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HUMAN VS. NON-HUMAN BONE: A NON-DESTRUCTIVE HISTOLOGICAL METHOD

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By

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Species identification is one of the first steps in the analysis of bone fragments in both forensic and archaeological contexts. Current methods for human vs. non-human taxa identification include morphoscopic, histological, and DNA analyses in order to determine forensic significance and assess what is present in an assemblage. This study will use an MA1000 AmScope camera microscope to examine the longitudinally fractured surface of cortical bone fragments to gauge if non-destructive taxa identification is possible from fragmentary remains without morphologically identifying features. This method is testing for a notable difference in human vs. bovid vs. cervid endosteal cortical bone without the use of destructive, histological cross-sections. The results of this study show there is a statistically significant association with positive bone identification between taxa, an accuracy measure of 65.6% for all taxonomic groups, 96.2% accuracy of identifying human bone correctly, and 7.9% misidentification of non-human bone as human. Expanding further on these results have implications for both forensic and archaeological contexts as an affordable, non-destructive analysis of fragments of various sizes when morphological identification isn’t possible.

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1.0 Introduction

As a collective discipline used in the justice system to build a preponderance of evidence and meet legal standards on the burden of proof, forensic science exclusively uses the scientific method to always corroborate and compare an unknown sample with a known or reference sample (Schanfield, 2007). This ensures the integrity of the evidence brought to court and removes bias of individual interpretation from the analysis and generation of reproducible results. Of particular focus for this paper is the application of the science of physical anthropology to the medicolegal system, called forensic anthropology. This subdiscipline specializes in the evaluation of the human skeletal system in order to develop a biological profile that includes the possible age, sex, stature, ancestry, and unique skeletal features of an individual in order to help police authorities identify them where decomposition or destructive conditions have removed all other identifying features (Bass, 2005; White and Folkens, 2005; Klepinger, 2006; DiGangi and Moore, 2013; Christensen et al., 2014). While it is not in their purview to decide cause and manner of death, this biological profile can additionally include the assessment of ante-, peri-, and post-mortem damage or alterations to the skeleton and assist forensic pathologists, medical examiners, and coroners with the medicolegal, forensic significance of remains (Christensen et al., 2014).

Before the forensic anthropologist can even begin a profile though, the first determination that needs to be made is whether or not the bones are human (Bass, 2005; Urbanová and Novotný, 2005; White and Folkens, 2005; Dominguez and Crowder, 2012; Nor et al., 2015; Croker et al., 2016; Johnson et al., 2017). This literature review will provide an overview of relevant bone anatomy, assess current human vs. non-human identification methods, and provide the relevant background for the non-destructive method developed in this paper.

1.1 Overview of Bone Anatomy

Within biological, anatomical systems, bone is a connective tissue that acts as the main supporting internal structure of the body and is one of the strongest biological materials in existence (White and Folkens, 2005). It is composed of an organic matrix of collagen fibers combined with an inorganic base of hydroxyapatite and other minerals which form a dense layer of cortical bone surrounding the medullary cavity, cancellous bone, and marrow. The cortical bone is arranged into matrices of osteons that form larger tube-like Haversian systems which run parallel to the shaft of the bone and are interwoven with vascular canals for blood vessels (Figure 1). Within the Haversian systems, the osteocytes (mature bone cells) sit within lacunae that are arranged in concentric rings, bounded by the lamellae, around the central Haversian canal that runs longitudinally to the length of the bone and contain the blood vessels and nerves of the periosteum. Finally, the cement line bounds the outer edge of each individual osteon which remain connected by the interstitial lamellae and blood vessels (Klevezal, 1996; White and Folkens, 2005; Katsimbri, 2017).

Across all vertebrate species, the skeleton is the primary weight-bearing mechanism allowing for movement, stability, protection of soft tissues, and mineral storage within the musculoskeletal system (Klevezal, 1996; White and Folkens, 2005). During juvenile growth, primary osteons are rapidly deposited in combination with high amounts of vascularization which creates dense bone matrices that will initially form woven bone tissue where the collagen fibers are randomly organized. This is soon replaced by lamellar bone tissue where the collagen fibers will rearrange into parallel-fibered orientation (White and Folkens, 2005; Straehl et al., 2013). Some species that grow rapidly, such as cows and sheep, have plexiform bone similar to woven bone that forms more rapidly than lamellar bone, but has more structural integrity due to

the combination of lamellar and non-lamellar bone which create a brick-like shape that grows first perpendicular and then parallel to the bone shaft. This tissue formation creates very stiff, inflexible bones and is rarely seen in humans (Dominguez and Crowder, 2012; Straehl et al., 2013; Kolb et al., 2015). Depending on the type of bone formed, osteons can have different shapes and cement line banding resulting from the collagen orientation and mechanical support for movement (Dominguez and Crowder, 2012; Dominguez and Agnew, 2016).

