

Comparative Mammalian Dental Enamel Histology

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A special thank you to all who made this possible! Without my constant support squad, I would never have been able to make it this far!

Love y'all.
Sarah-Wallace

ABSTRACT

Using light and scanning electron microscopy, this research documents similarities and differences in the enamel composition of human, dog, pig, and deer teeth. The main focus is on the Hunter-Schreger band (HSB) phenomena characteristic to mammalian dental enamel. The human teeth are controls to which comparisons are made. Deciduous and permanent maxillary and mandibular incisors, canines, premolars, molars were examined from labio-lingual and mesio-distal thin sections. HSBs in mammals compared to humans proved to show width and length differences while their enamel prism size did not vary across species. It is believed that human tooth enamel compared to other mammals can be a distinctive factor in the recovery process of comingled remains to determine to whom the tooth belongs.

PREFACE

Whether a single death or an unknown number of decedents, skeletal and dental fragmentation is not an uncommon crime scene discovery. From a mass disaster scene with widespread blunt-force trauma characterized at the Twin Towers (9-11) in New York City and bombing of the Alfred P. Murrah building in Oklahoma City, to a high-impact military aircraft loss with conflagration from southeast Asia a half a century earlier, diminution of these tissues confound identification and subsequent placement with a decedent. Further, the commingled nature of crime scenes with existing fauna, especially dog, deer, and pig, may complicate the identification process. The NASA Columbia disaster in 2003 resulted in a multiple, county-wide recovery scene resulting in the discovery of hundreds of commingled human and non-human bone and dental fragments. There is great importance in the identification of all remnants of the victim/decedent, human and non-human, soft and mineralized tissues mandate the forensic investigation.

This research documents, through the manufacture of dental thin sections of incisor, canine, premolar, and molar teeth, the histological nuances at the genera level of the above-mentioned animals, to clarify human and non-human identification when tooth crowns are fragmented. To date, only elementary comparative histological analyses exist on these specific mammals and not for the relevant forensic community charged with identification. This

research compares and contrasts enamel prisms and Hunter-Schreger banding in enamel.

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CHAPTER ONE

INTRODUCTION

Solitary or multiple homicide or accident victims and mass terrorist or natural disasters are unpredictable events. Such crime scene evidence may be discovered as a complete or partial body or unrelated parts including teeth, skin, hair, muscle and/or bone. Occasionally, as evidence is gathered, fragmented and commingled remains may be encountered requiring a differentiation between human or non-human and matching among victims for the purposes of identification.

These questions bring this research topic into focus. When a body is mutilated or fragmented, some parts of the recovered remains will represent oral anatomy. Perhaps a single tooth from a clandestine or carnivore-scavenged scene with a long postmortem time frame is the only evidence. Scene gathering and processing all remains and correct identification is a paramount goal. When the fatalities are multiple or reach a magnitude of hundreds, the identification process becomes monumental. In the event of mass or natural disaster, the gathering of non-human remains is unavoidable.

Other than human-made mass disasters like 9-11, environmental disasters like Hurricane Katrina in 2005 impacted not only humans but animals, plants, buildings, and houses were also damaged by the life-altering hurricane. When the high winds, waves and flooding ceased and search and rescue could begin, there was so much more discovered than human remains. Close to 2,000

people were pronounced deceased from the catastrophe that struck the southern states of Louisiana, Alabama, Mississippi, and Florida and nearly 90,000 square miles were affected by the tropical depression. The aftermath of a natural tragedy with the magnitude such as Katrina and the amount of wildlife and habitat loss, made the recovery process monumental.

Background

The teeth used in this study are human, dog, deer, and pig with the main goal being able to qualify their enamel on a microscopic level. The forensic goal is to diagnose fragmented remains from dental anatomy. Foundational knowledge on the creatures that are being examined in this experiment will help determine what animal the teeth belong to.

Human Teeth

Throughout life, we have twenty deciduous and thirty-two permanent teeth. A few individuals will keep all thirty-two teeth. Humans have incisors, canines, premolars and molars with the deciduous teeth beginning emergence around 6 months after birth and continuing until around 2-3 years. This stage is called the “teething” phase of infancy and childhood. The 3 to 6-7 year time frame is termed the “use” phase of the deciduous dentition. During this phase, the permanent crowns and roots are being completed beneath and lingual to the deciduous crowns and roots. From 6-7 years until 13-15 years, the follicles of the

underlying permanent crowns are dissolving the deciduous roots in a phase termed “mixed dentition.” Around 18 years, the “use” phase of the permanent dentition begins. This research project will evaluate the enamel prisms and Hunter-Schreger banding characteristics of our human (control), dog, deer, and pig dentitions.

Non-human Mammalian Teeth

Similar to humans, dogs (*Canus familiaris*) possess incisors, canines, premolars, and molars. A dog has fourteen deciduous teeth and twenty-one permanent teeth that have a much faster growth rate or “turnover” than the five human tooth phases. According to Reiter et al (2016), a dog’s deciduous dentition emerges within 4-6 weeks after birth with permanent teeth emerging between 3-7 months. A rare occurrence is the retention of deciduous teeth.

In research, white-tailed deer (*Odocoileus virginianus*) teeth are sectioned to estimate age from their cementum annulations. Deer have incisors, premolars and molars and on a rare occasion, a canine. At birth, a fawn has four mandibular incisors. Their deciduous teeth are soon replaced by thirty-two permanent teeth. A few weeks after birth, sixteen teeth emerge, totaling eight incisors and twelve premolars. At one-year, twelve molars emerge giving the deer a full set of 32 teeth. Past research has demonstrated the validity of estimating the age of deer from evaluation their cementum annulations.

Humans and pigs (*Sus scrofa*) are most closely related in dental development.

“As with humans, pigs feature molars, premolars, canines, and incisors and similar to most mammals, pigs and humans are diphyodont or develop and erupt two generations of teeth into their jaws. In the pig's deciduous formula of 3/3, 1/1, 3/3, there are three incisors, one canine, and three premolars on each side of the bottom jaw for a total of 28 teeth as compared to humans with 20 primary teeth growing into a mixed dentition of permanent and primary teeth that ends by age 13 years. For permanent teeth, pigs exhibit a dentition formula of 3/3, 1/1, 4/4, 3/3 or three incisors, one canine, four premolars, and three molars on each side of the top and bottom of the mouth for a total of 44 teeth.” (Davidson 2017).

Dogs are significant to this study given their association with humans and the scavenging of crime scenes by the ubiquitous coyote. Deer, as a hunted animal, is often immediately dressed in the field with hunting dogs and later, with coyotes scavenging the remains. Therefore, bones and teeth from a variety of animals are frequently found commingled at scenes with human skeletal and decomposing remains. Crime scene personnel seldom include a forensic anthropologist and even more seldom include a dentist. Most scene death investigators are poorly trained to recognize non-human from human bone and teeth. To err on the side of safety they collect everything.

CHAPTER TWO

LITERATURE REVIEW

Enamel is the hardest calcified matrix of the body (Nanci, 2013). Enamel is the hardest tissue in the vertebrate body and is found covering the crowns of teeth in mammals, reptiles, and amphibians (Lynch et al, 2010). Before enamel is created as the hard structure it exists during life, enamel is made by ameloblasts during development. Ameloblasts are enamel-forming cells that arrive during the bell stage of embryonic tooth development. At its simplest level, enamel is composed of inorganic and organic phases with the former predominating in mature enamel comprising 91% of the volume and 98% of the weight (Lynch et al, 2010). Enamel's underlying structure is extremely strong. Enamel is 96% inorganic while the other 4% is organic material and water. Tooth enamel is ectodermal in origin analogous to hair, skin, and nails. The inorganic content of enamel is a crystalline calcium phosphate (hydroxyapatite) substituted with carbonate ions, which is also bound in other ectodermal tissues such as: bone, calcified cartilage, dentine, and cementum (Nanci, 2013). Various ions--strontium, magnesium, lead, and fluoride—if present during enamel formation, may also be incorporated into the crystals (Nanci, 2013). These minerals within enamel make the substance extremely strong. As stated earlier, the ameloblasts are the enamel forming cells that are present during enamel development. According to Nanci, "... the ameloblasts, are lost as the tooth erupts into the oral cavity, and hence cannot renew itself." Knowing that enamel cannot renew itself supports the fact that this tissue has extreme integrity.

Enamel Prisms

The fundamental organizational units of mammalian enamel are the rods/prisms and interrod enamel/ interprismatic substance (Nanci, 2013). These structures can be viewed using a light microscope seen in Figure 2.2 and a scanning electron microscope seen in Figures 2.1.

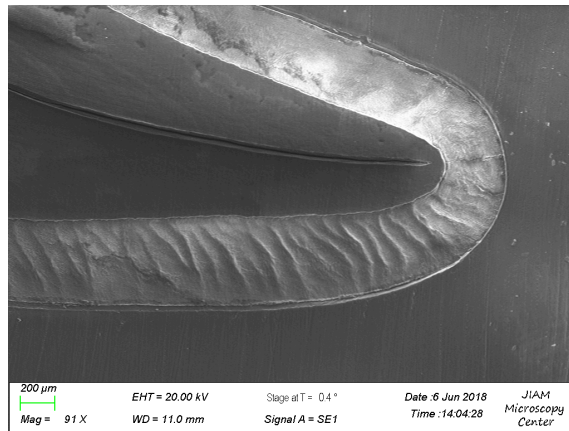


Figure 2.1 Above is a scanning electron micrograph of a pig canine at 91X.
Figure 2.2 Below is a light micrograph of the same pig canine at 50X.

Brand and Isselhard (2014) also characterize these two parts as the rod sheaths and the enamel rod. The rod is made of up hydroxyapatite crystals and is the primary unit of enamel's structure. Prisms are the common name for these rods and are interchangeably used in texts. The shape of the enamel rods is not always predictable. The enamel rods pack together tightly due to the cementing substance called the interrod substance and usually described as “keyhole shaped” (Brand and Isselhard, 2014; Nanci, 2013). “However, they may have different appearances in various areas of enamel or because of species differences,” state Brand and Isselhard, 2014. Figure 2.3 illustrates the different images that can be produced after sectioning the prisms in difference directions.

