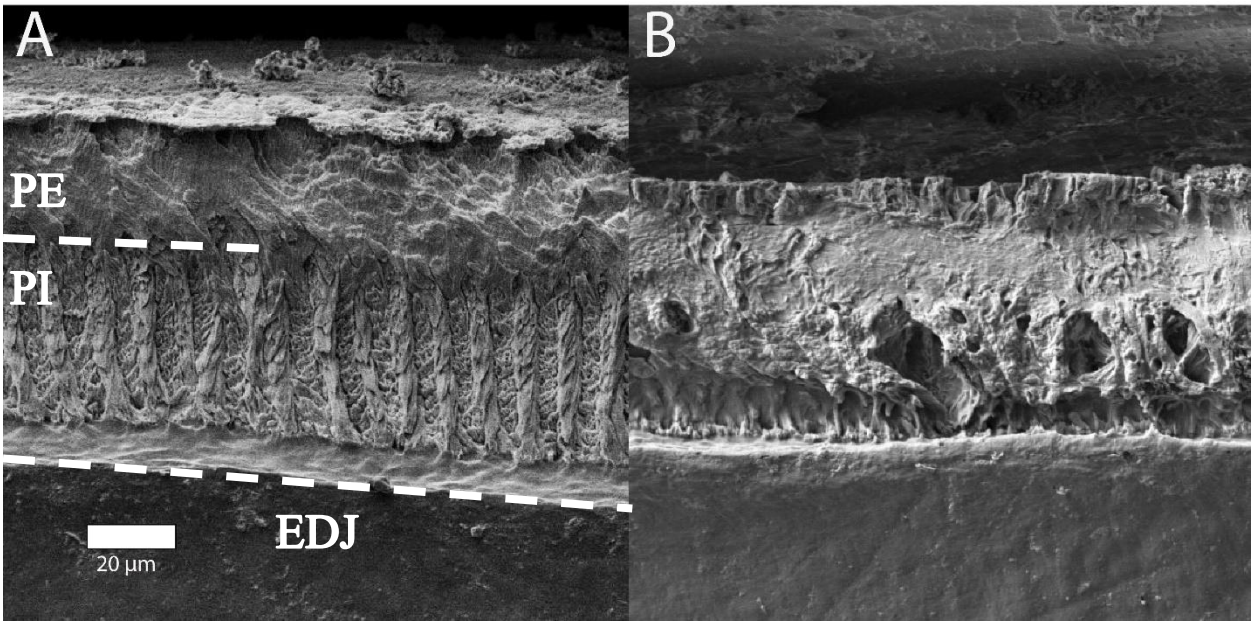

Figure 3. Transition zone. Pictures showing the transition in prism definition in another incisor. Incisor is oriented with the tip of the tooth on the left and the base of the tooth on the right. (A) Enamel microstructure from the tip of the tooth, shows clear definition of enamel prisms in the PI and PE. (B) Transition zone near the base of the tooth, clear definition near the EDJ but definition is lost in the PE and the upper half of the PI. Note: Both images were taken at the same magnification, the enamel where (B) was taken was thinner than at (A). (Specimen AU7984)