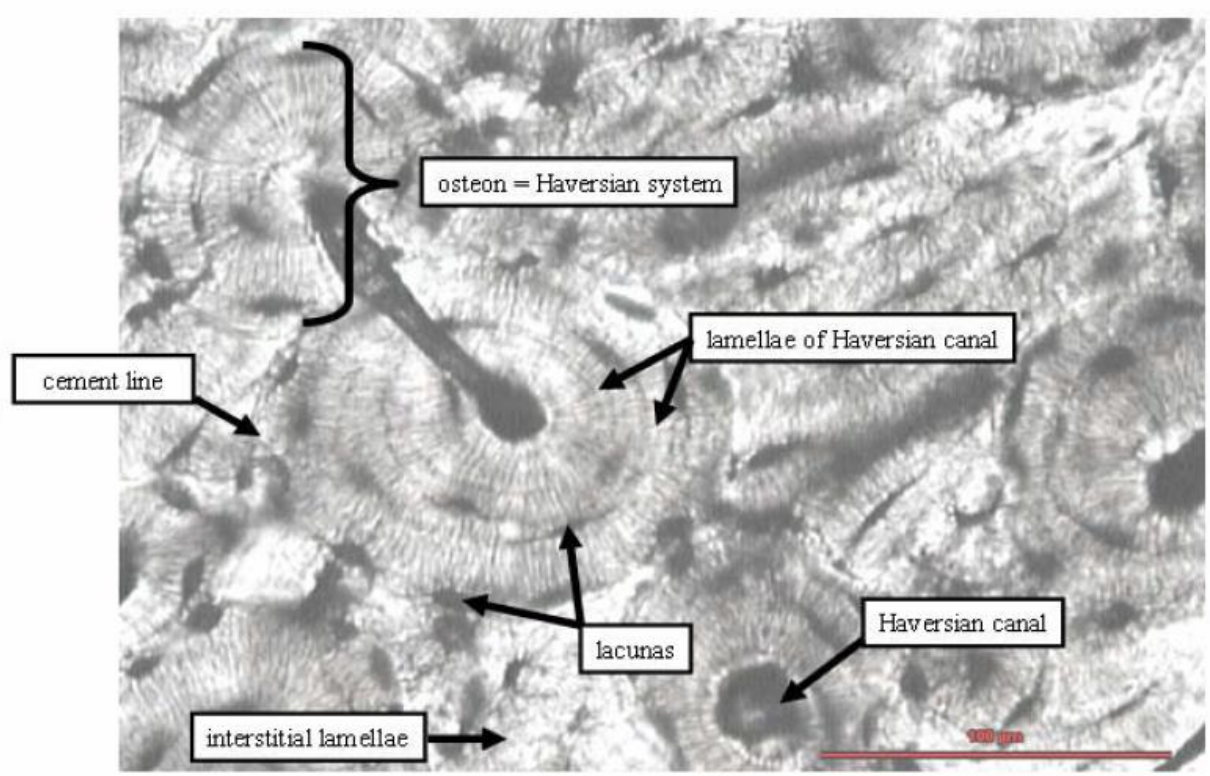

Figure 1: Cross-section of cortical bone and its internal structures (Urbanová and Novotný, 2005).

All long bone shafts are ossified at birth, but the combination of primary and secondary ossification centers around the epiphysial ends allow for healthy endochondral ossification stages to occur as the juvenile ages. This is performed by osteoclasts which will deposit bone longitudinally to increase length and radially to increase bone diameter and strength (Figure 2). During this process, long bones do not grow at the same rate at both ends and will preferentially

ossify at the end contributing to the growth in length. This can help with aging techniques in human biological profiles, but ultimately, the growth period terminates when the endocrine system transitions from the juvenile phase and the epiphyses fuse to the diaphysis (Bass, 2005; White and Folkens, 2005; DiGangi and Moore, 2013; Christensen et al., 2014; Katsimbri, 2017). It is important to note that human and non-human bones will ossify at different rates as humans have a longer juvenile development period and develop their cartilage models to primary bone over an extended stage of juvenile dependency (White and Folkens, 2005).

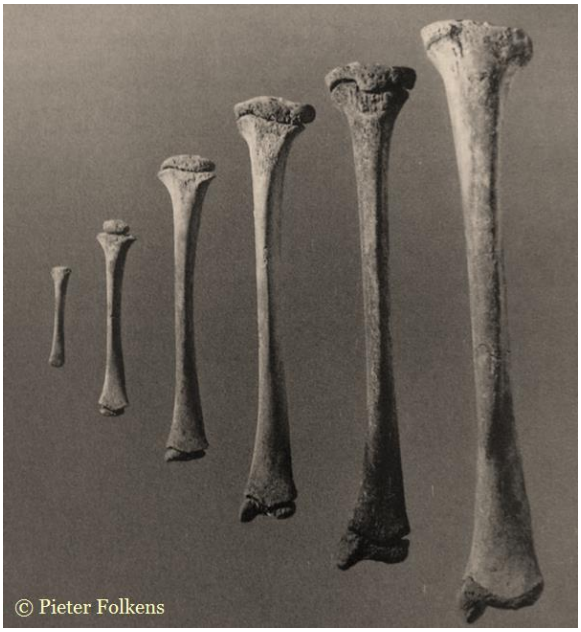

Figure 2: Different stages of growth and epiphysis fusion of human tibiae from newborn to 18 years old (White and Folkens, 2005)

After terminal growth is reached, an individual begins to age which is marked in the skeleton by the deterioration of processes instead of growth rates. This means that the epiphyseal lines will gradually disappear, secondary deposition and resorption of osteons will remodel the bone, and create Haversian bone composed of secondary osteons (Urbanová and Novotný, 2005; Hennig et al., 2015). Depending on the life history of the individual, the osteoclasts and osteoblasts will reshape the cortical bone differently (White and Folkens, 2005; García-Martínez et al., 2011; Kolb et al., 2015; Katsimbri, 2017). These processes are fairly consistent between humans and other mammals (Dominguez and Crowder, 2012), but will show differential

remodeling or deposition of secondary osteons in specific areas of the long bones depending on strain placed by differential locomotor strategies and weight-bearing needs (Kolb et al., 2015; Zedda et al., 2015, 2017).