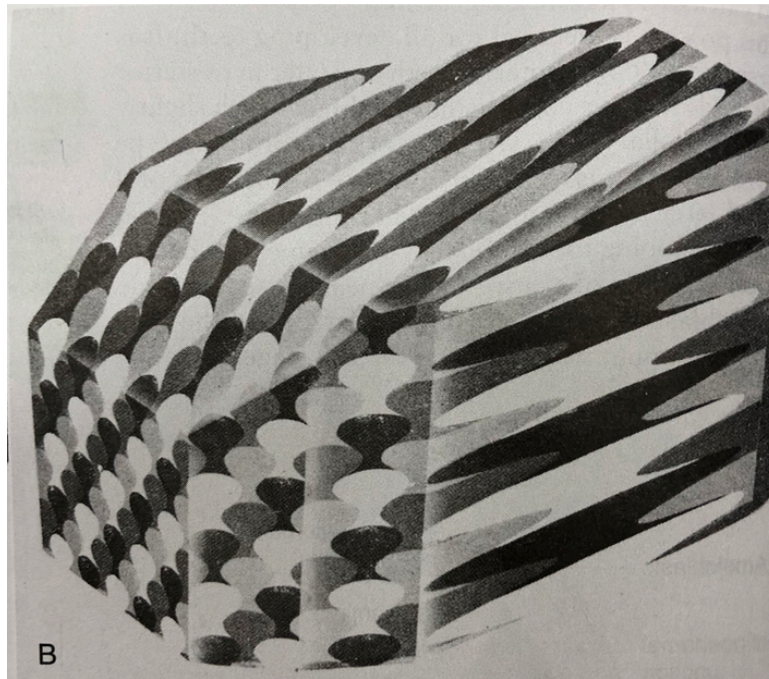


Figure 2.3 Picture B from Brand and Isselhard (2014), shows how cutting different angles can change the appearance of the rods.

“Enamel is built from closely packed and long carbonatoapatite crystals measuring 60-70 nm in width and 25 to 30 nm in thickness. Some investigators believe that the length of the crystals actually spans the entire thickness of the enamel layer,” (Nanci, 2013). Enamel prisms are measured in units of angstroms. An angstrom is $1/100,000,000^{\text{th}}$ of a centimeter. There are enamel prism/rod arrangements, seen in Figure B, that change upon the direction in which the tooth is sectioned. Dependent on the thin section of an enamel prism, their orientation is seen as a longitudinal or concave/ convex cross-sectional cut. This can also be seen in Figure 2.3. Although enamel prisms can be seen using a light microscope, a scanning electron microscope produces photos like Figure 2.4, which are necessary to visualize the enamel prisms in greater detail.

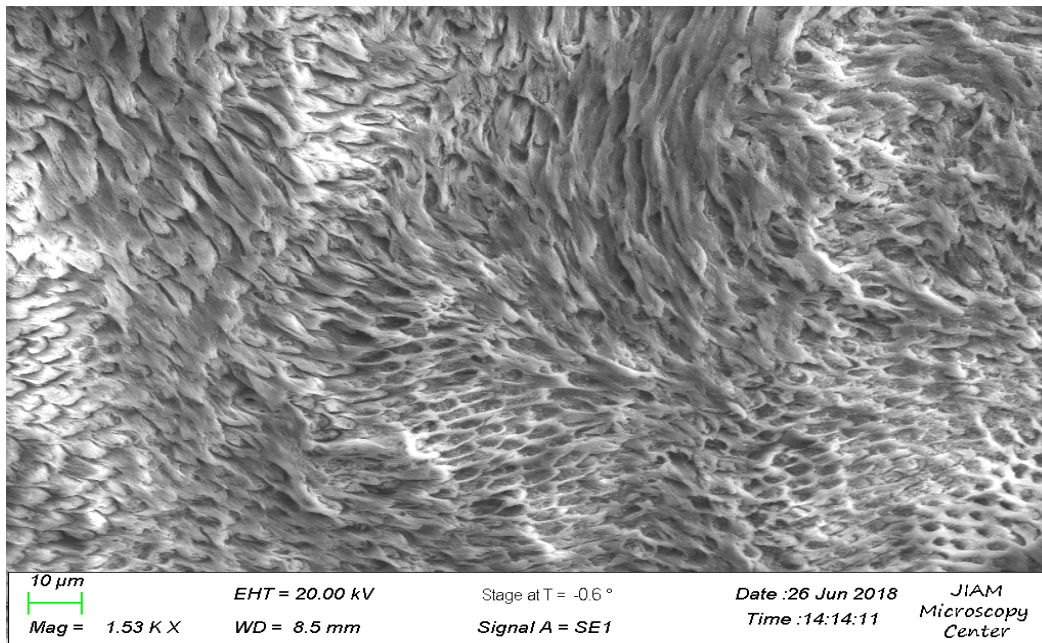


Figure 2.4 Scanning electron micrograph of the enamel prism arrangement of a pig tooth at 1.53 KX.

Striae of Retzius

The Striae of Retzius can be seen in histological thin sections of all of the mammals within this study. The striae of Retzius are generally identified as looking completely different from the other bands and lines seen in the enamel. In a longitudinal section of a tooth, they are seen as a series of dark lines extending from the dentinoenamel junction toward the tooth surface, while in a cross-section, they appear as concentric rings (Nanci, 2013). The dentinoenamel junction is the area in which the dentine and enamel meet. The striae of Retzius are believed to come from interruptions during enamel production. Another type of line seen within the enamel structure is the neonatal line. This is a line demarcated by the physiological changes that occur during birth. The neonatal line is an enlarged and well-defined stria of Retzius. Accentuated incremental lines are also produced by other systemic disturbances (ex: fevers) that affect amelogenesis (Nanci, 2013).

Hunter-Schreger Bands

The enamel prisms' arrangements are what give rise to the Hunter-Schreger bands. "The bands of Hunter and Schreger are an optical phenomenon produced by changes in direction between adjacent groups of rods," Nanci, 2013. These features are called Hunter-Schreger Bands (HSBs) in recognition of the first observers credited with reporting this curious phenomenon (Lynch et al, 2010). Neither of those investigators could suggest a reason for the presence of

the bands. Until the 1960s, the accepted explanation was that the appearances of HSBs were caused by differences in mineralization and hardness throughout the enamel, demonstrated by acid-etching, silver-staining, and microradiography techniques (Lynch et al 2010). Hunter-Schreger bands are seen in enamel when viewed under light microscopy at magnifications of 10X, 50X, 100X, and higher. The Hunter-Schreger bands can only be seen by the naked eye if the tooth has been thin-sectioned; however, to see the band detail, light or scanning electron microscopes are required. Current opinion suggests that the appearance of HSBs is related to the synchronous decussation of enamel prisms in the horizontal plane and is probably caused by reflection of light by inter-prismatic material (Lynch et al, 2010). Seen in Figure 2.5 are the light and dark banding of the enamel prism arrangements also known as Hunter-Schreger bands. The light and dark banding is also characterized as parazonies and diazones. Parazonies are the light bands whereas the diazones are the dark bands. The bands are more clearly seen in longitudinal ground sections seen below in Figure 2.5. This thin section is a labio-lingual section of a dog mandibular incisor.



Figure 2.5 Light micrograph of a dog-mandibular incisor's cusp tip at 100X showing the light and dark banding.

Gnarled Enamel

Gnarled enamel is a type of enamel that can be seen the crown region of most teeth, but primarily seen in molars. This type of enamel has a distinctive histological appearance. The Hunter-Schreger bands in gnarled enamel are more undulated resembling a wavelength design contrary to the normal straight bands seen in enamel. Over the tooth cusps, the rods appear twisted around each other in a seemingly complex arrangement known as gnarled enamel (Nanci, 2013). In this research, gnarled enamel was mostly seen in the cusp tips of pig and dog teeth as seen in Figure 2.6.

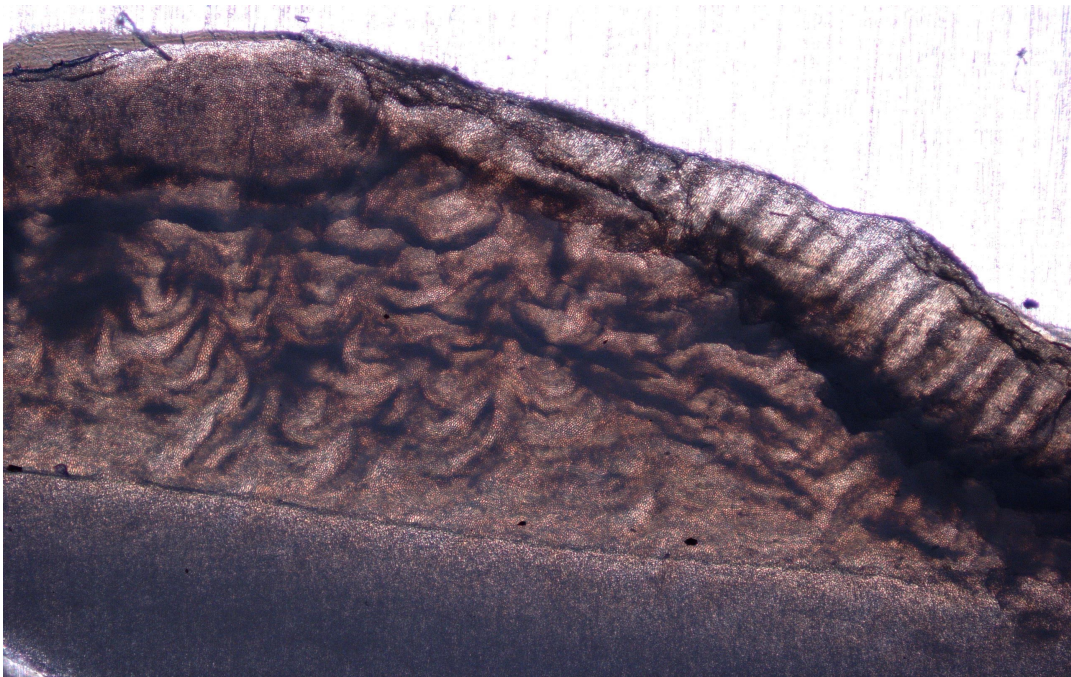


Figure 2.6 Light micrograph of a mesio-distal thin section of a dog-mandibular first molar with gnarled enamel at 50X.

CHAPTER THREE

MATERIALS AND METHODS

Histology is the science of looking at specimens under microscopes. This research is based off of features that can be seen on a microscopic level.. In order to capture all of the material for this research, histological thin-sections must be made. Beginning with obtaining the teeth from all of the chosen mammals, photographs for documentation are made of every tooth in multiple categories for each species shown in Figure 3.1. Once all of the photographs have been taken using a *Nikon Cool Pix* camera, we move on to sizing our teeth against the different molding compartments.



