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Dental Microwear Variation In Teleoceras Fossiger (Rhinocerotidae) From The Miocene (Hemphillian) Of Kansas, With Consideration Of Masticatory Processes And Enamel Microstructure

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DENTAL MICROWEAR VARIATION IN *TELEOCERAS FOSSIGER*
(RHINOCEROTIDAE) FROM THE MIOCENE (HEMPHILLIAN)
OF KANSAS, WITH CONSIDERATION OF
MASTICATORY PROCESSES AND
ENAMEL MICROSTRUCTURE

being

A Thesis Presented to the Graduate Faculty
of the Fort Hays State University in
Partial Fulfillment of the Requirements for
the Degree of Master of Science

by

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GRADUATE COMMITTEE APPROVAL

The graduate committee of Kelsie D. Abrams approves this thesis as meeting partial fulfillment of the requirements for the Degree of Master of Science.

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ABSTRACT

Dental microwear analysis is the study of microscopic features on the surfaces of teeth, and is used to reconstruct and analyze diet in extinct and extant animals. Microwear analysis on ungulates is typically conducted on the paracone or protoconid of the second molar, as these cusps are usually the first point of contact between upper and lower teeth during the chewing stroke. However, the exact method of mastication varies in different groups of ungulates, and the influence of mastication on the location and production of microwear features has been studied very little. Additionally, the role of highly specialized enamel microstructure in the production of microwear features has not been examined in many groups of animals. The goal of this project is to analyze central tendency of microwear features among cusps and between chewing facets in order to determine if a single cusp or facet type is more reliable for interpretation than other cusps or facet types in the North American Miocene rhinoceros, *Teleoceras fossiger*. This is accomplished through the testing of three main hypotheses. First, it is predicted that cusps that collide more frequently with other cusps will have higher numbers of microwear features than cusps that interact less frequently. Second, it is predicted that Phase 1 chewing facets will have more pits than Phase 2 facets, and Phase 2 facets will have more scratches than Phase 1 facets. Third, it is predicted that cusps constructed of normal, soft enamel will have a higher total number of features than cusps constructed of highly resistant enamel.

The lower second molars of 11 *T. fossiger* specimens were selected for analysis, as numerous complete dentaries were available for study. A total of 31 cusps from the 11

teeth were cleaned, prepared, and sampled in order to capture potential variation produced during the chewing stroke. Cusps were identified as Phase 1 or Phase 2 chewing facets, with each Phase associated with either normal enamel or enamel with specialized, resistant Hunter-Schreger Bands. Using low magnification microwear techniques, pits and scratches were identified and counted on all cusps and facets using 0.4 mm² areas, and the data were analyzed in R 3.1.1.

When testing the first hypothesis, eleven paired t-tests and one Wilcoxon paired sample test resulted in a single significant comparison between the hypoconid and the protoconid, with the hypoconid having significantly higher numbers of scratches than the protoconid. When testing the second hypothesis, a paired t-test and a Wilcoxon paired sample test comparing the number of scratches and pits between Phase types did not produce significant values. Finally, when testing the third hypothesis, a paired t-test comparing the total number of features between Phase types indicated no significant differences. Comparison of the characteristics of the hypoconid to other cusps indicates that mastication and enamel microstructure work in combination to preferentially produce more scratches on the hypoconid than on other cusps in *T. fossiger*, partially supporting the first hypothesis and the third hypothesis. Consequently, it is recommended that the hypoconid is not used for dietary analysis due to its higher variability in the number of scratches, which will affect the results of dietary reconstruction studies on *T. fossiger*.

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INTRODUCTION

Dental microwear analysis is the study of microscopic pits and scratches found on the surfaces of teeth, and is used to reconstruct diet in extinct and extant animals. While dietary reconstruction has been the focus of microwear studies in the past, there has been a recent shift to emphasize testing microwear methodologies to better understand variables involved with the production of pits and scratches (Archer and Sanson, 2002; Grine et al., 2002; Galbany et al., 2005; Fraser and Theodor, 2010, 2011; Fraser et al., 2009; Heywood, 2010; Kaiser et al., 2010; Beatty and Mihlbachler, 2012; Grine et al., 2012; Mihlbachler and Beatty, 2012; Erikson, 2013; Fraser and Rybczynski, 2014; Hoffman et al., 2015). Many of the variables that potentially affect the production of microwear features in ungulates, such as the role of mastication and enamel microstructure, have been explored, but how these variables affect feature distribution across a tooth has been little studied (Rensberger and Koenigswald, 1980; Boyde and Fortelius, 1986; Archer and Sanson, 2002; Kaiser et al., 2010, 2011, 2013; Fraser and Rybczynski, 2014; Mihlbachler et al., 2015). In addition, some taxa, like rhinoceros, have highly specialized enamel microstructure that forms on different parts of enamel bands (Fortelius, 1982, 1985; Koenigswald et al., 2011). The influence of enamel microstructure on the development and distribution of microwear features has never been examined in rhinoceros. The goal of this study is to evaluate the roles of mastication and enamel microstructure in the production of microwear features in a Miocene rhinoceros, *Teleoceras fossiger*.

Teleoceras fossiger is the largest of the teleoceratines and one of the most common Miocene North American fossil rhinoceros (Osborn, 1898; Prothero, 2005). While microwear studies have been undertaken on other teleoceratines (MacFadden, 2010; Hoffman, 2013), there are no published microwear studies on this species. Rhinoceros are frequently used in microwear studies due to their excellent fossil record and the five extant species available for ecological comparisons (Solounias and Semprebon, 2002; Muhlbachler et al., 2015; MacFadden, 2010; Hoffman, 2013; Taylor et al., 2013). Additionally, rhinoceros mastication, enamel formation, and enamel microstructure are well understood (Rensberger and Koenigswald, 1980; Fortelius, 1982, 1985; Pfretzschner, 1992; Herring, 1993; Popowics and Herring, 2006).

Microwear analysis is a method in the broader field of dental wear analyses. Once a tooth erupts in the jaw, it becomes subject to attrition (tooth on tooth wear) and abrasion (food on tooth wear) (Butler, 1952, 1972; Fortelius and Solounias, 2000). During mastication, abrasion of consumed materials across the occlusal surface of the tooth produces microscopic marks or features (Butler, 1972; Rensberger, 1978; Walker et al., 1978; Walker, 1981; Puech et al., 1986; Solounias et al., 1988; Janis, 1995; Fortelius and Solounias, 2000). The microscopic features are categorized as circular depressions called pits and linear depressions called scratches, and record the animal's last several meals prior to death, making it possible to reconstruct an animal's diet (Rensberger, 1978; Walker et al., 1978; Walker, 1981; Solounias et al., 1988; Teaford and Oyan, 1989; Janis, 1995; Fortelius and Solounias, 2000; Solounias and Semprebon, 2002; Solounias et al., 2010). Microwear has not only been used to differentiate among broad feeding

categories, but also to identify niche partitioning between species with similar diets, seasonal dietary fluctuations within species, and dietary trends over time within species (e.g. Puech et al., 1986; Teaford and Robinson, 1989; MacFadden et al., 1999; Merceron et al., 2004; Scott et al., 2005; Grine et al., 2006; Mainland, 2006; Rivals and Semprebon, 2011; DeSantis et al., 2013). Microwear research has been conducted on numerous taxa including dinosaurs, fish, marsupials, artiodactyls, perissodactyls, primates, and carnivores (e.g. Puech et al., 1986; Valkenburgh et al., 1990; Solounias and Hayek, 1993; Fiorillo, 1998; Semprebon et al., 2004b; Purnell et al., 2006; Joomun et al., 2008; Goillot et al., 2009; Christensen, 2014). While these studies typically focus on molar enamel, microwear features have also been described on incisors, dentine, non-occlusal canines, and non-occlusal surfaces of molars and premolars (Walker, 1976; Ryan, 1981; Ungar and Teaford, 1996; Goillot et al., 2009; Green, 2009; Rivals and Semprebon, 2011; Haupt et al., 2013).