1.2 Common Methods of Taxa Identification

Using this baseline of skeletal anatomy and physiology in forensic contexts, the first determination that needs to be made with skeletonized remains is whether or not the bones are human (Urbanová and Novotný, 2005; White and Folkens, 2005; Dominguez and Crowder, 2012; Nor et al., 2015; Croker et al., 2016; Johnson et al., 2017). This determines whether there is forensic significance to the remains and if further investigation or recovery is necessary for a case. Improper identification can cause a waste of both time and resources by the agencies involved (e.g. the police and researchers), so method reliability and proper training is necessary to mitigate needless losses (Bass, 2005; Langley et al., 2018). If a forensic anthropologist or other professionally trained individual is not readily available, photographs can be sent for an initial decision for the need of further recovery and analyzed in the lab at a later time (Dupras et al., 2012). Current methods of taxonomic identification include morphological, DNA, and histological analyses depending on the size and state of fragmentation of the remains.

1.2.1 Morphological

In response to differential weight-bearing and loading of different taxa, the functionality of bone remodeling and deposition varies between species depending on their locomotor strategies (Klevezal, 1996; Cubo et al., 2008; Dominguez and Crowder, 2012; DiGangi and Moore, 2013). This leads to morphological differences in bone shape/structure that acts as a baseline for basic taxonomic identification in cases where whole bones and/or identifiable features are available for analysis (Gilbert, 1990; France, 2009; Straehl et al., 2013; Broughton

and Miller, 2016). The first considerations of gross examinations are age, size, and morphological differences. Maturity of the bone, through epiphyseal fusion if the features are available, can help rule out smaller taxonomic groups (e.g. racoons) where terminal size is only comparable to a juvenile human individual that would have long bones with unfused ends. Next, if small taxa have been ruled out, larger mammals (e.g. horse or cow) can be considered where even juvenile individuals tend to be larger and more robust even when considering the size variability of adult humans. Adult specimens in larger mammals will be even more robust and exhibit denser cortical bone when compared with adult humans (White and Folkens, 2005; Dupras et al., 2012; Christensen et al., 2014).

Finally, overall morphology can be taken into consideration as humans tend to have relatively gracile muscle attachments and cortical bone thickness due to their bipedal locomotor strategy when compared with other species (Christensen et al., 2014; Croker et al., 2016). Some species may have comparable bone shapes (e.g. a bear paw vs. a human hand), but a professional forensic anthropologist or human osteologist is trained to recognize the morphological differences in epiphyseal shape, cortical bone robusticity relative to shaft size, and the presence, absence or fusion of some skeletal elements when compared with a human skeleton. While humans and other mammals do tend to share a similar overall body plan of skull, spinal column, axial skeleton, and four appendicular limbs attached to pelvic and shoulder girdles, some bones within this basic plan will evolutionarily fuse or change shape depending on the locomotor strategies applied by the taxonomic group (Bass, 2005; White and Folkens, 2005; Dupras et al., 2012; Croker et al., 2016). For instance, prey animals (e.g. deer, sheep, and horses) tend to have a fused radius and ulna with an accentuated diaphysis curvature to aid in weight displacement of the forelimb during their extended times spent running. In humans, the radius and ulna are

unfused, with long, narrow diaphyses, and a wider range of movement since they play no role in weight-bearing (Gilbert, 1990; Dupras et al., 2012).

In the field, the application of morphological identification is not always available, as taphonomic processes can damage the bone creating small fragmentary remains with no identifiable features. The completeness of the features can also have an impact on interanalyst variation and protocol drift due to training, experience, and availability of comparative sources (Lau and Kansa, 2018). The most significant benefit of applying this method first to the identification of skeletal remains lies in its non-destructive nature and ability to directly compare whole bones and fragments alike to larger comparative collections or photographic resources using a baseline of osteological training (Gilbert, 1990; White and Folkens, 2005; France, 2009; Dupras et al., 2012).

1.2.2 Histological Cross-Sections

Bone histomorphology can be defined as the structure of bone tissue at the microscopic level and has multiple uses in anthropological contexts for taxonomic identification, age estimation, pathology identification, taphonomic impacts on bone, nutrition, etc. (Bass, 2005; White and Folkens, 2005; Dupras et al., 2012; DiGangi and Moore, 2013; Christensen et al., 2014). As a next means of analysis, a number of studies have verified the ability to take a histological cross-section of a complete bone at specific points along the long bone shaft to establish human vs. non-human taxonomic identification. These thin cross-sections, or in one study cores (Stein and Sander, 2009), are then filled with resin, placed on slides, and examined under microscopes of varying resolution for cortical bone thickness ratios, osteon shape/consistency, directionality/type of vascularization, and a number of other measures to effectively determine human vs. non-human (Urbanová and Novotný, 2005; Cuijpers, 2009;

Dominguez and Crowder, 2012; Straehl et al., 2013; Kolb et al., 2015; Nor et al., 2015; Croker et al., 2016). Light microscopy specifically is ideal for histological analyses as the bone cross-sections should be thin enough to allow light to shine through the slide and easily highlights the microscopic cell structures for examination (DiGangi and Moore, 2013).

Using these methods, features such as plexiform bone, osteon banding, and circular osteon shape are relatively easily-identifiable measures that indicate non-human bone origin and don't require an extensive familiarity with bone histology (Dominguez and Crowder, 2012; Straehl et al., 2013; Kolb et al., 2015). In particular, osteon circularity is seen as one of the faster identifiers of non-human bone as human osteons tend to have a more elliptical shape. While human osteons do tend to decrease in size and become more circular with age, they remain distinct from non-human specimens in both 2D histological slides (Crescimanno and Stout, 2012; Dominguez and Crowder, 2012) and 3D renderings that assess the full shape of the Haversian canals (Hennig et al., 2015).