Figure 3.1 Photograph of the teeth before they were embedded in an epoxy resin mixture.

The molds used for this research are *Peel-A-Way* disposable plastic tissue embedding molds from *Polysciences Inc.* Most of the teeth were embedded in a 22X30 mm rectangular mold due to longer root lengths differing from some that were embedded in the 22X22X20 mm square molds. The same materials and

methods were used in Marks et al 1996. Before preparing the embedding mixture of *Buehler EpoThin™2 Epoxy Resin* with its compatible *Buehler EpoThin™2 Epoxy Hardener*, wrap copper transformer wire around the cements/enamel junction of the tooth as seen in Figure 3.2.

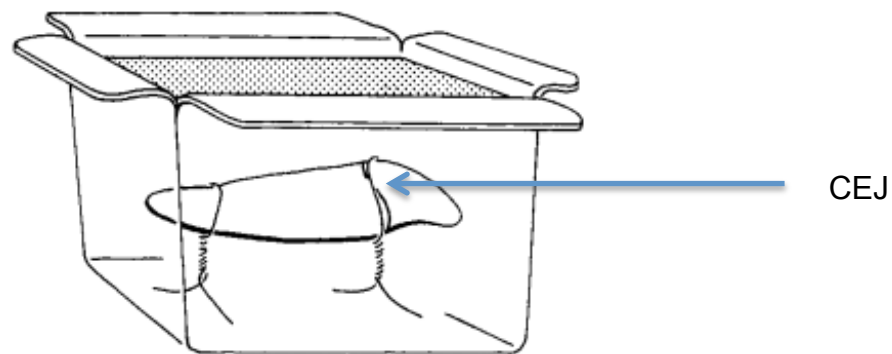


Figure 3.2 From Marks et al 1996, showing how the teeth were suspended in their *Peel-A-Way* molds before the Epoxy resin was poured over them.

There needs to be at least 1-inch of copper transformer wire wrapped tight around the cements/enamel junction/ cervix of the tooth. After wrapping the tooth's cervix with copper wire, the next step is to place the tooth in the *Peel-A-Way* compartment that is compatible with the tooth's size and shape. Once the selected tooth has been placed in the plastic compartment, a small drop of *Duro* super glue should be placed on the top of the tooth at the cervix, where the wire has been wound around, as well as the base wires that are hanging from the tooth. These small drops of superglue secure the positioning of the tooth inside

its selected *Peel-A-Way* mold. Allow the super glue to dry before going any further in the embedding process. After all of the teeth have been super glued in position, the experimenter mixes the *Buehler EpoThin™2 Epoxy Resin* with the *Buehler EpoThin™2 Epoxy Hardener* together. Once this step has begun, the embedding/“pouring up” process has been initiated. Prior to the pouring up process, paper identification tags are placed within the holding brackets of the *Peel-A-Way* molds. “Only pencil and certain inks will remain legible after the epoxy saturates the paper during mold filling,” (Marks et al, 1996). Figure 3.3 seen below illustrates the importance of labeling the identification tags with pencil during the pouring up process.



Figure 3.3 Photograph showing human teeth embedded in epoxy resin with labels that are easily readable.

The epoxy resin and epoxy hardener solutions are not measured by volume but rather by weight using a scale. This step requires a clean glass beaker for weighing and eventually mixing the two solutions. If any material other

than glass is used during this part of the experiment, the epoxy resin and epoxy hardener will burn completely through any other material due to the exothermic reaction caused by mixing the two solutions. Once the two solutions have been added to each other, the researcher has approximately 2 minutes to fill the molds before the mixture begins to set up. When pouring the molds, pour in a front to back direction, completely submerging the tooth in the epoxy resin/hardener mixture. A helpful hint: fill the mold more than necessary. As the mixture hardens, the air escapes the mixture and therefore tightens the mold around the tooth. Give the just poured blocks 24 hours for a full curation before the cutting and mounting process of the newly created blocks can begin.

Cutting The Blocks

When the molds are ready to be thin sectioned using the *Buhler Isomet slow-speed saw*, the first task is to take your hardened blocks out of the *Peel-A-Way* mold chambers. As the researcher takes the blocks out of the molds, it is seen that the paper identification tag that was placed in the holding brackets is also embedded in the block. Once the *Peel-A-Way* molds have been removed, the next step is to mount the block on to the aluminum holder arm, which is attached to the *Isomet* saw. After the block has been attached to the holder/arm, the cutting process can begin. The initial cut needs to be made almost through the middle point of the block and specimen. In order to get an accurate slide with the correct tissues affixed, the first cut should be done this way. Following the

first cut on the block, the fresh cut side of the block should be cleaned with 70% isopropyl alcohol. Cleaning the fresh side with isopropyl alcohol is to remove the oil and residue that is gathered from cutting the block. After cleaning with isopropyl alcohol, dry the new surface off as well as guarantee there is no residue from the oil remaining on the block. If there is remaining oil, when the glass slide is mounted, the glass will not be able to stick to the fresh cut surface. The next step is to have an *Abco 3"X1" Micro Slide* with rounded corners as well as a 1.10 mm thickness. Since the mammals that were used for this experiment have larger teeth than normal human teeth, these larger slides are more valuable. Before the mounting process takes place, use a diamond engraving pencil as used in Marks et al (1996), to etch on to the glass surface the label for which the tooth that will be mounted on that specific slide. Figure 3.4 shows a label that was etched on to the glass slide. Figure 3.4 also shows what the end product of making a thin section should look like prior to the grinding and etching processes.

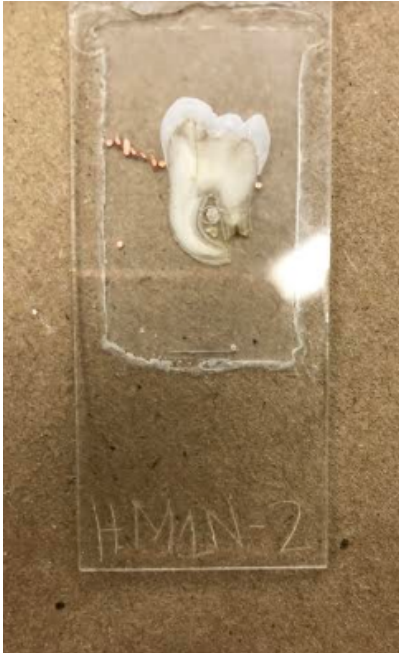


Figure 3.4 Photograph of a thin-section slide, showing how the label is etched on to the glass for variation of the teeth from one another.

After the label has been etched to the slide, the mounting process is ready to begin. *Duro* super glue is necessary for this next step. Put the super glue on the side opposite your label and then place the slide on the clean surface of the block very quickly. Hold the slide on the block for roughly 10 seconds and then release the slide. Within those 10 seconds, attempt to skate the glass slide on the surface of the block to eliminate air bubbles. The glass slide should be attached to the block after that step. After mounting the glass, the cutting can begin. Cranking the thickness dial to cut at a .35 mm thickness is optimum for getting the proper amount of tissue on the slide. After ensuring the correct thickness of the slide, turn on the Isomet saw. The speed should be set at a slow speed of 3-4. Gradually crank the cutting speed up to 6-7 to speed the cutting

process of the slide for faster production time. The mounting and cutting step should be repeated twice, producing two slides made for each individual tooth. This allows for mistakes to happen and there be a second slide able to evaluate.

Grinding and Acid Etching a Slide

After the slides have been made, the next step is to grind the slides down. Grinding slides down is very important in enamel microscopy. If the slides have been cut too thick, then the evaluation of the Hunter-Schreger bands will be impossible. The grinder used in this research was a normal polishing and grinding piece of equipment. In order to grind down the slides, the use of 600-grit paper was needed as well as a *System Abele* slide holder. When grinding slides, periodically check on the thickness of the slide to make sure that all of the material is not being ground away. The optimum slide thickness is 15-50 microns, but if calipers are not accessible, hold the slide up to light and see if when the light shines through the enamel that the enamel is an amber color. When the amber color can be seen, then the etching step can take place.

“Wilson and Shroff (1970) and Rose (1977) recommend sections be etched in one normal solution of hydrochloric acid for 15 sec with moderate agitation of the slide. After etching, rinse slides in tap water (do so thoroughly or acid etching will continue), dip in 95% alcohol, and air dry,” (Marks, 1996). This was done using the exact same recommendation of one normal solution of hydrochloric acid but rather dip the slide for 15 seconds, it was dipped for 30-45

seconds. Some times the grinding and etching process had to be repeated if the enamel features were not seen under the light microscope.

Light Microscopy

The purpose of this research is histological examination enamel composition at 10X, 50X and 100X using a *LEICA* model *DMRX* light microscope. *Image-Pro Express* software with a *SONY DXC-S500* mixing system for digital imagery of the thin sections was used. All slides were viewed in the same order with micrographs taken at 50X and 100X. The Hunter-Schreger bands seen at 10X, but to ensure clarity, 50X and 100X were used to examine the cusp tip, mid coronal region on both labial and lingual surfaces as well as the cemento-enamel junction.

Scanning Electron Microscopy

A *ZEISS EVO | MA 15* scanning electron microscope seen in Figure 3.5 from the University of Tennessee Cherokee Farms' Joint Institute for Advanced Materials was utilized to capture the structural reality of the enamel. The same slides utilized for light microscopy were used for SEM analysis after coating them with 25 nanometers of gold palladium. The gold plating allows surface electron movement and image clarity. Copper tape affixed to the slide corner the corner provided anchorage of the slide to the platform while inside the vacuum chamber.

Magnifications of 89X-626X produced micrographs revealing the big picture to narrowing focus on single and grouped prisms.