Microwear studies interpret diet by looking at the frequencies and ratios of pits and scratches on teeth in extinct animals and comparing those to extant animals with known diets (Walker et al., 1978; Teaford, 1988, 1991; Solounias and Semprebon, 2002). Plants with higher phytolith concentrations (grasses and forbs) are thought to produce more scratches compared to plants with lower phytolith concentrations (twigs and shrubs) (Walker, 1976; Walker et al., 1978; Gügel et al., 2001; Solounias and Semprebon, 2002; Merceron et al., 2005). These proportions (many scratches and few pits, or few scratches and many pits) are called feeding, dietary, or microwear signals (Solounias and

Semprebon, 2002; Semprebon et al., 2004b; Organ et al., 2006; Rivals and Solounias, 2007; Rivals and Semprebon, 2011; Schulz et al., 2013).

High magnification microwear (HMM) and low magnification microwear (LMM) approaches are used to analyze dental microwear. The HMM method is well established, well understood, and relatively standardized compared to the more recently developed low magnification approach. However, HMM is time-consuming and expensive, requiring highly specialized training, expensive scanning electron microscopes, and rental charges to operate equipment (Semprebon et al., 2004a; Joomun et al., 2008; DeSantis et al., 2013). Consequently, HMM studies often have small sample sizes, weakening the robustness of results (Solounias and Semprebon, 2002). Low magnification microwear uses relatively accessible and inexpensive stereo light microscopes to study microscopic features at 3x to 50x magnification (Solounias and Semprebon, 2002). Although LMM is a relatively new technique, there has been research supporting that this method can identify microwear features and interpret feeding behavior as accurately as HMM studies (Solounias and Semprebon, 2002; Semprebon et al., 2004a; Goillot et al., 2009; DeSantis et al., 2013). Research (Solounias and Semprebon, 2002; DeSantis et al., 2013) suggests this technique may allow for better dietary interpretations than HMM because low magnification can be used to study large features, such as puncture pits and gouges caused by seeds and nuts, features that are too large to be observed at higher magnifications. Low magnification microwear entails less equipment and supplies, requires less training, and allows for quicker analysis of tooth surfaces than HMM studies, often resulting in larger sample sizes (Solounias and

Semprebon, 2002; DeSantis et al., 2013). As a consequence, this technique is becoming increasingly popular, particularly because comprehensive microwear studies require large sample sizes from multiple taxa (including extant and extinct organisms) in order to make strong dietary comparisons and conclusions (Solounias and Semprebon, 2002; Fraser et al., 2010; Goillot et al., 2009; DeSantis et al., 2013).

While microwear analyses provide significant insight into the diet and ecology of animals, the variety of methods used to quantify microwear features stresses the need for strict standardization (Grine et al., 2002; Galbany et al., 2005; Scott et al., 2008; DeSantis et al., 2013). More importantly, there are many variables in the study of microwear that are unexamined. These variables include, but are not limited to, variability in the number and type of microwear features found on cusps and chewing facets, ontogenetic changes in feeding behavior, differences in enamel microstructure on a single tooth, and the effects of masticatory processes on the production of microwear features. For example, microwear studies typically use the upper second molar (M2) or lower second molar (m2) for the reasoning that it is in the middle of the molar row and more likely to exhibit medial amounts of tooth wear (Walker, 1976; Fortelius, 1982; Solounias and Semprebon, 2002). Once the M2/m2 has been selected, a cusp is then selected for analysis. The major cusps on the upper molar are the protocone, hypocone, metacone, and paracone (Fortelius, 1982, 1985). The major cusps on lower molars are the protoconid, hypoconid, entoconid, metaconid, and paraconid (Fortelius, 1982, 1985) (Fig. 1). Microwear analysis of ungulates is typically conducted on the paracone of the M2, or the protoconid of the m2, because these are the initial sites of contact between upper and lower second molars

during the chewing stroke in most ungulates (Fortelius, 1982, 1985). This first contact with the lower protoconid is not universal in ungulates, however, and in rhinoceros it is the hypoconid of the m2 that first contacts the upper molar rather than the protoconid (Fortelius, 1982, 1985). Additionally, evidence suggests that mastication can cause significant differences in the frequency of pits and scratches on different cusps of a tooth, across molar rows, and across all teeth in the mouth, potentially affecting accurate dietary interpretations depending on what part of the tooth is selected for analysis (Gordon, 1984a; Grine, 1986; Joomun et al., 2008). Therefore, examining the frequency and central tendency of pits and scratches among different cusps and teeth is necessary in order to understand which tooth and cusps should be used for dietary analysis.

Many mammals, and most ungulates, have a two-phase chewing stroke (Mills, 1967, 1973; Fortelius, 1982, 1985) (Fig. 2). During Phase 1 of the chewing stroke in rhinoceros, upper and lower teeth collide, creating Phase 1 facets on the protoconid, hypoconid, and on the buccal enamel band of upper molars, which includes the paracone (Fortelius, 1982, 1985) (Fig. 1, 2). Phase 1 is associated with greater speed and pressure during occlusion than Phase 2, as the force vector between the points of contact is nearly perpendicular. A study on extant rhinoceros, *Ceratotherium* and *Diceros*, demonstrated that Phase 1 facets have more pits than Phase 2 facets, and that this is likely caused by the crushing phase of chewing (Mihlbachler et al., 2015). After the initial point of contact, the force vector remains the same throughout the chewing stroke, though the area on which the force is distributed changes between Phase 1 and Phase 2. Force is distributed over all five lower cusps and on the lingual upper cusps during Phase 2.

Phase 2 is when the majority of food abrasion occurs, as the plant material is ground across highly resistant rows of enamel prisms called Hunter-Schreger bands (HSB) (Rensberger and Koenigswald, 1980). Hunter-Schreger bands are aligned horizontally in most ungulates, but are aligned vertically in rhinoceros along the enamel-dentine junction (EDJ). This is likely an adaptation to the large forces generated by rhinoceros during mastication, as the HSB in a vertical orientation are more resistant to abrasion than when aligned horizontally (Rensberger and von Koenigswald, 1980; Fortelius, 1982, 1985). Phase 2 facets tend to be rounded in shape, rather than flattened like Phase 1 facets, because abrasion by food polishes the surface, exposing the HSB.

Throughout the two-phase chewing stroke, cusps on both lower and upper teeth have a different number of cusp-to-cusp collisions, and interact with either one or two different teeth. For example, the hypoconid and metaconid interact with two different upper teeth and have between four and six interactions with upper enamel bands. Other cusps, like the protoconid and entoconid, interact with less than four enamel bands and only with one upper tooth.

The variation in speeds, angles, areas over which force is distributed, differential hardness of enamel microstructure, and the number of cusp interactions encountered during the chewing stroke suggests the number of pits and scratches found among cusps and between facet types may vary. This study will analyze the central tendency of microwear features on the lower second molar (m2), focusing on central tendency of features among cusps and between Phase 1 and Phase 2 facets, in order to determine if a single cusp or facet type is more reliable for interpretation than other cusps or facet types.

If the number or type of microwear features found among cusps and between facet types is highly variable, then dietary interpretations may depend on which cusp or facet type is used for study.

This study has three hypotheses: two pertaining to mastication, and one pertaining to enamel microstructure. First, when analyzing the effect of mastication on the production of microwear features, it is expected that cusps interacting more frequently with masticated material and other cusps (the metaconid and hypoconid) will have a higher number of total features than cusps that interact with material and cusps more infrequently (the entoconid and protoconid). Second, when examining the role of two-phase mastication on microwear feature production, it is expected that cusps with Phase 1 facets will have higher numbers of pits due to the vertical collision of teeth during Phase 1 of the chewing stroke. Cusps with Phase 2 facets are expected to have higher numbers of scratches due to the translational motion of the teeth during Phase 2 of chewing. Third, to examine the role of enamel microstructure, this study will analyze central tendency of the total number of microwear features found between facets of two different types of enamel microstructure: Phase 1 facets with normal enamel, and Phase 2 facets with enamel containing HSB. Between facets, it is expected that the more resistant Phase 2 facets will have fewer numbers of total features than the Phase 1 facets with softer enamel.