In forensic and archaeological contexts, this method has traditionally used as a means of estimating age at death, pathology, and measuring degree of bone preservation for DNA analysis. For human vs. non-human taxa identification, histomorphology assessments have been strongly corroborated and validated by multiple sources but is destructive in nature, requires equipment not always available to researchers, and cannot be utilized in the field.

1.2.3 DNA

DNA analysis is another destructive technique that can applied to bone through a crime lab or molecular anthropology lab. Preservation is key in this process where the DNA has not been degraded via poor environmental or taphonomic conditions. The protocols are standardized and consistent between labs which is an optimal benefit of using this technique in forensic cases

when it is possible (White and Folkens, 2005; DiGangi and Moore, 2013). First, the sample is prepared by washing exposed surfaces multiple times to remove contamination of external DNA, then reduced to as small of pieces as possible via smashing, chemicals, or a grinding tool of some kind. Next, the sample goes through chemical reactions in order to break open the cell (i.e. cell lysis) so that the DNA can be separated from the rest of the cell body via extraction standardized extraction protocols. In order to account for conditions where the preservation is poor or only trace amounts of the sample were recovered, the next step involves the amplification or replication of what genetic material is present in order to have a viable quantity of genetic material for further testing and to account for the loss of the original sample material. This is done through polymerase chain reaction (PCR) protocols that go through very specific heating and cooling cycles to copy the DNA over and over. Finally, the DNA is ready for sequencing where a genetic profile can be created and specific regions can be targeted for analysis and interpretation (White and Folkens, 2005; Schanfield, 2007; DiGangi and Moore, 2013). This is a gross oversimplification of molecular methods but lays a foundation for understanding the nature of DNA testing and its current use in forensic and archaeological contexts.

In terms of taxonomic identification, DNA is not commonly used in forensic contexts due to cost, time constraints, and the destructive nature of the method. Other methods like those discussed above are preferentially used to determine forensic significance of the remains. While it is possible to do a more specific species identification, the cost and destructive constraints outweigh the benefits for identification and must include a reference library of possible taxa to compare the genetic profile to. Beyond the initial human vs. non-human identification process though, forensic DNA testing is increasingly being used to assess non-human evidence such as

hair or saliva for evidential value (Schanfield, 2007). As these methods are developed further for expediency, accuracy, and cost-effectiveness, there are future implications for the use of DNA taxonomic identifications of forensic significance. For now, though, the use of DNA analysis will focus on developing genetic profiles of known human samples for identifying perpetrators, victims, and developing databases such as the National Missing Persons DNA Database (NMPDD) and the Combined DNA Index System (CODIS) (Christensen et al., 2014).

For similar cost and destructive restraints in archaeological contexts, DNA analysis is more commonly used to address the potential sex, pathology, ancestry, individuation, and diet of a known human specimen. This is usually done in tandem with other osteological analysis techniques for the sake of the biological profile provided by bioarchaeologists and forensic anthropologists (White and Folkens, 2005). In some instances where species identification was used to trace subsistence strategies, the analysis was applied to stone tools (Kimura et al., 2001; Shanks et al., 2001, 2004) as faunal records are not always representative of the full suite of species being consumed (Kimura et al., 2001). For its accuracy, this method is still collectively expensive and time consuming and is not widely used for the identification of unspecified bone samples.

1.3 Common Species Represented

Approximately 20-30% of cases examined by forensic anthropologists are determined to be of non-human origin (Bass, 2005; Klepinger, 2006; Dupras et al., 2012; Christensen et al., 2014). Common species confused with adult human remains include large mammals such as bear, deer, large dogs, and pigs, but in contexts where people (e.g. farmers and hikers) with no osteological training submit bones to the crime lab for possible forensic significance, cows and horses may also be submitted to forensic anthropologists for analysis (Dupras et al., 2012). It is

important for forensic anthropologists and other professionally trained specialists to know the distribution of non-human species in the geographic region in order to narrow down the possibilities and use proper comparative resources for positive identification (Gilbert, 1990; Bass, 2005; Dupras et al., 2012).

1.4 Research Goals and Significance

This research attempts to create a non-destructive method of identification using a notable, non-random difference between human and non-human internal cortical bone structures of fragments. The null hypothesis stands that no difference can be observed between human and non-human samples and that the additional destructive step of histological cross-sections is necessary for species identification. If there is a failure to reject the null hypothesis, the analyst will be able to non-randomly differentiate between human and non-human bone fragments using the methods created in this research.

The results of this study could provide an accessible, affordable, and fast identification method which, in turn, could save time and resources if non-human determinations are made early in a forensic investigation. By applying this at a crime lab, someone without extensive histological knowledge in osteology could look for quantitative and basic morphological features to establish forensic significance of remains. In an archaeological context, this method could be useful for similar cost, non-destructive, and efficiency reasons with the additional caveat that most faunal fragments found are regularly too small for taxonomic or element identification beyond possible long bone shaft fragments. This would additionally help give archaeologists and biological anthropologists a method for human vs. non-human taxonomic identification in known archaeological sites that is respectful to tribal beliefs on how to handle the deceased.

2.0 Materials & Methods

Collectively, this paper examined fragmented remains of human, cow, and deer long bone fragments where the internal surface of the cortical bone has been exposed. The non-human species were chosen due to their appearance in potential forensic cases that have come through the University of Montana Forensic Anthropology Lab (UMFAL). The samples for this project (Table 1) come from the University of Montana Forensic Collection (UMFC), University of Montana Archaeology Lab (Historic Fort Missoula Site: 24 MO 1100), and the Montana State Crime Lab- Missoula (FSD).