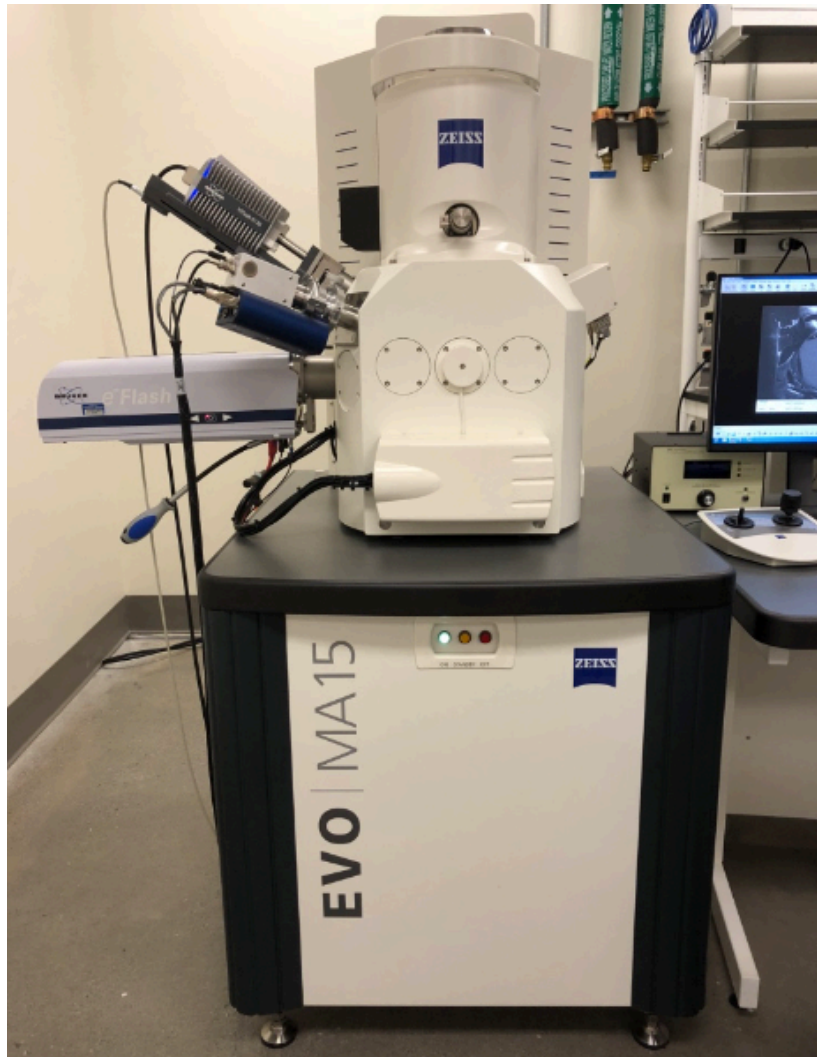


Figure 3.5 A *Leica EVO/MA15* scanning electron microscope from the University of Tennessee Cherokee Farms' Joint Institute for Advanced Materials.

CHAPTER FOUR

RESULTS AND DISCUSSION

After using light and scanning electron microscopy, the objective was to determine if the enamel band measurements in the teeth of the human, dog, deer, and pig are different. Examination revealed differences in the lengths of the bands of human teeth compared to the other mammals. Human Hunter-Schreger bands do not span the entire length of the tooth shown in figure 4.1, whereas other mammalian HSBs span the entire length of the enamel also seen in figure 4.1.

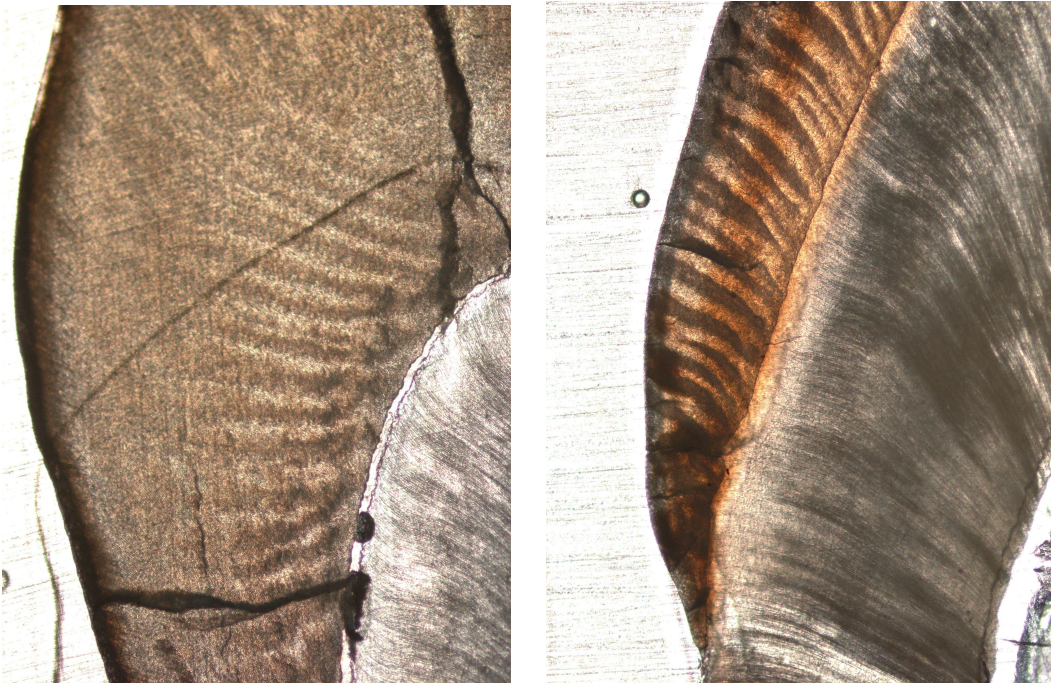


Figure 4.1 The left micrograph is the distal side of a human-mandibular first molar mid-coronal region compared to the right micrograph, which is the lingual side of a deer mandibular first molar in its mid-coronal region. Both images were micrographed at 50X.

Light microscopy was not only used to determine structural differences, but the mechanical differences of the microscopes as well. Viewing the samples under the power of 50X was more diagnostic than using 100X. The HSBs were condensed to too small of a plane of vision when evaluated at 100X. Additionally, the lines were more distinct in the mid-coronal regions of each tooth than in the regions of the CEJ and cusp tip.

After examining 28 total slides of teeth all with examples from human, dog, deer, and pig, measurements were taken on the band lengths and widths. In the measurements, each category of mammal: human, dog, pig, and deer, had one representative tooth. The single tooth measured from each mammal was all mandibular M1's or mandibular first molars. The distance between cusp tip to CEJ were measured in millimeters (mm) using calipers. The results were: human 7.11mm, dog 8.88mm, deer 8.98mm, and pig 5.99mm. This showed that they were all similar in this respect. Additionally, using an Image Pro Express computer system, the distance between each of the enamel bands were measured in pixels. The term "pixel" is actually short for "Picture Element." According to TechTerms.com, "These small little dots are what make up the images on computer displays, whether they are flat-screen (LCD) or tube (CRT) monitors. The screen is divided up into a matrix of thousands or even millions of pixels" The results from these measurements are as followed in Tables 4.1-4.4.

Table 4.1

Human Hunter-Schreger Banding

Count	Statistic	Length	Angle
1	Min. Val.	114.1253	94.83042
2	Max. Val.	312.1969	111.1613
3	Range	198.0717	16.33064
4	Mean	238.1679	104.0247
5	Std. Dev	64.66621	5.861157
6	Sum	2619.547	1144.272
7	No. of Sample	11	11

Table 4.2

Dog Hunter-Schreger Banding

Count	Statistic	Length	Angle
1	Min. Val.	46.21803	125.3112
2	Max. Val.	258.3352	141.3402
3	Range	222.1172	16.02898
4	Mean	148.736	132.0416
5	Std. Dev	61.96231	4.848954
6	Sum	2379.776	2112.667
7	No. of Sample	16	16

Table 4.3

Pig Hunter-Schreger Banding

Count	Statistic	Length	Angle
1	Min. Val.	91.30435	1.789911
2	Max. Val.	216.0858	180
3	Range	126.7815	178.2101
4	Mean	132.4373	109.115
5	Std. Dev	32.05428	85.80903
6	Sum	2648.746	2182.3
7	No. of Sample	20	20

Table 4.4

Deer Hunter-Schreger Banding

Count	Statistic	Length	Angle
1	Min. Val.	43.04128	16.43495
2	Max. Val.	124.5787	51.84277
3	Range	81.5374	33.40782
4	Mean	95.69151	34.44145
5	Std. Dev	18.85871	8.536629
6	Sum	3157.82	1136.588
7	No. of Sample	33	33



Figure 4.2 Shows the technique used for recording the measurements produced by the excel spreadsheets.

Figure 4.2 shows how the numbers in tables 4.1-4.4 were recorded using the measuring software within the *Image Pro Express* program. The green numbers represent the distance between the middle of the each band from one another. Starting at the bottom with 156.5217, this measurement started in the middle of the last band and spanned until the middle of the band directly above it. The values are measured in units of pixels. According to TechTerms.com, a pixel is a measurement of the resolution of an electronic image device such as a computer; hence they make up a computer's image. Pixels can be measured in PPI pixels per inch or PPCM pixels per centimeter. After taking measurements of the enamel bandwidths, it was determined that with the larger the tooth crown is, there will be more bands to count. Shown in our table on the number of samples row, humans had the fewest number of bands to measure between whereas deer has the most with 33 bands. All of these teeth were measured at the same mid-coronal region at 50X magnification to keep consistency.

It was also noted that deer enamel bands seem to be thinner in comparison to dog bands. Does this mean that dog bands can't sometimes be skinnier? No, but mostly, when lingual side of the tooth was examined, the dog's bands were much thicker than any other animal observed in this research.

All four mammals were evaluated using different scanning electron microscope magnifications at different regions of the tooth. The enamel prisms/rod arrangements were assessed to determine mammalian differences. After comparing micrographs of the enamel rod arrangements, it was revealed that

there are species differences. Human tooth arrangements are shown in Figure 4.3 and pig tooth arrangements are shown in Figure 4.4.