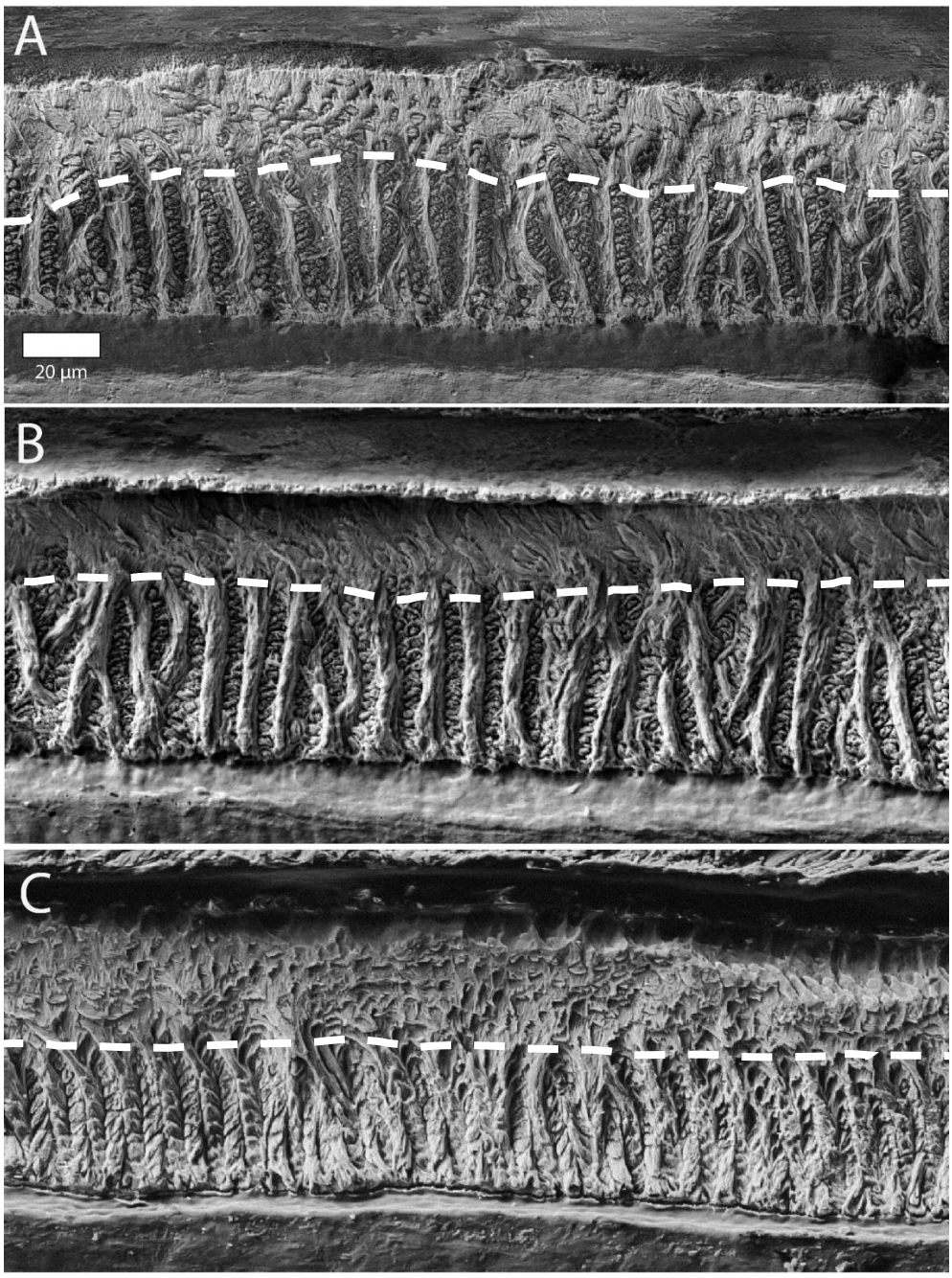

Figure 4. Comparison of hibernation and non-hibernation enamel. Series of images depicting the hibernation and non-hibernation (active season) enamel from a single specimen. The images are arranged from closest to the tip at the top to closest to the base at the bottom; in each image, the tip of the tooth was toward the left and the base of the tooth was toward the right. (A) Hibernation–mark enamel at the middle of the tooth, shows a distorted junction between the portio interna and portio externa. (B) Active season enamel slightly past the hibernation mark, shows clear and linear junction between the portio interna and externa. (C) Active season enamel in the basal half of the tooth, also shows a clear and linear junction between the portio interna and externa. (Specimen AU7984)