Table 1: Specimen Overview of Photos (as of 3/1/19)

Species	**Specimens**	**Elements**
Human	• FSD 0-5174 • UMFC 158 • John Byrd • UMFC 28 • UMFC 68	• R. Tibia, P+D • L. Femur Shaft, P • L. Tibia, Mid • L. Humerus, P • L. Tibia, D R. Tibia, D
Cow (Bovid)	• UMFC 85.3 • UMFC 85.4 • UMFC 86.2 • FSD 18-246 (1) • FSD 18-246 (2) • FSD 18-246 (4) • 24MO1100 (1)	• R. Femur, D • R. Femur, D • R. Femur, D/M • L. Femur, D • IN Radius, D • L. Tibia (Broken w/ hammer) • R. Humerus, P
Deer (Cervid)	• UMFC 85.1 • UMFC 85.2 • FSD ## • UMFC 86.1 • UMFC 86.3 • UMFC 85.5 • FSD 18-246 (3)	• R. Humerus, M • R. Humerus, M • L Humerus, M • L. Humerus, M • R. Radius (Broken w/ hammer) • L. Metacarpal, M • R. Humerus (Broken w/ hammer)

The cortical edge of the bone was cleaned with a toothbrush to take off the worst of the dirt, but as an important note: no water was used. This ensures the integrity of the evidence if applied to a forensic case. AmScope LED-144 light settings were kept between 2 and 5 for

consistency and the AmScope contrast setting was left at +1 so no later adjustments were needed to clarify the structures for analysis. Using the matte black velvet as both a solid background and a prop for proper orientation, the bone was positioned under the microscope and the boom arm manipulated so the camera could get close enough to the specimen for clear resolution. The fracture edge had to be as flat as possible for the microscope to focus properly, areas with attached cancellous bone or transitionary foramen were targeted if possible, and internal vs. external cortical edges were noted for photo orientation. An MA1000 AmScope camera microscope was used to photograph longitudinal images of the internal surface of fragmented long bone samples of the taxa listed above under 2-250x resolution. Once the bone was properly in-focus, images were saved as TIFF files which maintain a professional photography quality of higher resolution and no file compression. These factors properly maintain the integrity of the photo for forensic contexts.

2.1 The Research Sample

Long bones were chosen for their robusticity, histologic consistency between elements, relatively simple growth pattern and morphology, and because they tend to survive in both archaeological and forensic contexts (Urbanová and Novotný, 2005; Stein and Sander, 2009; Straehl et al., 2013; Nor et al., 2015). All specimens are from adult individuals in order to maintain consistency of bone type, but specific ages were not available to account for the possibility of secondary bone remodeling/deposition. Specimens did not exhibit any obvious pathologies that may have altered the cortical bone structures. All specimens are from historic or contemporary contexts, so pre-historic, archaeological age did not contribute to differences in features within this study. Some non-human specimens did exhibit butchery marks, but such marks did not obscure features and were noted in the photo log (Addendum A).

Due to lack of bovid and cervid remains with proper fracture edges and no taphonomic properties that would obscure the cortical bone, specimens FSD 18-246 (3) and FSD 18-246 (4) were initially whole bones broken with a hammer for the purposes of this project to expose the longitudinal cortical surface. Each bone was placed individually in a plastic bag to collect all fragments and struck with a metal-head hammer at mid-shaft until the long bone broke.

Collectively, 83 photos were taken of known human, bovid, and cervid specimens verified by morphological features. Microscopic photos of linear fractures that were viable for use in this analysis included 21 human, 22 cervid, and 23 bovid. The additional 17 photos were excluded due to photo orientation of transverse fractures or included post-mortem damage that obscured qualitative features such as glue or Dremel sample cuts. Analysis of transverse fractures were excluded in this analysis due to lack of human remains that could be clearly photographed in this way and can be addressed in future research.

2.2 Qualitative Features

Once the collective microscope photo samples were taken, they were compared and assessed for consistent features within taxonomic groups using the background information provided in the literature review. The histological features targeted and analyzed include: shape, size, and number of foramina from vascularization and descriptive appearance/consistency of the cortical bone surface. Other features (e.g. linear ridges in human specimens that run parallel to the shaft) were additionally noted in the comparison stage, but it was found that the type of fracture and conditions of the bone would obscure or confuse these features for identification by individuals with no osteological experience to be able to differentiate fracture patterns vs. structural differences. Figure 3 shows the features flagged for this pilot study and their taxa-identifying distinctions. Some of these features did exhibit in other taxonomic groups, but a

preponderance of presence, absence, and number of all the features helped decide the most likely taxa classification. All photos here are oriented with the external cortical edge on the left and the internal cortical edge on the right.

Figure 3: Flow chart of feature distinctions and taxonomic identification choices created for blind test participants

2.2.1 Human

Collectively, human samples tended to exhibit long, ovular foramina from vascularization that were encased in bone independent of the transition from cortical to cancellous bone. Additionally, the cortical surface looked slightly reflective under the microscope's LED light and in some specimens, there were long, parallel ridges of bone if the fracture type did not obscure the structures. Figure 4 highlights the shape of the foramina (red outlines) and additionally shows the light spots described as being slightly reflective.