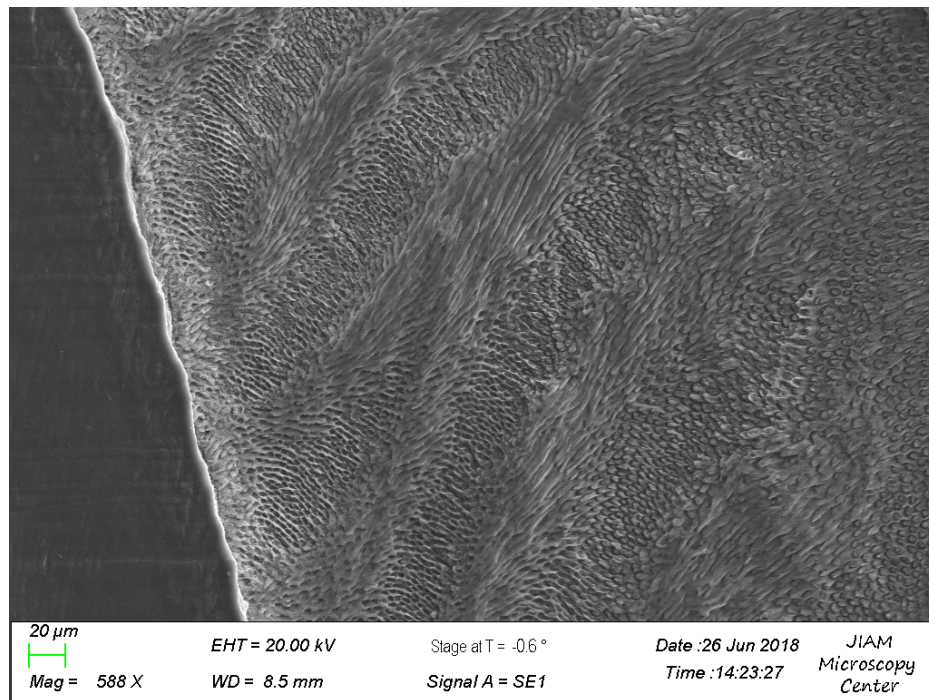


Figure 4.3 Scanning electron micrograph of human tooth enamel prism arrangement at 588X.

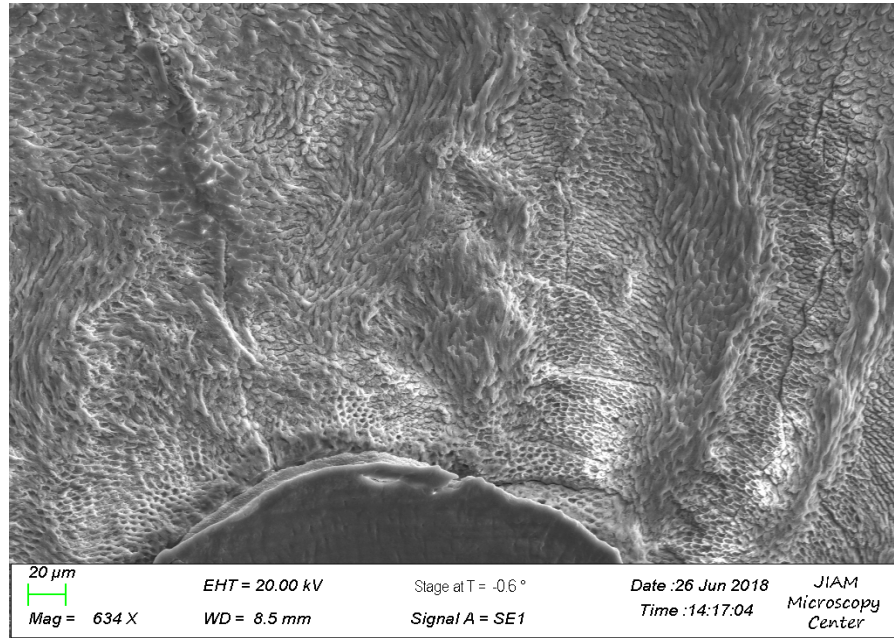


Figure 4.4 Scanning electron micrograph of pig tooth enamel prism arrangement at 634X.

The pig tooth was photographed at a slightly higher magnification than the human tooth (less than 50X). The human tooth's cusp enamel rod arrangements are seen as straighter lines. While the pig tooth's cusp enamel rod arrangements appear more undulated in contrast to the human's.

The scanning electron microscope also revealed that the Hunter-Schreger bands in human teeth shown in another micrograph in Figure 4.5, do not span the entire length of the tooth's enamel, while in dogs, shown in Figure 4.6, span the entire length of the enamel.

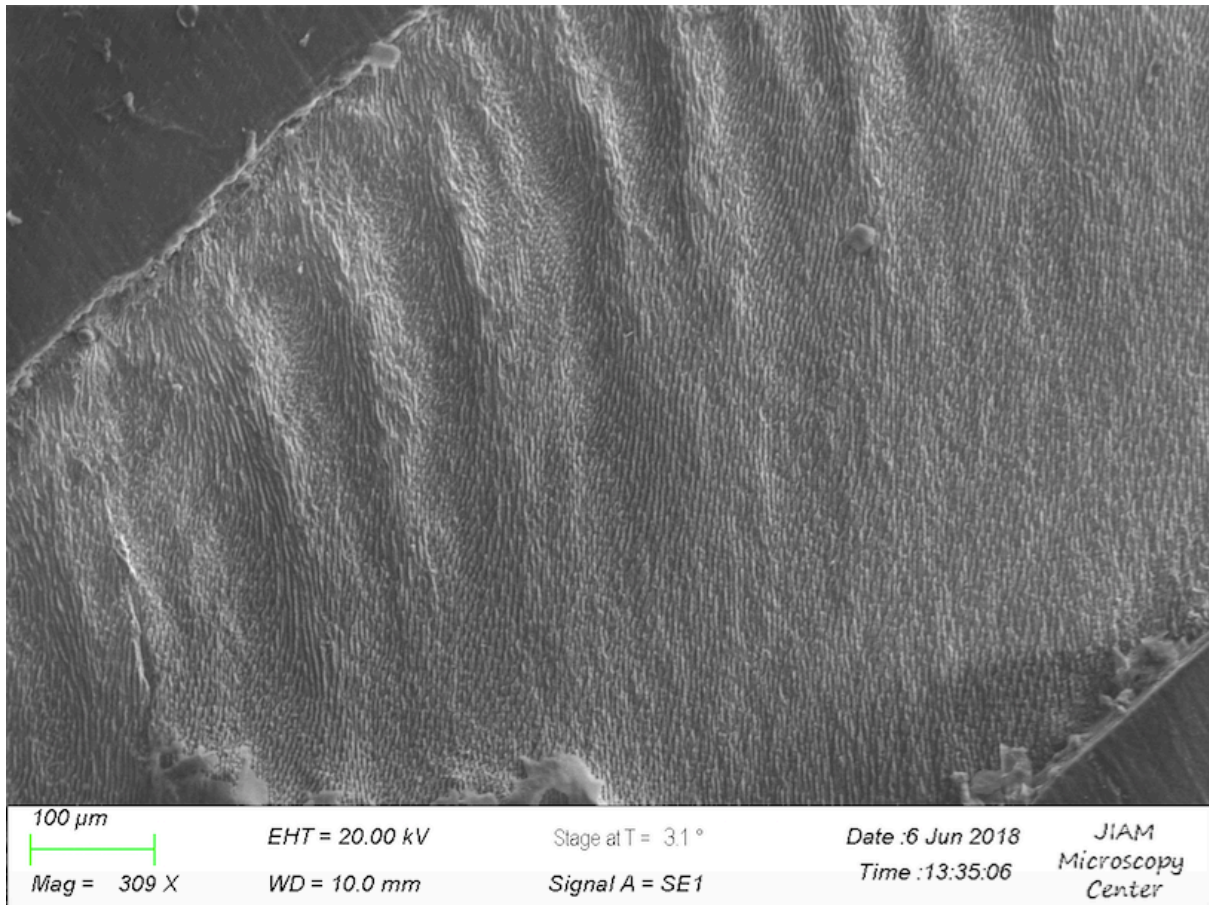


Figure 4.5 Scanning electron micrograph of human Hunter-Schreger band length at 309X.

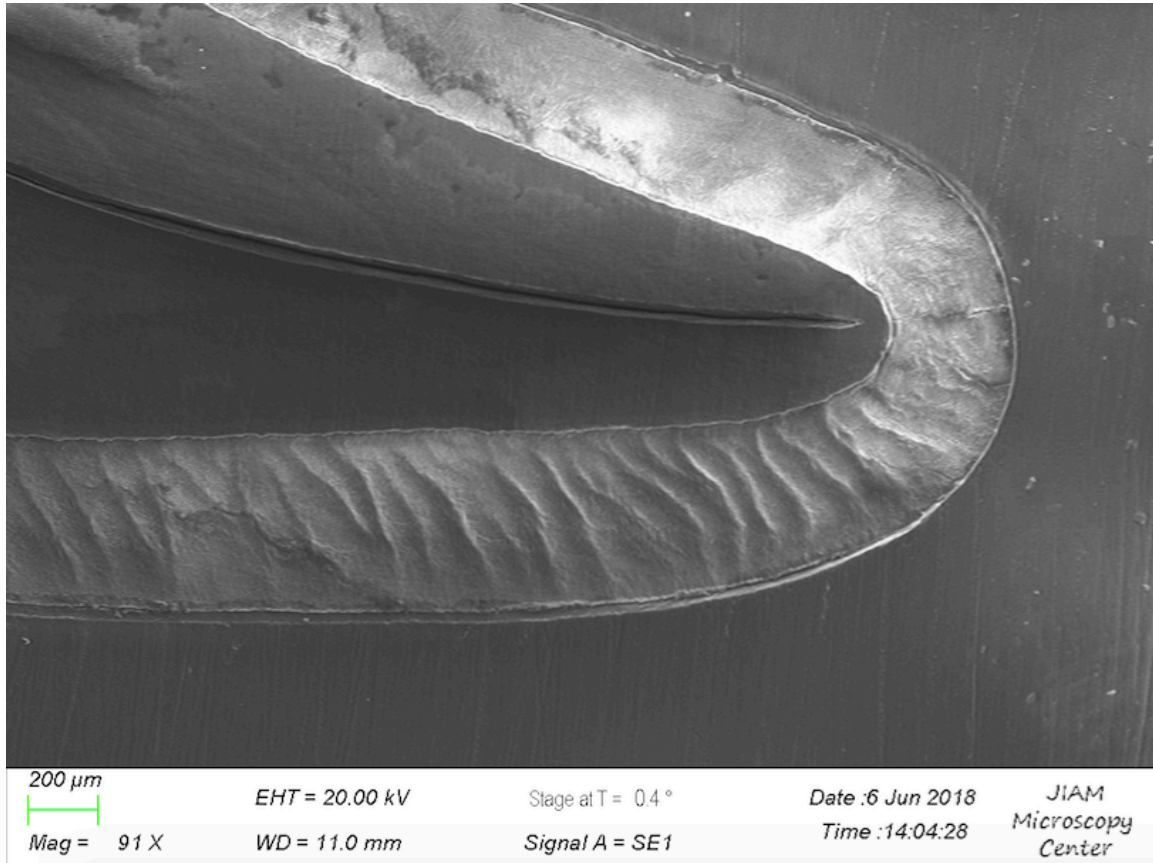


Figure 4.6 Scanning electron micrograph of pig Hunter-Schreger band length at 91X.