MATERIALS AND METHODS

Materials

Eleven adult m2s of *T. fossiger* were sampled, as many complete lower jaws were available for study. The m2 is sometimes used for this reason and has been shown within herbivorous primates (which have a similar chewing stroke to rhinoceros), to have no statistical difference in microwear patterns to upper molars (Rensberger, 1978; Gordon, 1984a; Fortelius, 1985; King et al., 1999; Schmidt, 2001; Semprebon et al., 2004a). Adult specimens are typically used in microwear studies in order to eliminate ontogenetic variation in feeding behavior (Solounias and Semprebon, 2002) and are identified by a fully erupted M3/m3 that had begun to occlude.

Specimens came from three Miocene Ogallala Formation localities across Kansas: the Jack Swayze Quarry (JSQ), the Minium Quarry (MQ), and the Long Island Quarry (LIQ) (Fig. 3). There were two specimens from JSQ, one from MQ, and the remaining eight specimens came from LIQ. The JSQ and MQ specimens are housed at Fort Hays State University's Sternberg Museum of Natural History (FHSM), and the LIQ specimens are housed at the Smithsonian Institution (USNM).

Thirty-one cusps from 11 m2s were sampled (Tables 1, 2). Phase 1 facets of the protoconid and hypoconid, and Phase 2 facets of the metaconid and entoconid (Fig. 1, 2), were sampled to examine the entire chewing stroke. Ten of the 11 paraconids (a Phase 2 facet) were heavily cracked or broken off, and therefore the paraconid was discarded from the study. The paraconid has the same number of cusp-to-cusp interactions as the

entoconid (Fortelius, 1982, 1985), another Phase 2 facet with HSB, and therefore the loss of this data is not considered a critical flaw for this study.

Methods

Cleaning, molding, and casting procedures followed the original LMM methods described by Solounias and Semprebon (2002). Teeth were soaked in Klean Strip premium stripper in 15 minute intervals to remove surface detritus. The MQ and JSQ specimens required up to three soakings to remove old shellac. Stripper was then removed with cotton balls, Q-tips and 91% isopropyl alcohol. Vigorous scrubbing to remove the consolidant was avoided so specimens would not be damaged or altered by the cleaning process. Teeth were examined beneath a hand lens at 15x magnification to determine if all residue had been removed. Shellac residue appears as jagged, crystalline structures on the teeth, and is clearly distinguishable from clean enamel. Cusp molds were made with Sultan genie regular body polyvinylsiloxane. The walls of the molds were built using Plastalina clay and the casts were made with Epo-Tek 301 two-pound epoxy resin. Resin was centrifuged for five minutes to reduce air bubbles in casts, and was manually poured into the molds, starting first at the side of the mold, and then the mold was tipped to allow resin to flow evenly across the mold.

Specimen casts were observed underneath the microscope to determine cast quality and if the non-occlusal surfaces had scratches and pits. To reduce taphonomic biases in the dataset, specimens exhibiting prominent non-occlusal surface pitting and scratching, or a complete lack of features due to either abrasion, poor specimen quality,

or poor conservation, were removed from this study. Identification of taphonomic alteration follows Grine (1977, 1986), King et al. (1999) and Teaford et al. (2008). Examination of enamel textures and cast quality indicated that 16 of the 31 cusps were in good condition, with microwear features appearing on polished, smooth enamel surfaces (Fig. 4). The remaining 15 cusps exhibit early stages of taphonomic alteration, but were retained because of the presence of a number of easily visible features, and because they did not show signs of extensive tumbling (Grine, 1977, 1986; King et al., 1999) (Fig. 5).

LMM image capture and processing followed Fraser et al. (2009). Lighting is well documented to affect the appearance of pits and scratches (Fraser et al., 2009). Standard oblique lighting was used in this study, meaning that two lights were projected onto the specimen at roughly 45° to the surface being observed. In order to provide optimal appearance of features, specimens were rotated underneath the light and examined for up to an hour before analysis sites were selected and photographed. Analysis sites were selected on the point of each cusp, with each study area being as close to the center of the enamel band as possible. Images of cusps were captured at 3.2x using an Olympus SZX16 microscope and camera, and CellSensStandard software. Four to six images were taken of each cusp under different exposures, and were merged using Photomatic Pro 5.0.5. Images were cropped to standard 0.4 mm² areas (Solounias and Semprebon, 2002) using Jasc Paint Shop Pro 8 and imported into ImageJ for data collection; digital resolution is 1.2 pixels/micron for all images.

Counts of pits and scratches were taken from photomicrographs, following the methods outlined in Fraser et al. (2009). Previous research suggests that reducing the

number of observers and the time spent during counting sessions lowers observer error, producing more precise results (Fraser et al., 2009; Muhlbacher et al., 2012). Counts were performed by a single observer and counting sessions lasted less than five hours to reduce errors associated with mental, physical, or eye fatigue. Pits and scratch counts were recorded using Image J. Identification of pits and scratches follow the definitions of Solounias and Semprebon (2002).

The data obtained are not independent because multiple samples were taken from a single tooth in each individual, meaning that cusps on that individual's tooth were exposed to the exact same food material and taphonomic processes. Therefore, it was appropriate to conduct multiple paired t-tests to test hypotheses with the dependent data. All statistical tests were conducted in R 3.1.1 statistical software. Pit and scratch count data were first tested for a normal distribution with Shapiro-Wilks tests before assigning a paired t-test for normally distributed data or a Wilcoxon paired sample test for non-normally distributed data. In order to increase the likelihood of significant results, a Benjamini-Hochberg false discovery rate (B-H FDR) was used in place of the highly conservative Bonferroni correction to determine significant values for all tests in which multiple comparisons were necessary (Nakagawa, 2004). Tests where the B-H FDR was required to determine significant values are listed in Tables 3 and 4.

In order to test hypothesis one and examine the effects of variable numbers of cusp-to-cusp interactions, two comparisons were conducted. First, the numbers of pits and scratches found on each of the four cusps were compared with 11 paired t-tests and one Wilcoxon paired sample test. The comparison of the multiple tests required a B-H

FDR to determine a critical or significant value. The hypoconid-entoconid scratch comparison was non-normally distributed and this comparison was conducted with a Wilcoxon paired sample test. Second, to test central tendency of the total number of features among cusps, pit and scratch counts for each cusp in each individual were totaled and then compared using six paired t-tests. These tests also required a B-H FDR value to determine critical values.

To test the second hypothesis on mastication (that Phase 1 facets will have more pits and Phase 2 facets will have more scratches), a paired t-test and a Wilcoxon paired sample test were used. The scratch data for Phase 1 and Phase 2 facets was normally distributed, and therefore a paired t-test was used to compare counts of scratches found on Phase 1 facets to counts of scratches found on Phase 2 facets. The pit data for the phases was normally distributed for Phase 2 cusps, but was not normally distributed for Phase 1 cusps, and therefore a Wilcoxon paired sample test was used to compare counts of pits found on Phase 1 facets to pit counts on Phase 2 facets. These tests were single comparison tests and did not require a B-H FDR to determine a significant value: therefore, their results were compared to a standard 0.05 significance value.

In order test hypothesis three and examine the role of enamel microstructure on the production of microwear features, each cusp was assigned as a Phase 1 or Phase 2 cusp, with Phase 1 cusps corresponding to normal enamel and Phase 2 cusps corresponding to enamel with Hunter-Schreger Bands. The total number of features for each cusp of a certain Phase type were calculated, and these totals were compared with a

paired t-test. The results of this test were compared to the standard 0.05 significance value.