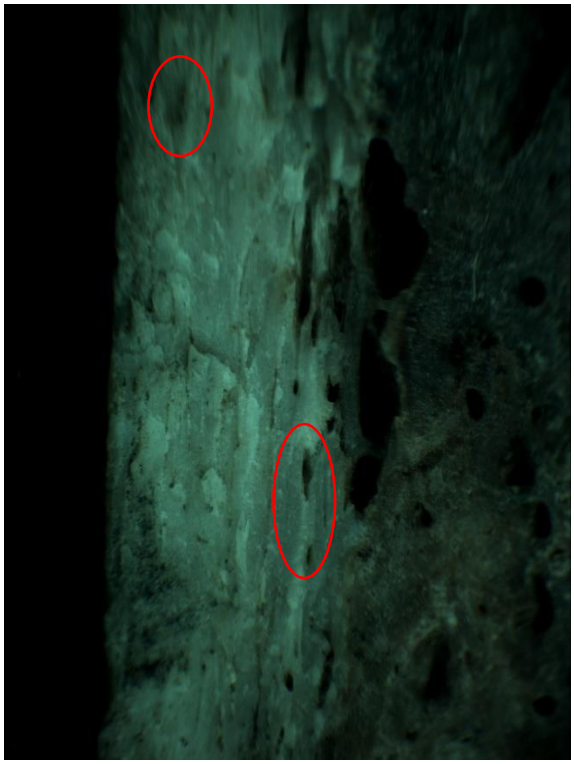

Figure 4: Photo 39 from John Byrd (S11, E3), a left tibia at the midshaft

2.2.2 Bovid

Bovid samples exhibited small, round foramina (red outlines) that tended to be clustered together (marked densely saturated in Figure 3's flow chart). This gave the overall cortical bone surface a porous, granular appearance comparable to course sand paper. Figure 5 does exhibit some dirt that could not be removed without water, but the shallow porous nature of the cortical bone surfaces is still visible.

Figure 5: Photo 68 from UMFC 86.2, a right femur at distal midshaft

2.2.3 Cervid

Cervid samples tend to exhibit similar round foramina (reds outlines) to bovids, but they are slightly larger and spread farther apart. The cortical bone surface is additionally more reflective and has a smooth, plastic appearance under the microscope. Some cervid samples (e.g. Photo 84) did tend to have some ovular foramina in the transition between cortical and cancellous bone, but these features were not encased in the cortical structure as the vascularization of human bone shows.

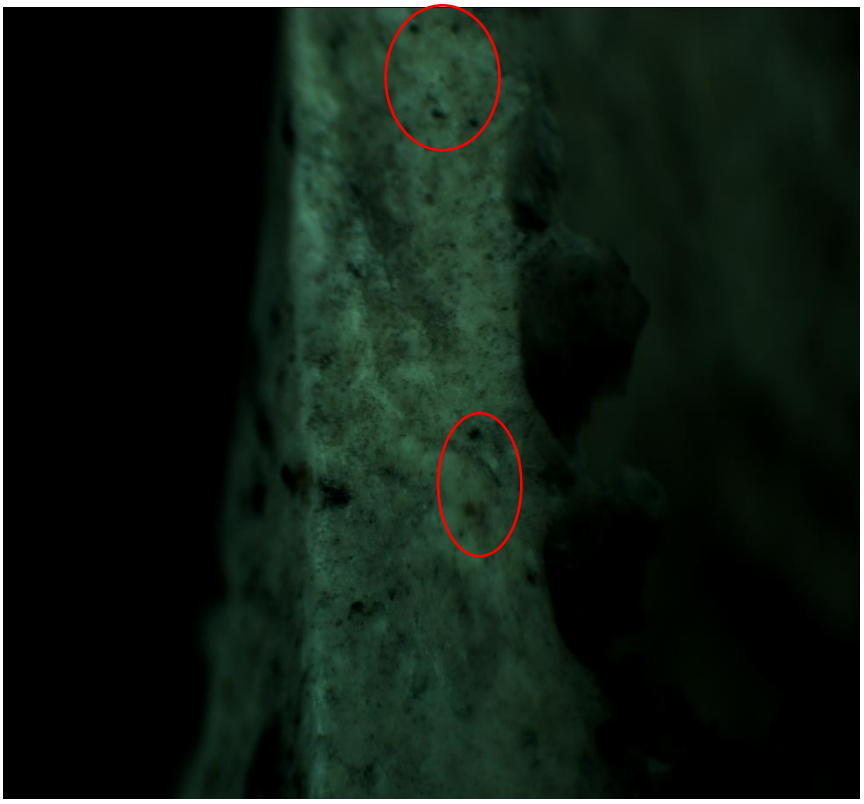

Figure 6: Photo 63 from UMFC 86.1, a left humerus at midshaft

2.3 Blind Test

The blind test aimed to assess initial communicability, usability, and preliminary accuracy of this method. Nine graduate students from the University of Montana Anthropology Department were given ten unspecified photos, the flow chart of qualitative features provided in Figure 3, and a small amount of background to understand the orientation of the photos. Due to the varied backgrounds of the students, the foramina were circled to differentiate proper vascular structures from residual dirt or fracture patterns on the bones (Addendum C).

Students downloaded the word document with Figure 3 and used it to assess the flagged features in the ten provided photos, made their guesses for taxonomic identification, and saved their answers as a new word document in the provided file folder with their names. A double-blind study was not conducted in order to account for the level of osteological experience amongst the subjects which ranged from forensic anthropologists with osteological knowledge, to archaeologists with exposure to bone identification, but not faunal analysis, to no osteological background whatsoever.

Answers were compiled into an Excel (Office 365) sheet and assessed using Chi-Square and Fisher Exact Test statistics for significance values as well as basic percentages of right and wrong identifications per photo. Significance statistics were run for both the random chance that one out of three answers would be correct due to the low number of taxonomic groups to choose from as well as the upward bound if the expected values were 100% accuracy.

3.0 Results

The original hypotheses of this study assert that there is a notable, non-random difference between human and non-human internal cortical bone structures of fragments. The null hypothesis stands that no difference can be observed between human and non-human samples

and that the additional destructive step of histological cross-sections is necessary for species identification. If there is a failure to reject the null hypothesis, the analyst will not be able to non-randomly differentiate between human and non-human bone fragments using the methods created in this research. Overall, blind study participants were able to differentiate between human and non-human cortical bone with minimal background information (Addendum C). Differential identification between cervid and bovid photos was not as accurate (Table 2). The results of this study were deemed statistically significant independent of random chance that participants chose the right answer. In all significance tests the null hypothesis was rejected, showing there is a non-random association between positive human vs. non-human taxa identification.