Figure 4.6 shown above, illustrates the Hunter-Schreger bands spanning the enamel length in the pig tooth. Similar results were found in the deer and dog teeth. In this research, the labial side of the anterior teeth show HS banding more prominently than on the lingual surfaces. An example is also shown in Figure 4.6. The same goes for buccal sides. In the second photograph of the pig's tooth seen in Figure 4.6, if evaluated closely, the bands span the length of the enamel. These bands are little more undulated than the human teeth. Seen below in Figure 4.7, the micrograph was taken with the HDBSD camera lens while using

the scanning electron microscope. As seen in the previous pictures, the topography of those micrographs is more three-dimensional when compared to this micrograph where the image is more flat. This camera style is called HDBSD whereas most of the other photos were taken using SE1. SE1 stands for Secondary Electron Image, which originate from the vicinity of where the SEM beam interacts with its sample. Contrary to SE1, HDBSD stands for High Definition Back Scatter Electron Detector and ultimately produces a less topographical image.

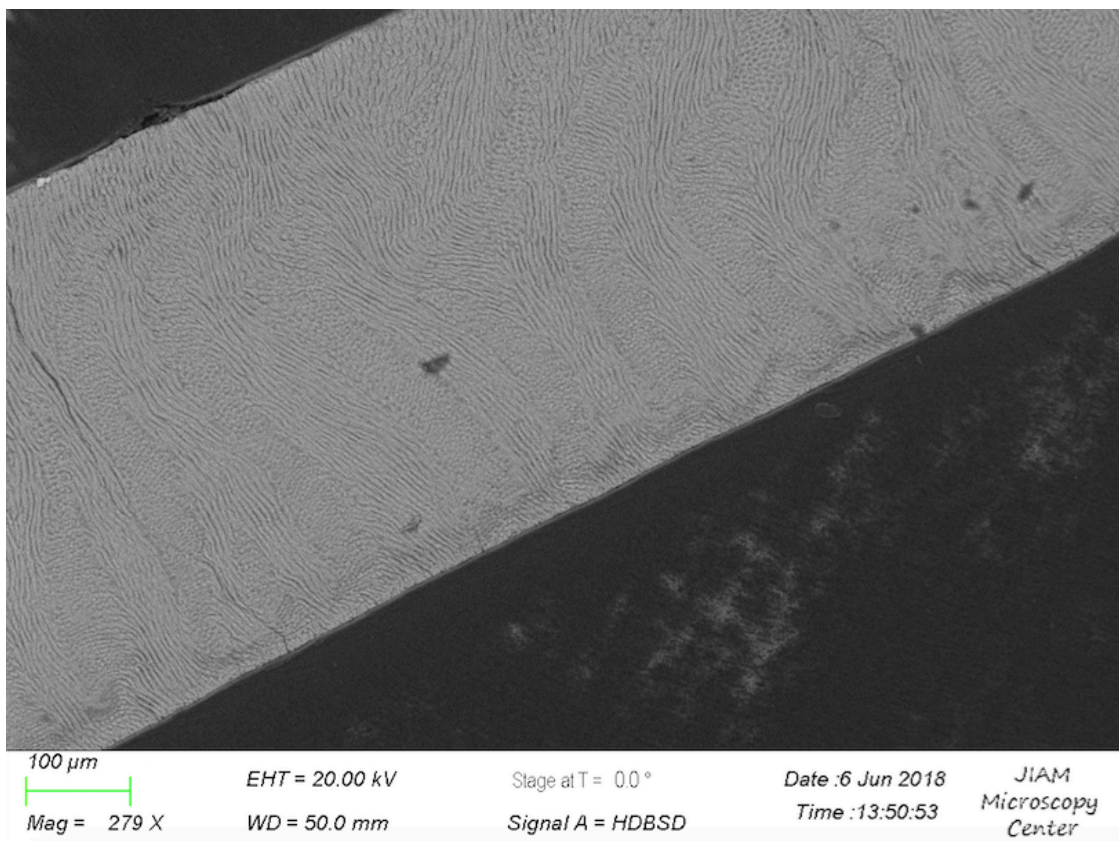


Figure 4.7 Scanning electron micrograph showing the difference in HDBSD micrographs compared to SE1 micrographs.

After comparing the animals to the human teeth using SEM, the next step was to compare all three non-human species to one another. The same tooth location was viewed and evaluated for differences; beginning with dog in Figure 4.8, then pig in Figure 4.9, and finally deer in Figure 4.10, using the same location on all the teeth, the goal was to capture the differences in this comparison.

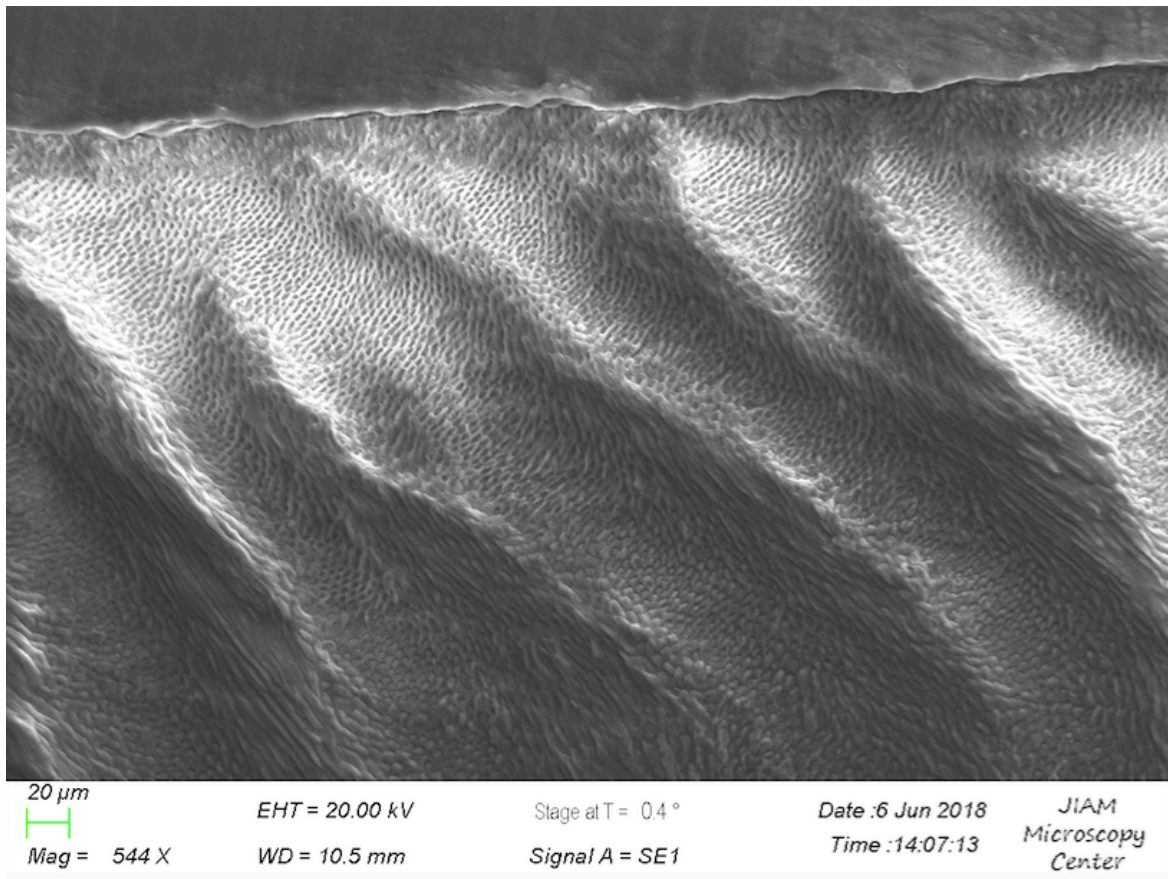


Figure 4.8 Scanning electron micrograph of the cusp of a dog canine tooth at 544X.

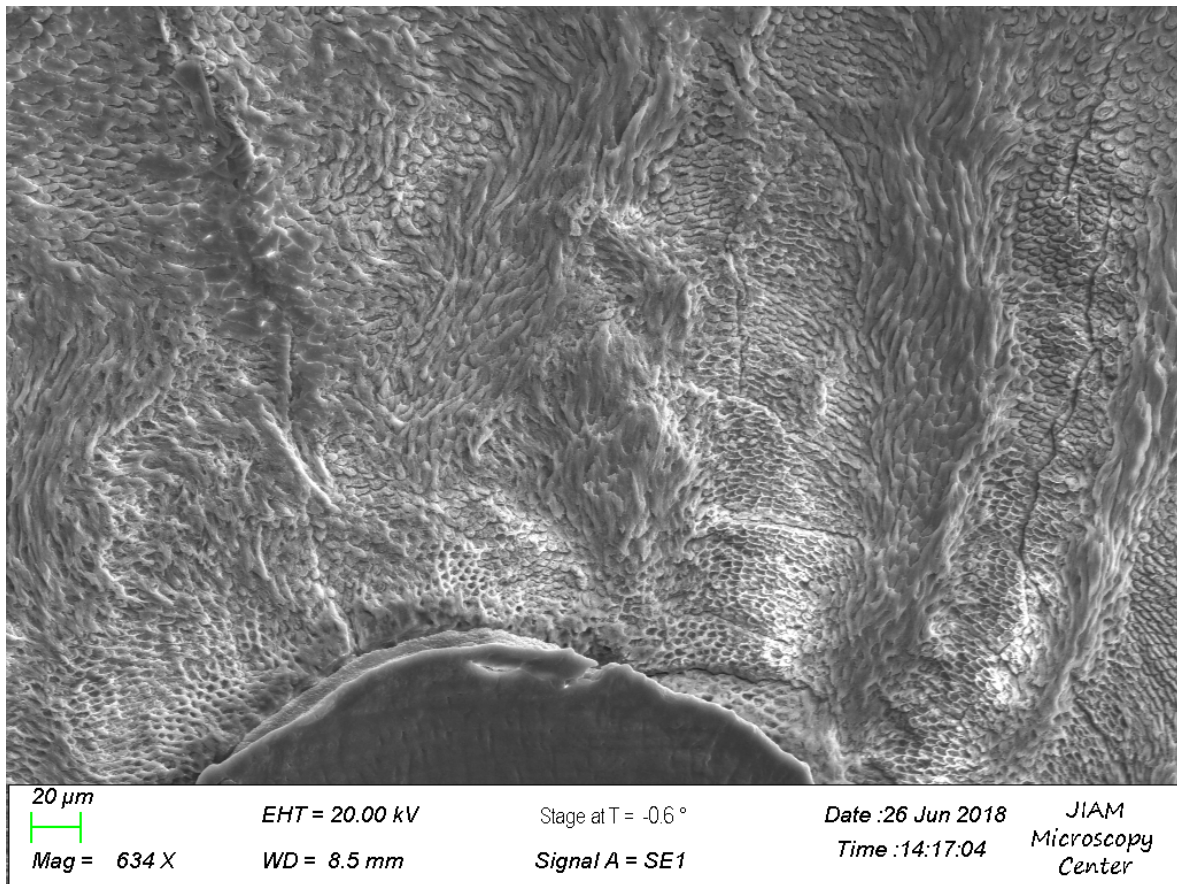


Figure 4.9 Scanning electron micrograph of the pig cusp tip at 634X.