Last, and unrelated to testing the three hypotheses, a single Wilcoxon paired sample test was conducted to test the total number of pits in individuals to the total number of scratches in individuals, in order to indicate whether a certain type of feature was more prevalent than the other. This test did not take in account cusp or facet type, but was simply interested in investigating whether or not a certain type of feature was more prevalent.

RESULTS

All pit and scratch counts are recorded in Table 1, with the total number of examined cusp counts recorded in Table 2.

To test the first hypothesis on mastication looking at cusp interactions, two comparisons were conducted. Of the 11 paired t-tests and one Wilcoxon paired sample test (the hypoconid-entoconid scratch comparison) used to compare pit and scratch counts among cusps, only the comparison between the number of scratches on the hypoconid and protoconid is significant (B-H FDR $0.0083 < p\text{-value } 0.017$) (Table 3). In order to determine the difference in scratches between the cusps, a histogram was produced (Fig. 6), demonstrating that the hypoconid has significantly more scratches than the protoconid. When testing the second part of the first hypothesis by analyzing the total number of features found among cusps using the six paired t-tests (Table 4), there are no significant results, with all cusps having the same relative distribution of features.

Next, two comparisons were conducted to test the second hypothesis on mastication regarding pit and scratch counts between Phase 1 and Phase 2 facets. The paired t-test used to compare the scratch counts between Phase 1 and Phase 2 facets produces a p-value of 0.4491, much greater than the significance value of 0.05 (Table 5) (Fig. 7). The Wilcoxon paired sample test used to compare the pit counts between Phase 1 and Phase 2 facets produces a p-value of 0.7545, again greater than the significance value, and therefore indicating no substantial differences.

A single comparison was used to test the third hypothesis analyzing the influence of the two types of enamel found on Phase 1 and Phase 1 facets. The paired t-test

comparing total number of features found between Phase types (Fig. 8) also does not have any significant results, with each Phase type having similar frequencies of features.

Last, and unrelated to the three hypotheses, a Wilcoxon paired sample test comparing overall numbers of pits to scratches (regardless of cusp or facet type) indicates significant differences ($p < 0.05$), with there being more scratches than pits in the dataset (Fig. 9).

DISCUSSION

Initial hypotheses predicted: (1) highly variable numbers of microwear features among cusps, with cusps most active during mastication having a higher total number of features; (2) the two-part chewing stroke produces more pits on Phase 1 facets and more scratches on Phase 2 facets; and (3) Phase 2 facets have significantly fewer overall features than Phase 1 facets, due to the presence of HSB in Phase 2 facets. For all three hypotheses, only one comparison test is significant. The comparison between the hypoconid and protoconid pertains to the first hypothesis of this study, and the hypoconid has significantly more scratches than the protoconid (Fig. 6). Consequently, it is important to compare the characteristics of the hypoconid to the other cusps of the m2 in order to understand its significance.

Neither the hypoconid nor protoconid differ in total number of features from the other cusps (Table 4). Additionally, neither the hypoconid nor the protoconid have significantly different numbers of scratches or pits than the metaconid and entoconid (Table 3). Thus, the comparison between the hypoconid and protoconid indicates that something unique is happening to this pair of cusps that significantly affects the hypoconid and not the protoconid. Scratch counts for both cusps are located on Phase 1 facets, meaning that both cusps occur on the same crushing phase of the chewing stroke, and that both cusps have the same type of normal enamel. The only difference between the two cusps is the number of cusp-to-cusp interactions, with the hypoconid interacting more frequently with other cusps and masticated material than the protoconid (Fortelius, 1982 1985).

During Phase 1 of the chewing stroke, the hypoconid of the m2 initially collides with the protocone of the M1, before falling into the posterior basin of the M2 (Fortelius, 1982, 1985). Upon falling into this basin, the hypoconid of the m2 slides across four to six upper enamel bands depending on the wear stage of the M1 and M2 (Fortelius, 1982, 1985). This movement is then followed by the collision of the protoconid of the m2 with the paracone of the M2, the location most popular for microwear studies. While the protoconid is typically used in most ungulate microwear studies because it is the cusp that contacts the M2 paracone first, in rhinoceros it is the hypoconid of the m2 that first interacts with the M2, not the protoconid (Fortelius, 1982, 1985). Since the hypoconid has a greater number of cusp interactions (four to six collisions with other enamel bands), it is more active in the breakdown of food than the protoconid, which has fewer cusp (less than four collisions with other enamel bands) (Fortelius, 1982, 1985). This study's first hypothesis predicted that the number of interactions a cusp participates in during mastication will influence the distribution and number of microwear features formed, with more active cusps having more features. In this case, the hypoconid in *T. fossiger* is more active during mastication than the protoconid, and consequently has a proportionately higher number of scratches, supporting the first hypothesis that active cusps have more features, and explaining why the hypoconid-protoconid scratch comparison indicates significant differences.

However, if mastication were to truly influence the distribution and number of microwear features formed, logic would dictate that the metaconid, which is equally as active as the hypoconid (Fortelius, 1982, 1985), should also have an increased number of

features: but it does not. During Phase 2 of the chewing stroke, the lower molar slips into the upper molar in an almost concentric motion to finish the chewing stroke (Fortelius, 1982, 1985). The metaconid participates with four to six enamel bands, and like the hypoconid, interacts with both the M1 and M2 (Fortelius, 1982, 1985). While the number of cusp interactions are the same for the hypoconid and the metaconid (Fortelius, 1982, 1985), there is a significant enamel microstructure difference between the two cusps. Although the influence of enamel microstructure is the focus of the third hypothesis and not the first, enamel microstructure does become relevant when determining why the hypoconid is significant. The hypoconid cusps sampled for this study were Phase 1 facets, made of normal, soft enamel. The metaconid cusps sampled for this study were Phase 2 facets, constructed of much harder enamel containing HSB. This difference in enamel microstructure indicates that, while both being equally active during mastication, the harder enamel of the metaconid prohibited extensive scratching as is seen in the protoconid. This suggests that enamel microstructure does play a small role in the production and distribution of microwear features in *T. fossiger*, supporting the third hypothesis of this study. The HSB in the metaconid counteract the increased number of cusp-to-cusp interactions of the metaconid, and result in a reduction of total number of features that would have otherwise been produced from the increased collisions with other enamel bands. The first hypothesis predicting an increased number of features due to an increased number of cusp-to-cusp interactions is also supported, but only as long as the active cusp in question is constructed of normal enamel and not highly resistant enamel.

It is crucial to examine how the two-phase chewing stroke (hypothesis two) affects the central tendency of pits and scratches between phase types, because any deviation from central tendency would significantly affect the results of dietary analyses, depending on which phase type is analyzed. Dietary analyses require comparison of the frequency of pits and scratches found on a cusp in order to infer diet. If a cusp selected for a dietary study had significantly higher numbers of pits or scratches due to differences in the two phases of the chewing stroke, results of that study would not be reporting an accurate dietary inference. In order for a pit to be produced on enamel, there must be a vertical crushing component to the chewing stroke (Fortelius, 1982, 1985). This vertical, high-pressure motion is produced during Phase 1 of the chewing stroke, supporting the second hypothesis that Phase 1 facets may have more pits than Phase 2 facets (Fortelius, 1982, 1985). Muhlbachler et al. (2015) demonstrated that modern rhinoceros *Ceratotherium* and *Diceros* both had more pits than scratches on Phase 1 facets. To produce a scratch, the masticated material requires a translational motion between two teeth. This translational motion mainly occurs during Phase 2 of the chewing stroke, again supporting the second hypothesis that there may be more scratches on Phase 2 facets (Fortelius, 1982, 1985). Muhlbachler et al. (2015) also found that *Diceros* has more scratches on its Phase 2 facets than on Phase 1. However, the second hypothesis of this study, and the results of Muhlbachler et al. (2015), were not supported by the data in this study: there was no significant difference in the number of pits and scratches between Phase types in *T. fossiger*. This means that the two-phase chewing stroke does not preferentially produce certain features on certain facets in *T. fossiger*. The hypoconid,

which is a Phase 1 facet, was shown to have *more* scratches than any other cusp, rejecting the second hypothesis that the vertical motion of Phase 1 chewing produces more pits on these facets. Yet, as discussed above, the high number of scratches on the hypoconid is due to a combination of increased activity during the chewing stroke and soft enamel, and is not related to the initial collision with the upper teeth during Phase 1 of chewing. If a higher number of scratches was produced by Phase 1 chewing, then it would be expected that the other Phase 1 facet, the protoconid, would also have more scratches than other cusps. The protoconid has significantly less scratches than the hypoconid, therefore ruling out the two-phase stroke as being significantly influential in the production of highly variable microwear features between facets of different phase types.