Table 2: Answers provided by blind study participants, green boxes are the correct answers. "Original P#" corresponds to the original research sample photo log (Addendum A) and "Photo" corresponds to the order of the blind test images (Addendum C)

Original P#	Photo	Human	Bovid	Cervid
39.1	1	8	1	0
70	2	0	1	8
84	3	1	6	2
96	4	0	2	7
25	5	9	0	0
68	6	0	8	1
81	7	4	1	4
21.1	8	9	0	0
63	9	0	7	2
43	10	0	7	2

3.1 Accuracy of Identification

Osteological background did not seem to have a significant impact on number of correct answers as most individuals (5/9) in the blind study identified 7/10 correctly with only two receiving the lowest score of 5/10, one individual received 6/10, and the highest score was 8/10. Only one individual misidentified a human bone as a bovid specimen (Table 2, Photo 1). The

other two human photos (Table 2, Photos 5 & 8) were identified with 100% accuracy. The average percentage of correct answers across all photos was 65.6%, but proper identification of human photos specifically was 96.2%. Among the non-human photos, 7.9% of responses were cervid specimens misidentified as human and no bovids were improperly identified in this way. Values of correctness did drop for cervid vs. bovid identification where bovids were correctly identified 63.0% of the time and cervids were correctly identified 44.4% of the time.

3.2 Significance Values

Chi-Square tests were run to test the significance of taxa identification where failure to reject the null hypothesis would show that there is no association between positive/negative identification and type of bone. In the initial Chi-Square test with taxa identification for all groups (Table 3), the results showed a significant association positive and negative identification and the type of bone and did reject the null hypothesis.

Table 3: Chi-Square test of positive vs. negative taxa ID for all groups

	Positive ID	Negative ID	Total
Human	26	1	27
Bovid	17	10	27
Cervid	16	20	36
Total	59	31	90

df = 2
Chi-Square value = 18.49
The result is significant at p< .001

In another Chi-Square run, the positive ID responses were compared to an expected value of 100% accuracy, meaning 27 Human, 27 Bovid, and 36 Cervid positive identifications, to see if the observed values remained significant even with deviation from the expected (albeit unrealistic) accuracy measure (Table 4). The test showed that the results were significant at p< .01 and there is a notable deviation from the expected 100% accuracy distribution.

Table 4: Chi-Square test of observed positive ID vs. expected values of 100% accuracy for all groups

	Human	Bovid	Cervid
Observed	26	17	16
Expected	27	27	36

df = 2
Chi-Square value = 14.85
The result is significant at p< .01

Finally, a Chi-Square test of observed positive ID was run against the random chance that correct answers were selected since there was a 1-in-3 possibility of this occurring for every picture (Table 5). The test shoed the results were significant at p< .01 and there is a notable deviation from the expected random distribution. Between Table 5 and Table 4, the observed results maintain statistical significance between the two extremes of 100% accuracy and random chance of selecting a correct answer, so the null hypothesis is rejected in both instances.

Table 5: Chi-Square test of observed positive ID vs. random chance of correct answers for all groups

	Human	Bovid	Cervid
Observed	26	17	16
Expected	30	30	30

df = 2
Chi-Square value = 12.7
The result is significant at p< .01

Lastly, a Fisher Exact Test was run to assess the significance of human vs. non-human positive and negative identification to corroborate the Chi-square findings due to the small sample size (Table 6). The test showed that the result was significant at p< .01 and rejects the null hypothesis.

Table 6: Fisher Exact Test run for p< .01

	Positive ID	Negative ID	Total
Human	26	1	27
Non-Human	33	30	63
Total	59	31	90

Fisher Exact Test Statistic = 0
The result is significant at p< .01

4.0 Discussion

Collectively, the results of this study showed that it is possible to identify human vs. non-human fragments regardless of osteological background knowledge based on basic qualitative features. These are outlined in Figure 3, but initial comparisons of the collective photo samples (Addendum A) showed the most difference between foramina shape/number and qualitative observations of the cortical bone surface when bone fragments showed differential fracture patterns on the edge being photographed. Based on the results of the blind test, the null hypothesis was rejected through multiple tests of significance, so there is a discernible difference between bone type in the photographs taken with a high-resolution camera AmScope microscope. It should be taken into consideration that the small sample size combined with the human identification results alone may have skewed the significance values, but considering this study was attempting to prioritize the identification of human vs. non-human bone, that goal was achieved. Random chance of correct guesses was accounted for and the results remained significant, so while this study does not yet have the validation and corroborative tests to use in the field, it is a promising pilot study for future research.

With the results as they stand, specific identifications within non-human taxonomic groups will need further analysis and refining in order to improve the poor accuracy found in this study. One consideration to understand the difficulty of differentiating cervids and bovids could lie in their shared evolutionary trajectory as prey animals with a number of anatomical similarities and skeletal morphologies (Gilbert, 1990; France, 2009). They are from distinct family groups but share locomotor strategies and histological structures where the main significant (skeletal) difference lies in the bovids' size and robusticity. By including additional taxonomic groups in future studies and possibly expanding non-human categories to broader

distinctions (e.g. two-toed ungulates vs. predators) for initial identification purposes, the lack of accuracy could possibly be mitigated in future studies.