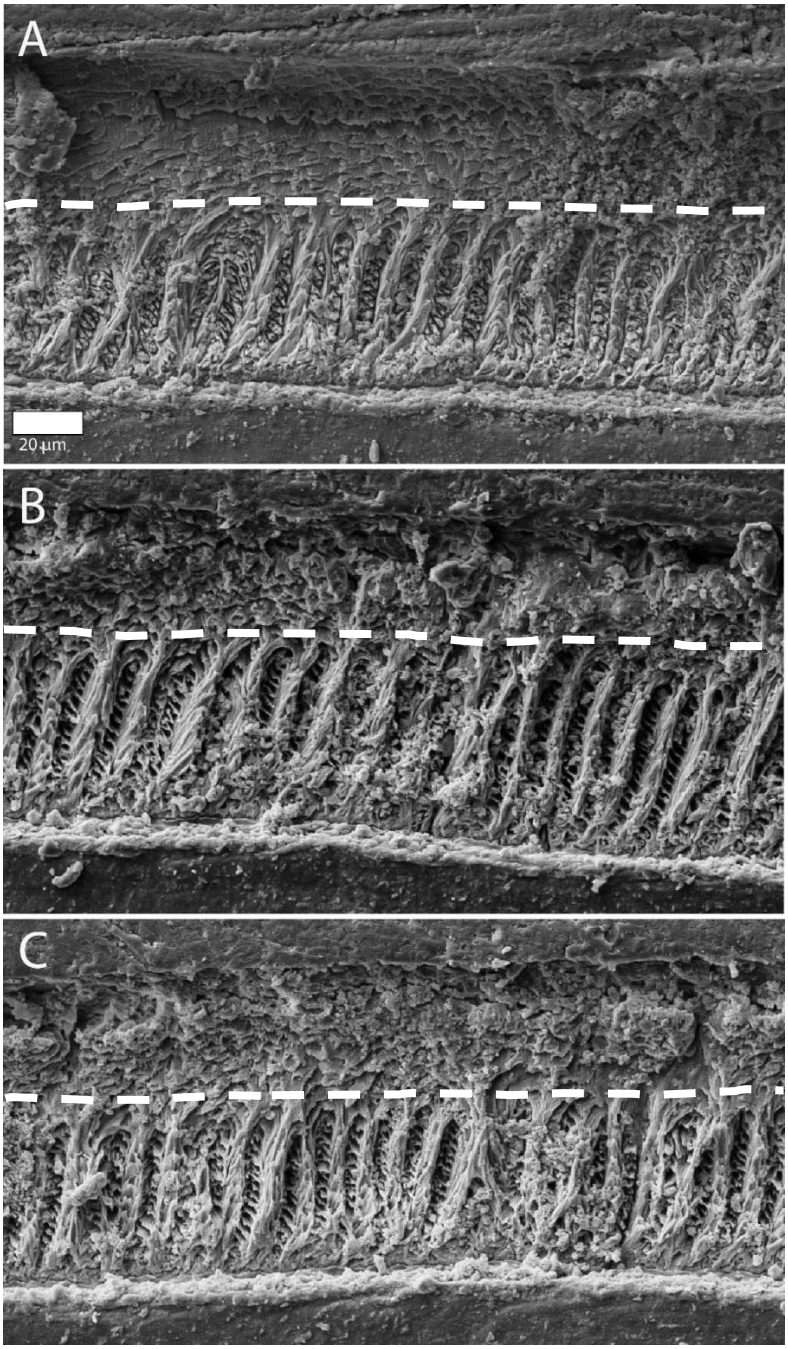

Figure 5. Hibernation and non-Hibernation Enamel. Images showing the comparison of hibernation to active season enamel. The images are arranged from closest to the tip at the top to closest to the base at the bottom; in each image, the tip of the tooth was toward the right and the base of the tooth was toward the left. (A) Active season enamel from the middle of the tooth, shows a clear and linear distinction between the portio intera and portio externa. (B) Hibernation mark enamel from the base half of the tooth, shows a clear and linear junction between the portio interna and externa. (C) Hibernation mark enamel a little farther towards the base of the tooth, shows clear and linear junction between the portio interna and externa. Note: this tooth was pitted with a laser and thus may show distortions in the enamel. (Specimen AU7982)