Finally, the third hypothesis tested in this study predicted that enamel microstructure would influence microwear feature distribution across a tooth. Enamel microstructure could potentially affect the location and production of microwear features by having differential hardness across the tooth. This was tested by comparing the total number of features found on Phase 1 facets (soft enamel) to Phase 2 facets (HSB enamel). The results of the paired t-test comparing total number of features suggest that enamel microstructure does not influence the location or production of features across a tooth, and that both types of enamel respond to the chewing stroke and mastication of food material equally (Tables 4). However, comparison of characteristics found in the protoconid to the metaconid tentatively suggests that enamel microstructure does play a conservative role in the formation and distribution of microwear features, at least on cusps where increased numbers of interactions are occurring. Ultimately, it is the

combination of increased cusp-to-cusp interactions with normal (softer) enamel microstructure that contributes to the deviation from central tendency of scratches on the hypoconid in *T. fossiger*. When increased masticatory processes are coupled with HSB, the HSB are more resistant to the high number of interactions and therefore prohibit extensive formation of microwear features, as is seen in the metaconid.

Variability of microwear features across the m2 of non-equine perissodactyls has only recently been described (Mihlbachler et al., 2015), but there have been several studies on primates, proboscideans, and suids that found microwear features vary within molar rows, between chewing facets, and along enamel bands (Gordon, 1982, 1984a, 1984b; Grine, 1986; Hunter and Fortelius, 1994; Merceron et al., 2005; Palombo et al., 2005; Todd et al., 2007; Calandra et al., 2008; Joomun et al., 2008). Mihlbachler et al. (2015) analyzed microwear feature distributions found between the lingual (corresponds to Phase 2 facets in this study) and buccal side (corresponds to Phase 1 facets) of M2s in extant grazing and browsing rhinoceros. The authors found that the grazing rhinoceros *Ceratotherium* had a homogenous scratch distribution across the M2, but had more pits on the buccal sides of the tooth. They also found that the browsing rhinoceros *Diceros* had significantly different distributions of both pits and scratches across the M2, with more pits on the buccal side and more scratches on the lingual side. While Mihlbachler et al. (2015) concluded that mastication does play a role in the production and distribution of microwear features, both the grazing rhinoceros and the browsing rhinoceros had unique signatures, or signals indicative of diet, evident in the distribution of microwear features found across their M2.

The only significant difference in scratch and pit distribution in *T. fossiger* was the high number of scratches on the hypoconid, a cusp on the buccal side of the mouth, while buccal cusps had more pits than scratches in both *Ceratotherium* and *Diceros*, (Mihlbachler et al. 2015). The higher number of scratches, and not pits, on the hypoconid in *T. fossiger* suggests that *T. fossiger* may have unique circumstances that cause its microwear feature distribution to be distinguished from the microwear patterns of modern rhinoceros. These unique circumstances are due either to the slight taphonomic alteration associated with this study's specimens, a different dietary ecology than *Ceratotherium* and *Diceros*, or a combination of these two factors. Taphonomic alteration can never be truly discounted in fossil microwear studies, but for the current study every effort was taken to remove specimens that showed significant alteration. While there may be a small amount of influence from taphonomic alteration on the results of this study, it is not considered to be strong enough to alter the results and conclusions of this study.

Although paleodiet reconstruction is outside the focus of this study, hypsodont crown morphology of *T. fossiger* has led to the tentative suggestion that it was a grazer (Prothero, 2005), and more like *Ceratotherium* in dietary ecology than the browsing *Diceros*. The low numbers of scratches (mean of 7.9 scratches per cusp) in *T. fossiger* do not suggest a strict grazing ecology, as grazers are typically found with 20 or more scratches on average (Solounias and Semprebon, 2002). This low number of overall scratches, combined with the high number of scratches on the buccal hypoconid cusp (contradictory of results found in modern rhinoceros), tentatively supports the hypothesis that *T. fossiger* had a different dietary ecology than *Ceratotherium* and *Diceros*,

suggesting a mixed-feeding diet. However, further research is needed to properly understand the diet of this animal, and more research on rhinoceros mastication and enamel microstructure is needed to help contribute an understanding to the biology of this group. The Muhlbachler et al. (2015) study examined upper rhinoceros molars as opposed to lowers, and was more interested in relative location in the mouth rather than cusp or facet type. Therefore, it is important that the methods of this current study are repeated on modern taxa, like *Ceratotherium* and *Diceros*, in order to better understand the exact role and function of mastication and enamel microstructure in the formation and distribution of microwear features across second molars in rhinoceros. A thorough understanding of these variables will contribute to more accurate dietary interpretations for extinct rhinoceros taxa.

CONCLUSION

The goal of this study is to test three hypotheses regarding the influence and role of mastication and enamel microstructure in the production of microwear features. When testing the first hypothesis, the results of a paired t-test indicate that the hypoconid has more scratches than other cusps. Comparison of the hypoconid to the metaconid and protoconid suggests that mastication and enamel microstructure do influence the production and distribution of microwear features when working in combination. These results are somewhat conservative due to a small sample size (11 teeth, 31 cusps). The high number of scratches found in the hypoconid indicates that it is not the best cusp to use for dietary analysis. Selection of other, more homogenous cusps like the metaconid, entoconid, and protoconid may allow for more accurate dietary analyses than use of the hypoconid in *T. fossiger*.

Going forward, it is important to study more fossil and modern rhinoceroses in order to compare the results of this study to results produced in similar species. Repeating the study on *T. major* found in the Nebraskan Ash Falls bed would make excellent fossil comparison to the results of this study, because those specimens died suddenly and were not subject to post-mortem transport (Voorhies, 1985). Further research is also critical for understanding of how, where, and why microwear features are formed, and it is critical that these variables are examined on large ungulates. More work is needed to better understand how taphonomic processes affect large ungulate teeth, and additional methods of quantitatively identifying taphonomic alteration must be developed. Continuing to rigorously test the LMM method in different taxa will greatly increase the power and

robustness of future dental microwear dietary analyses. Once there is a thorough understanding of masticatory processes and enamel microstructure in rhinoceros, it may be possible to conduct a robust dietary analysis on *T. fossiger*, in order to better understand the unique microwear patterns discovered in this study.