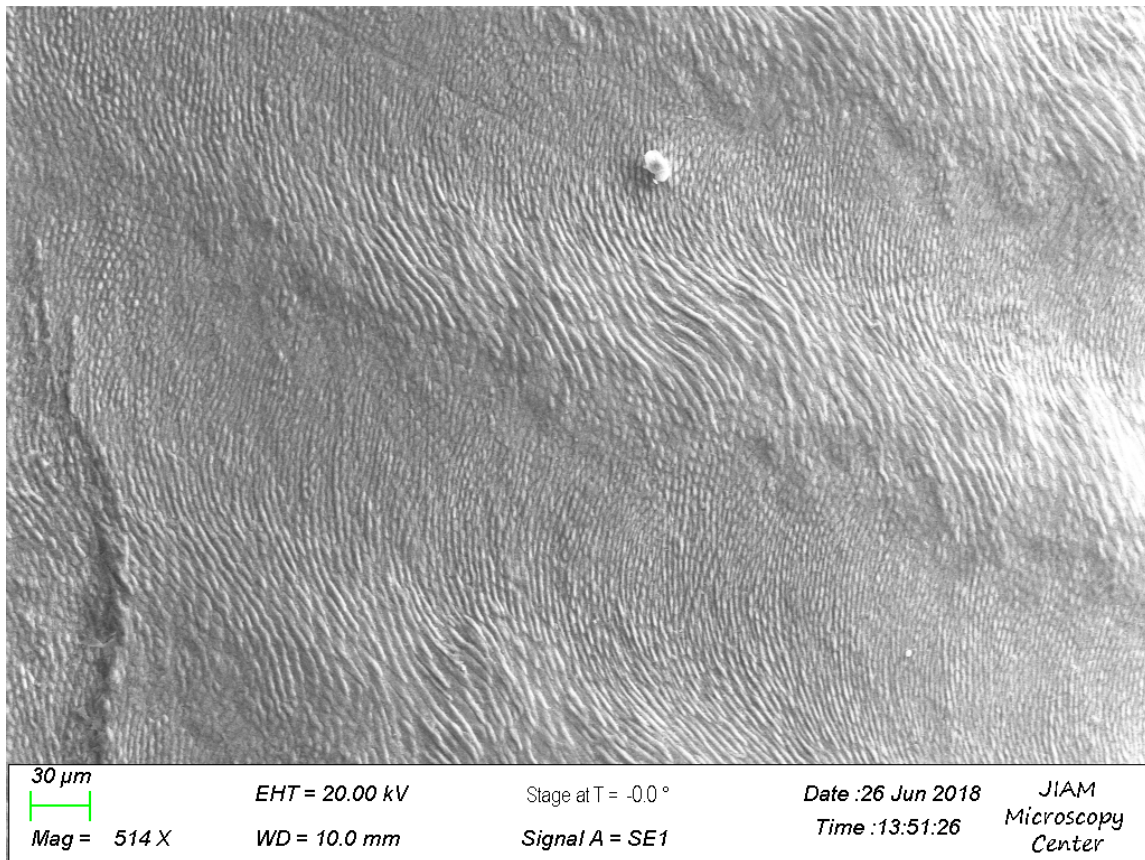


Figure 4.10 Scanning electron micrograph of a deer-mandibular first molar cusp at 514X.

After looking at the different scanning electron micrographs of the three different animals, the pig tooth stands out the most. In Figure 4.9, the micrograph of a pig tooth, shows the most defined, undulated Hunter-Schreger bands. Dog and deer teeth also show undulations but not as dramatic as the pig's tooth. This was interesting due to other texts saying that pig and human teeth share the most similarities.

In the mesial-distal thin sections, the bands were much easier to see and both surfaces showed distinct Hunter-Schreger bandings. Towards the cementoenamel junction; the mesial-distal and labio/bucco-lingual sections

showed that the lines either became thicker in widths or were not as noticeable. The bands are still there, but become thicker and not as distinct when getting close to the CEJ.

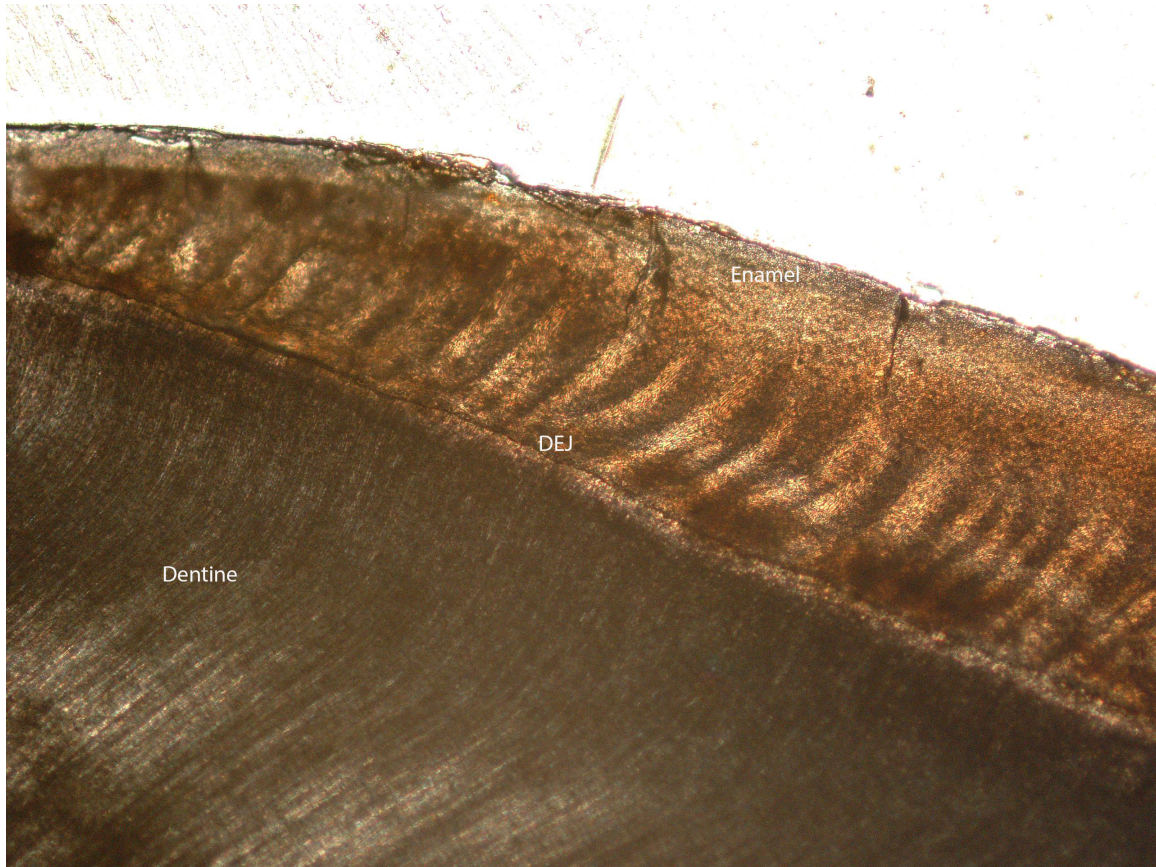


Figure 4.11 Light micrograph of the distal side of a deer-mandibular first molar CEJ at 50X.

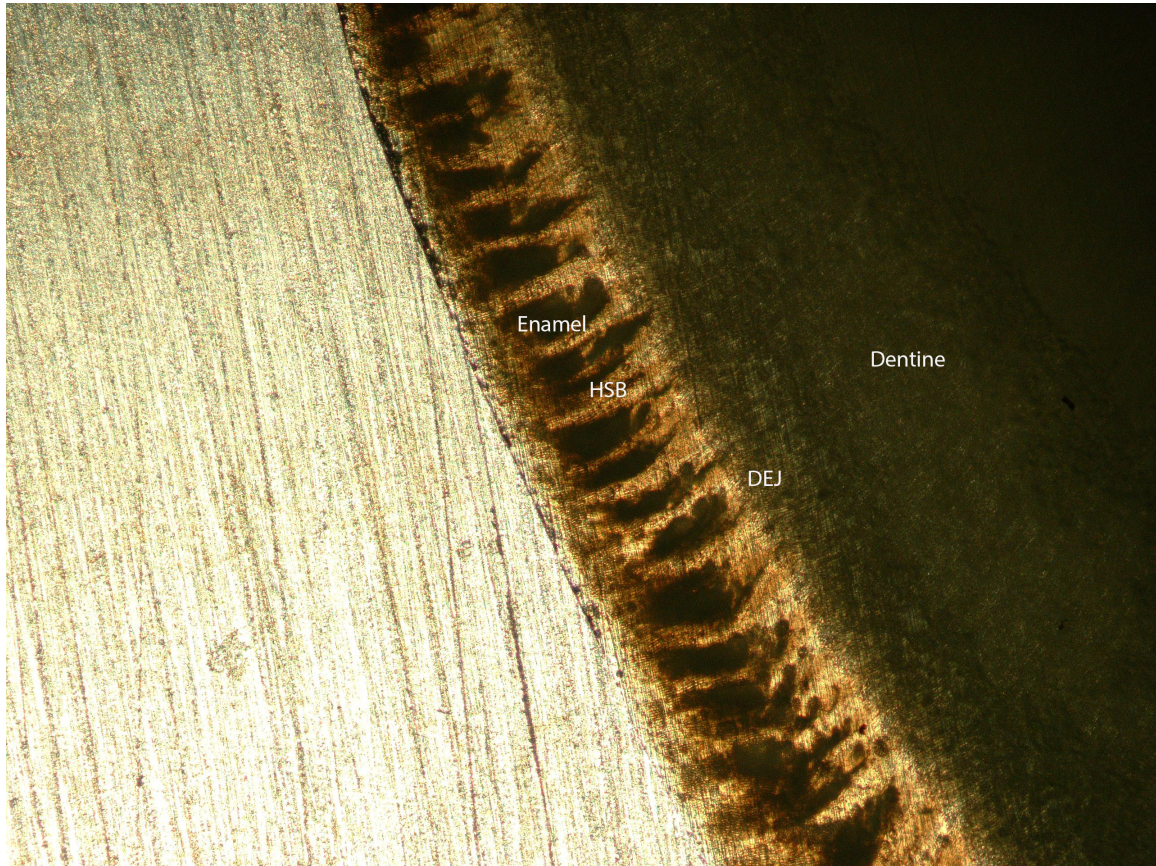


Figure 4.12 Light micrograph of the mesial side of a dog-mandibular first molar at its mid-coronal region at 50X.