While the verified accuracy of this method remains to be seen, when compared with other analyses outlined in the literature review, this study has implications for next-step analysis in order to gauge forensic significance of morphologically unidentifiable skeletal remains. This could provide a methodological bridge before destructive analyses such as histological cross-sections or DNA are used on forensic evidence or an archaeological assemblage. In forensic contexts, if the remains are deemed human and further destructive analysis is necessary, that will remain independent of compromising the integrity of the original specimen for purely identification purposes. Similarly, in archaeological contexts, the taxonomic identification of the sample could have implications for further destruction of the sample through migration studies or dating techniques, but those analyses will remain independent of the original identification goal of the specimen.

Overall, this method was quick, straightforward and communicable to individuals without extensive osteological background using equipment readily available in a university setting. These qualitative human vs. non-human features could be flagged using a standard microscope as well, but the application of a camera microscope additionally adds a form of evidence that could be presented in court for corroboration of evidentiary findings. If the method can be developed further to include more species, samples, and use a more portable camera microscope, such as a DinoLite, there are additional implications for practical use in the field to quickly identify determine forensic significance and whether further recovery needs to be performed. While the analyst should always err on the conservative side in order to ensure forensic integrity of identification, this method could save time and resources when developed further and applied

in geographically remote investigations where a forensic anthropologist or professionally trained osteologist may not be readily available for on-site analysis.

5.0 Conclusion

As has been outlined in the literature review, species identification is one of the first steps in the analysis of bone fragments in both forensic and archaeological contexts. Current methods for human vs. non-human taxa identification include morphoscopic, histological, and DNA analyses in order to determine forensic significance and assess what is present in an assemblage. This study used an MA1000 AmScope camera microscope to examine the longitudinally fractured surface of cortical bone fragments to gauge if non-destructive taxa identification is possible from fragmentary remains without morphologically identifying features. This method tested for a notable difference in human vs. bovid vs. cervid endosteal cortical bone without the use of destructive, histological cross-sections. Based on the results of a blind test where participants were given minimal background information, there is a statistically significant association with positive bone identification between taxa, an accuracy measure of 65.6% for all taxonomic groups, 96.2% accuracy of identifying human bone correctly, and 7.9% misidentification of non-human bone as human. Expanding further on these results have implications for both forensic and archaeological contexts as an affordable, non-destructive analysis of fragments of various sizes when morphological identification isn't possible.

5.1 Broader Impacts

On the broader scale, this project has implications for both forensic and zooarchaeological contexts in the application of non-destructive taxa identification for small bone fragments with no identifying morphological features. This method could provide an

accessible, affordable, and fast identification method that could save time and resources if non-human determinations are made early in a forensic investigation. By applying this at a crime lab, someone without extensive histological knowledge in Osteology could look for quantitative and basic morphological features to establish forensic significance of remains. This method would not substitute the need for an osteological expert in advanced analyses (especially if the remains turn out to be human), but it is a good starting point to determine where the best allocation of resources lies for a case.

In an archaeological context, this method could be useful for similar cost, non-destructive, and efficiency reasons with the additional caveat that most faunal fragments found are regularly too small for taxonomic or element identification beyond possible long bone shaft fragments. This would additionally help in possible NAGPRA protocols as a non-destructive analysis for unknown skeletal remains. It would give archaeologists and physical anthropologists a method for taxonomic identification in known archaeological sites that is respectful to tribal beliefs on how to handle the deceased.

5.2 Limitations

As it stands, limitations to this research include a small sample size and insufficient precedence on the accuracy and applicability of such a method. This is especially relevant for use in forensic contexts where reliability has significant medicolegal implications (Langley et al., 2018). Interanalyst error (Lau and Kansa, 2018) is an additional concern in the analysis of faunal remains and should be controlled for in the verification and corroboration of this pilot study.

In terms of the ability of the microscope to focus properly, limitations included difficulties getting the camera close enough to the bone with the extended edges of the light attachment. In tandem with this, there were difficulties focusing the bone properly if there was a

lot of peripheral bone in the background or the irregularity of the fracture edge made it difficult to level the cortical surface. Being able to calibrate the microscope for scale would additionally add the ability to compare cortical bone thickness towards the preponderance of evidence for one taxonomic denomination over another.

A final limitation of this study was the ability to properly clean some of the bones without use of water, causing some photos to have residual dirt obscuring the features. Along with this, while the bones are all from contemporary sources, they do show differential levels of preservation, taphonomic damage, and are from individuals of different ages. These additional factors could impact the accuracy of taxonomic identification and application of this method in a lab setting.

5.3 Future Research

Future research to expand this project should include more species and start by focusing specifically on Equid, Canid, and Ursid specimens as they tend to show up consistently in forensic cases and expand the sample size to collections outside of the University of Montana. It should additionally compare more fracture types (e.g. transverse) for fragments with differential breakage patterns where the longitudinal edge may not be observable under the microscope.

Finally, another area for future research is to examine bones in different levels of preservation and age to assess the integrity/observability of the structures. The degradation of cortical bone through burning, exposure to water, variable soil compositions, and other taphonomic conditions will undoubtedly have significant impacts on the appearance of different structures under the microscope. Age additionally builds into these degenerative considerations as pre-historic fragments buried for an extended period of time may appear dissimilar to contemporary specimens.

Collectively, this study examined the possible accuracy and utility of an otherwise untested, non-destructive human vs. non-human identification technique using a high-resolution camera microscope to examine linear fracture edges of cortical bone from human, cervid, and bovid specimens. Based on the results of a blind study given to other anthropology and archaeology graduate students at the University of Montana, this method has significant potential for the identification of human vs. non-human bone in forensic contexts. Future research can help elucidate and improve the low accuracy of identification between non-human groups, but as an initial pilot study for possible methods development, this project has promise for next-step identification purposes in both forensic and archaeological contexts where the use of morphological features is not possible.

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