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TABLES

TABLE 1
Specimens, Samples, and Microwear Counts

Quarry Name	Specimen Number	Sampled Cusps	Scratches	Pits	
Jack Swayze Quarry	FHSM-737	Protoconid	11	5	
	FHSM-739	Protoconid	4	1	
		Hypoconid	12	5	
		Metaconid	7	0	
Minium Quarry	FHSM-8115	Protoconid	1	0	
		Hypoconid	13	14	
Long Island Quarry	USNM-6791	Protoconid	4	0	
		Hypoconid	7	2	
		Entoconid	5	6	
	USNM-6836	Protoconid	8	0	
		Hypoconid	7	0	
		Metaconid	10	4	
		Entoconid	5	5	
	USNM-6843	Protoconid	4	0	
		Hypoconid	5	0	
		Metaconid	4	1	
		Entoconid	3	0	
	USNM-6858	Hypoconid	10	1	
		Protoconid	6	7	
		Metaconid	14	10	
	USNM-6928	Protoconid	6	7	
		Hypoconid	7	2	
		Metaconid	15	4	
	USNM-6941	Protoconid	7	0	
		Hypoconid	15	6	
		Metaconid	14	2	
		Entoconid	10	5	
	USNM-6947	Protoconid	2	0	
		Hypoconid	4	0	
	USNM-7878	Hypoconid	2	1	
		Metaconid	24	3	
	Average:			7.9	2.9

TABLE 2

Cusp Sample Size Totals

Cusp	Number Sampled
Metaconid	7
Hypoconid	10
Entoconid	4
Protoconid	10
Total	31

TABLE 3

Results from 11 Paired T-tests and Wilcoxon Paired Sample Test, Comparing Microwear Number of Scratches and Pits in Cusps with B-H FDR Critical Values (Wilcoxon Paired Sample Indicated with *, Significant Tests Indicated with **)

Cusps Compared	P-value for Scratches	B-H FDR Critical Value for Scratches	P-value for Pits	B-H FDR Critical Value for Pits
Metaconid - Hypoconid	0.248	0.0416	0.501	0.05
Metaconid - Entoconid	0.109	0.03	0.478	0.0416
Metaconid - Protoconid	0.023	0.016	0.295	0.03
Hypoconid - Entoconid	0.089*	0.025	0.267	0.025
Hypoconid - Protoconid	0.017	0.0083**	0.203	0.016
Entoconid - Protoconid	1	0.05	0.060	0.0083

TABLE 4

Results from Paired T-tests Comparing Total Number of Features on Cusps, with No Significant Critical Values

Cusps Compared (Total Features)	P-value	t	Degrees of Freedom	B-H FDR Critical Value
Metaconid-Protoconid	0.026	2.9384	6	0.0083
Metaconid-Hypoconid	0.03479	2.7169	6	0.016
Metaconid-Entoconid	0.3702	1.0516	3	0.05
Protoconid-Hypoconid	0.225	-1.2029	9	0.03
Protoconid-Entoconid	0.1727	-1.7823	3	0.025
Entoconid-Hypoconid	0.2754	1.3308	3	0.0416

TABLE 5

Results from Paired T-test and Wilcoxon Paired Sample Test Comparing Scratch and Pit Counts Between Phase Types

Data	Shapiro-Wilks Normality P-value	Comparison Test Used	P-value	t or V	Degrees of Freedom
Scratch Counts Phase 1	0.2081	Paired T-test	0.4491	t = -0.7848	11
Scratch Counts Phase 2	0.3305				
Pit Counts Phase 1	0.0002189	Wilcoxon Paired Sample Test	0.7545	V = 29	0.7545
Pit Counts Phase 2	0.2356				

FIGURES

Lingual

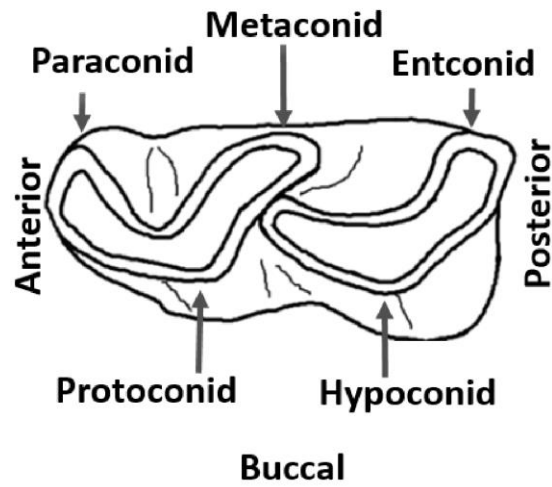


Figure 1: Schematic of the five major cusps on a m2 of a rhinoceros. Protoconids and hypoconids display both Phase 1 and Phase 2 facets, while paraconids, metaconids, and entoconids only display Phase 2 facets.

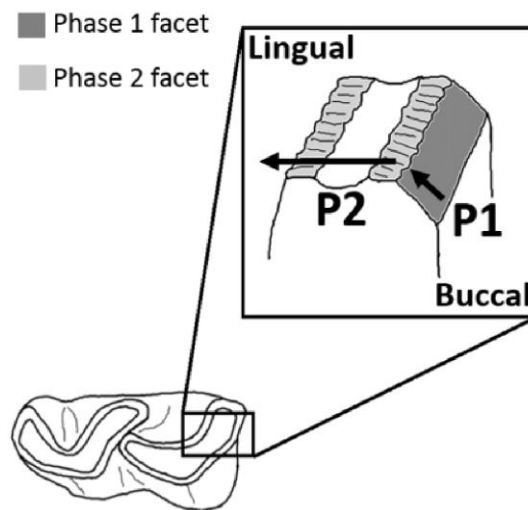


Figure 2: Schematic showing the motion and both phases of the chewing stroke in rhinoceroses. Phase 1 facets occur as flattened, inclined planes, while Phase 2 facets occur as horizontal planes with the wavy texture of HSB.

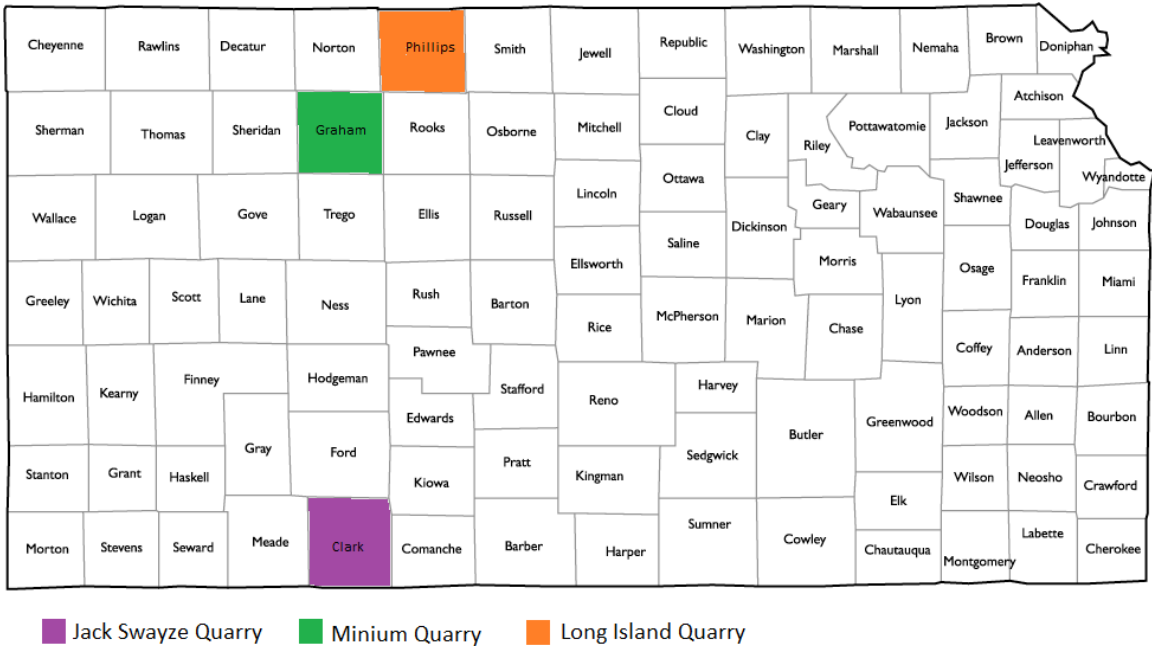


Figure 3: Specimens used in this study came from three Miocene Ogallala formation quarries in Kansas. The Minium Quarry material from Graham County and the Jack Swayze Quarry material from Clark County are housed at the Sternberg Museum of Natural History. The Long Island Quarry material from Phillips County is housed at the Smithsonian Institution.