In dog teeth, the bands seem to be much thicker than in the other animals. The deer and pig HSBs tend to be thinner, but for unknown reasons. In Figure 4.13 deer HSBs are shown in the mid coronal region as well as in Figure 4.14 the pig HSBs are shown in the mid coronal region.



Figure 4.13 Light micrograph the buccal side of a deer-mandibular first molar at its mid-coronal region at 50X.



Figure 4.14 Light micrograph of the mesial side of a pig-mandibular third premolar at its mid-coronal region at 50X.

Another measurement performed in this research was measuring the enamel prisms and comparing them among the different species. This was done using the same calipers used in measuring the crown heights. Scanning electron micrographs were used to measure the enamel prisms. Micrographs such as Figure 4.15 were used to measure the enamel prisms in all types of orientations.

Calipers were used to measure the enamel prisms/rods. The prisms were measured in different orientations such as longitudinal, convex cross-sectional, and concave cross-sectional. Their measurements for each mammal are as followed in Table 4.5. Table 4.5 displays the measurements of the enamel prisms measurements at different directionality: longitudinal, concave and convex cross-section. Measurements on columns 1-5 are of the longitudinal prisms whereas 6-10 measurements are of the convex cross-sectional prisms, and 11-15 are of the concave cross-sectional prisms.

After taking the measurements of the enamel rods/prisms, it was determined that enamel prisms across species were almost identical. In Table 4.5, it is noticed that the measurements are all almost 1.50 mm within each other. The deer did not have as many concave cross-sectional cuts on the micrographs; therefore, those measurements are not present in the table.

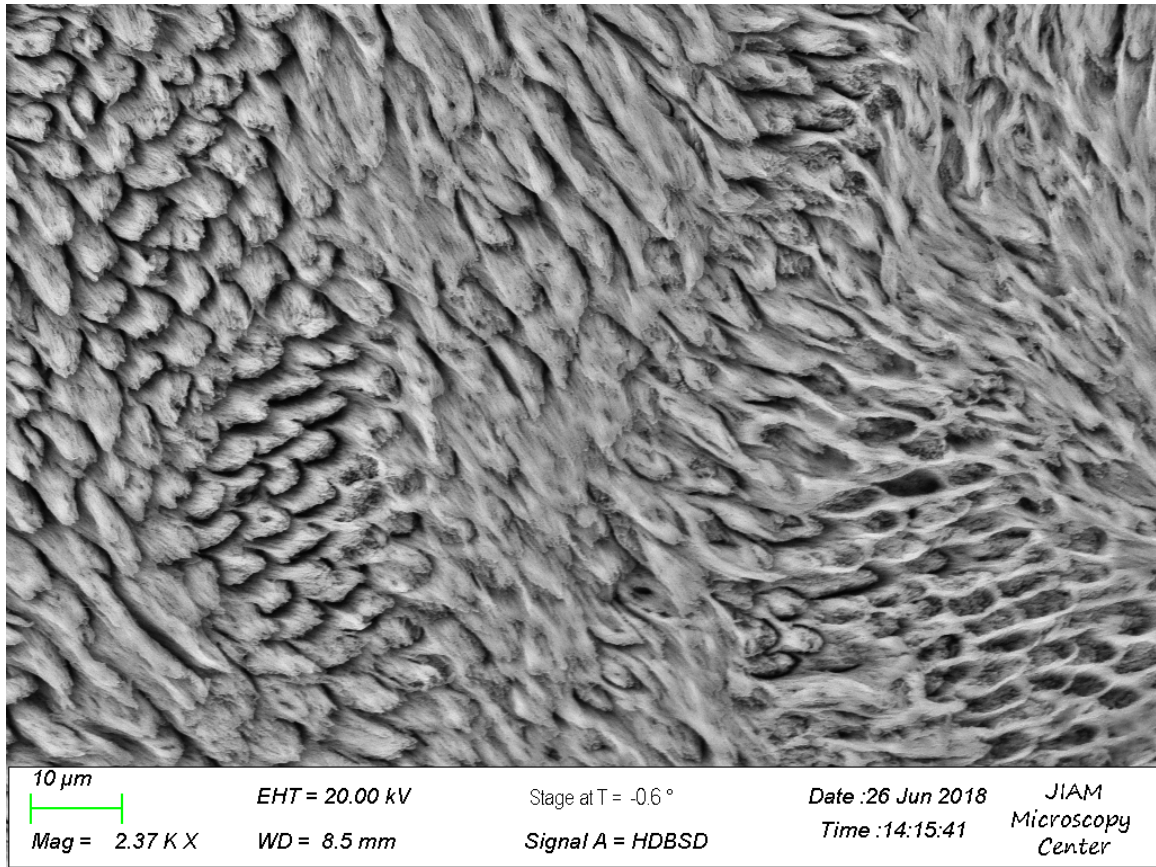


Figure 4.15 Scanning electron micrograph showing pig enamel prisms at 2.37 KX.

Table 4.5

Enamel Prism Measurements in Microns and Millimeters

Mammal	Human	Dog	Pig	Deer
1	2.5um/2.40mm	3.0um/3.66mm	3.0um/4.24mm	2.5um/3.30mm
2	2.5um/2.85mm	2.5um/2.89mm	3.0um/3.79mm	3.0um/3.64mm
3	2.5um/3.13mm	2.0um/2.31mm	3.0um/3.87mm	2.5um/3.37mm
4	2.5um/3.20mm	2.2um/2.38mm	3.0um/4.02mm	2.5um/3.37mm
5	3.0um/3.63mm	2.2um/2.61mm	3.2um/4.64mm	3.0um/4.05mm
6	2.5um/3.16mm	3.0um/3.61mm	3.0um/3.65mm	2.5um/2.96mm
7	2.5um/2.85mm	2.5um/3.36mm	3.0um/3.64mm	2.5um/3.20mm
8	2.5um/3.08mm	3.0um/3.83mm	2.5um/2.89mm	3.0um/3.56mm
9	3.0um/3.58mm	3.0um/4.23mm	3.0um/3.81mm	2.5um/3.21mm
10	2.5um/3.22mm	3.0um/3.54mm	2.5um/2.98mm	2.5um/2.81mm
11	3.0um/3.93mm	2.5um/2.85mm	2.5um/2.79mm	
12	2.5um/3.64mm	2.5um/2.76mm	2.5um/3.14mm	
13	2.5um/3.45mm	2.5um/2.81mm	2.5um/3.02mm	
14	2.5um/3.33mm	2.5um/2.96mm	2.5um/3.38mm	
15	2.5um/3.51mm	2.5um/2.89mm	2.5um/3.58mm	

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Fragmented and comingled remains are commonly found at crime scenes, mass, and natural disasters leaving investigators to put the pieces back together. This research helps assist in differentiation of human from non-human remains.

Using dissecting, light, and scanning electron microscopy differences in mammalian dentition can conclude that there are differences besides the obvious gross anatomical variances in a large pig canine compared to a smaller human canine. Since most laboratories that receive human remains only have dissecting or light microscopes, this research focused on the variances observed using these instruments versus the scanning electron microscope. As stated in the results chapter, it was observed that human bands do not span the length of the enamel where as the other animals' bands span the length of their enamel. The data also revealed that deer teeth have more enamel bands than any other species and their bands are thinner.

The scanning electron microscope revealed that the enamel prisms are more undulated in the pig than the dog or deer; and in human teeth, the bands are contrarily straight. Additionally, the scanning electron microscope aided in clarifying whether enamel prisms are relatively the same size across different species. Those measurements concluded that enamel prisms are only 1.50 mm larger and smaller from one other.

Settling on whether these small disparities completely differentiate one mammalian species from the next is the driving factor in which more research

should be performed on this topic. There are many other organisms that should be examined in whether their enamel composition resembles humans or other animals. This study could have been improved if a larger number of teeth were evaluated. Additional research to determine chemical composition and cemental annulation differences would be useful as well.

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VITA

Sarah-Wallace Ward was born in Knoxville, Tennessee on June 15, 1994 to the parents of John and DeAnna Ward. She is the oldest of two daughters and the older sister to Caroline Ward. Prior to attending graduate school at the University of Tennessee at Knoxville, she received her undergraduate degree at the University of Tennessee at Chattanooga in Pre-Professional Biology. Before college, she went to A.L Lotts Elementary School, West Valley Middle School, and Bearden High School all in Knoxville, TN. Upon returning home from undergrad, she was persuaded by Dr. Chris O'Rourke DDS to apply for the University of Tennessee's Forensic Odontology masters program from which she graduated from in August 2018.

During graduate school, she decided to write a thesis instead of a capstone project. After accepting the challenge of a thesis, she began research on comparative mammalian dental enamel histology from interest given to her by Dr. Murray Marks in the lackluster field. In her thesis, she compared the tooth enamel composition of humans to dogs, pigs, and deer to see their similarities and differences under the use of a light and scanning electron microscope. She concluded that there were structural differences between the different species used in her research.

She hopes to further her research one day to determine if there are species differences among animals using the same techniques along with attending dental school in the future.