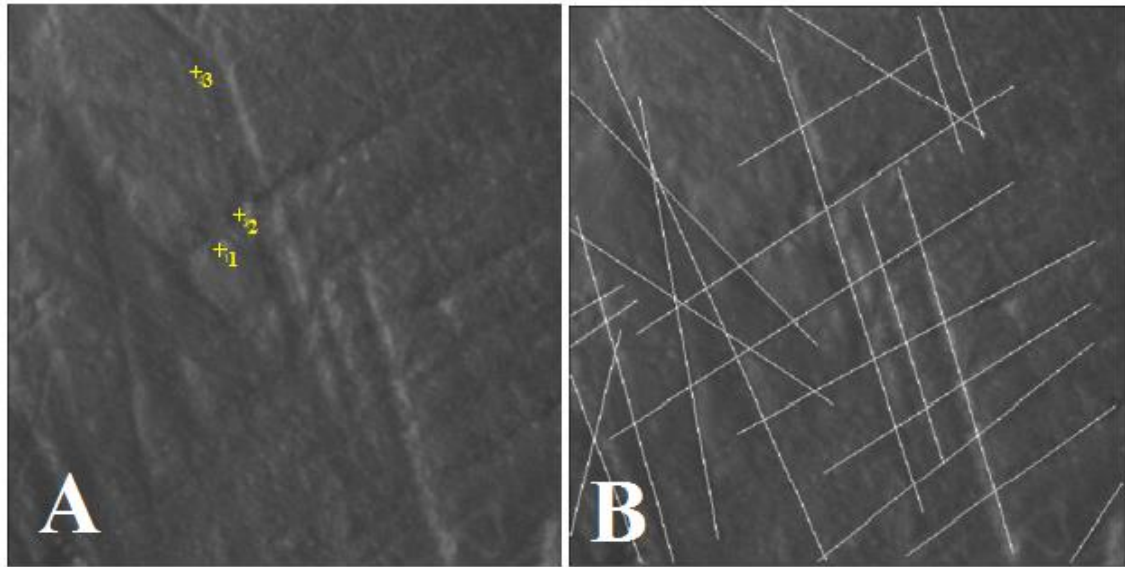


Figure 4: USNM-7878 metaconid. This specimen underwent minimal taphonomic abrasion and tumbling, and the features appear on smooth enamel. The metaconid has three well defined pits, A, and the highest number of scratches, B. Images are of 0.4 mm² count areas.

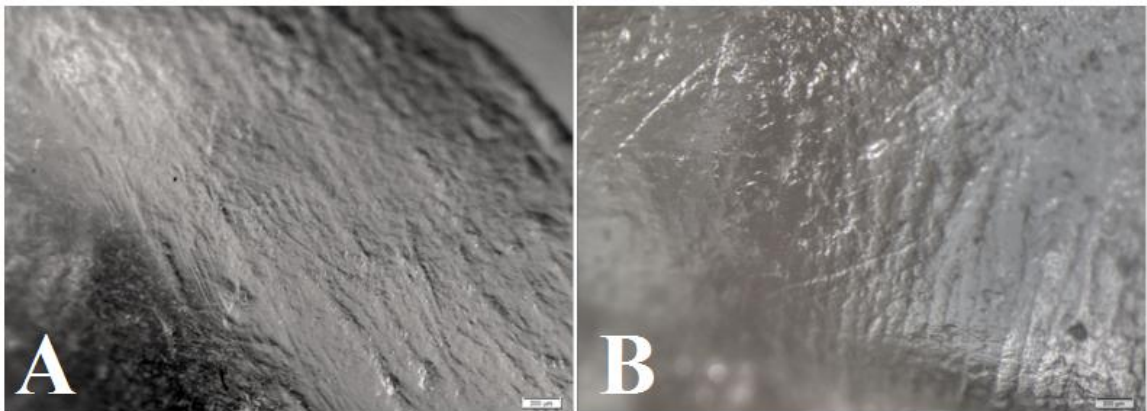


Figure 5: Images of taphonomic alteration.

FHSM-739, A, displays the protoconid Phase 1 facet with a pitted surface indicative of the early stages of taphonomic abrasion and tumbling. However, the pitting is mild and a number of features still remain: therefore, the cusp was retained for use in the study.

USNM-6791, B, displays heavy pitting and mottled texture on metaconid that is indicative of moderate abrasion with sediments. Remaining scratches are the deepest of the features, and all superficial features have been removed. Cusps of this quality were discarded from the study. Scale bars represent 200 μ m. Both pictures shown at 3.2x magnification.

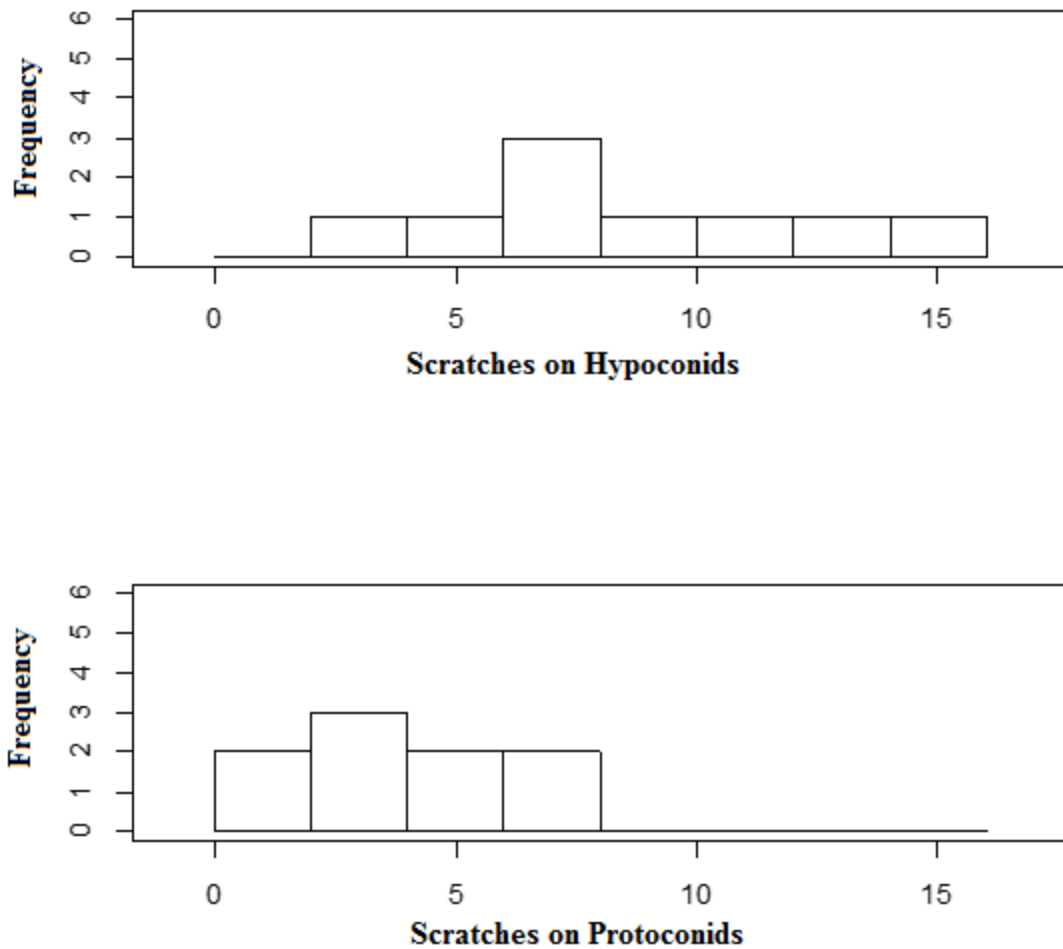


Figure 6: Histogram from the paired t-test comparing the scratches found on protoconids and hypoconids. This test produced significant results, with a B-H FDR value of 0.0083, which is less than the p-value for this test (0.017). The histogram indicates that the hypoconids have significantly more scratches than the protoconids.

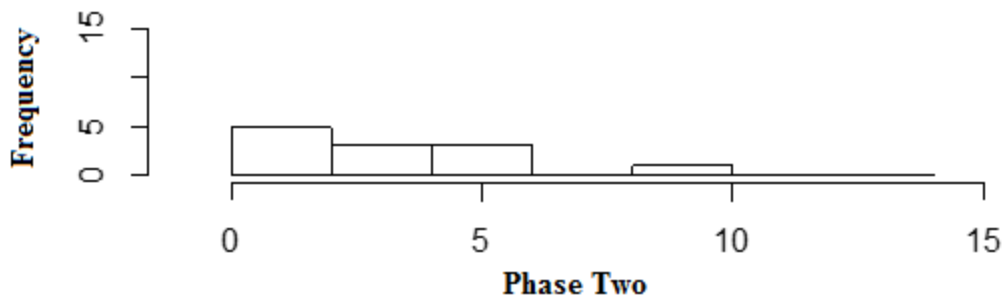
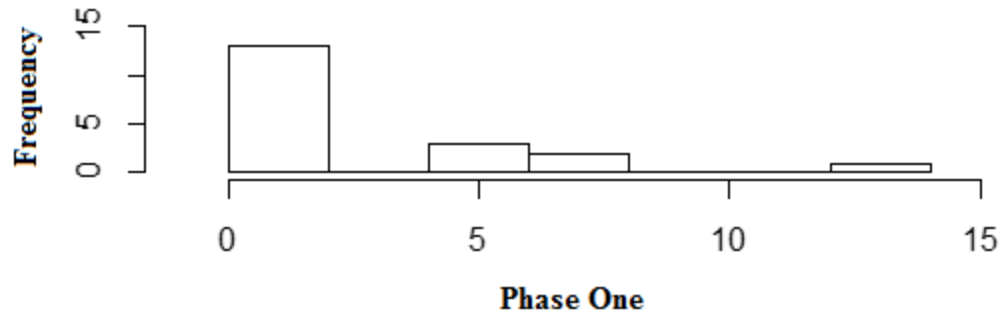


Figure 7: Histogram of pit count data distribution for Phase 1 and Phase 2 facets. Data for Phase 1 was not normally distributed.

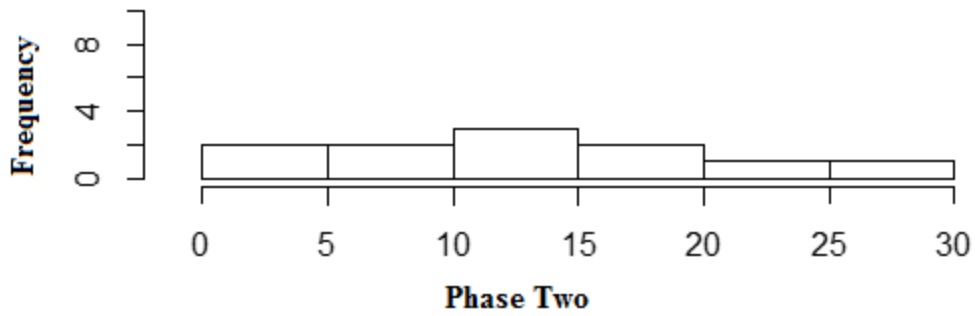
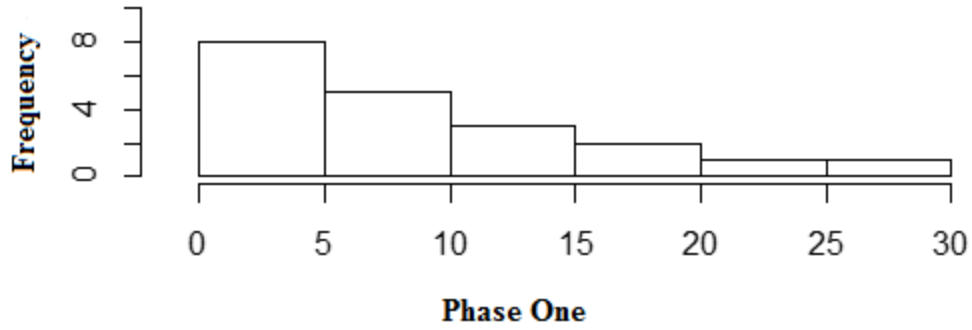


Figure 8: Histogram from a paired t-test comparing the total number of microwear features found on Phase 1 and Phase 2 facets. Phase 1 facets correspond to normal enamel, and Phase 2 facets correspond to stronger enamel with vertically aligned Hunter-Schreger Bands. The results of this test ($p\text{-value} = 0.1388 < 0.05$) are not significant, and therefore the total number of microwear features found on the two different enamel microstructures is homogeneous.

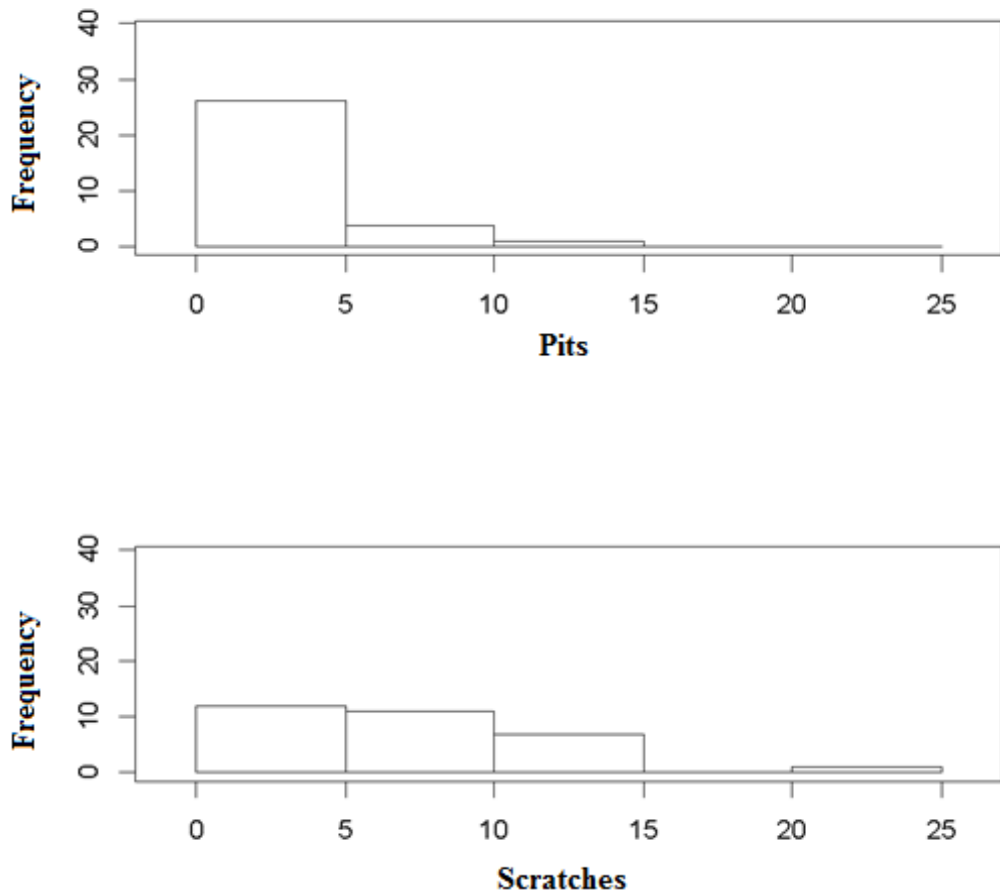


Figure 9: Histogram of the Wilcoxon paired sample test showing the frequency of pits and scratches on the 31 sample sites. There were significantly higher numbers of scratches than pits (p-value = 0.0055).