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Differentiation of Fragmented Bone from South East Asia: The Histological Evidence

Derek Christian Benedix
University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Derek Christian Benedix entitled "Differentiation of Fragmented Bone from South East Asia: The Histological Evidence." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Anthropology.

Murray K. Marks, Major Professor

We have read this dissertation and recommend its acceptance:

Walter E. Klippel, William M. Bass III, Sandra K. Elkins

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Acceptance for the Council:

Anne Mayhew
Vice Chancellor and Dean of
Graduate Studies

(Original signatures are on file with official student records.)

DIFFERENTIATION OF FRAGMENTED BONE FROM SOUTH EAST ASIA: THE HISTOLOGICAL EVIDENCE

A Dissertation
Presented for the Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Derek Christiaan Benedix
August 2004

DEDICATION

This dissertation is dedicated to my teacher, my mentor, and my friend: Dr. Murray K. Marks. I thank you one hundred and four times for telling me to head west to Can-tuck-ee, instructing me in this field, nurturing my academic pursuits, and finally, teaching me to appreciate the night sky and the Cameron's monument.

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Attempting to write an acknowledgment section is trying. When you sit down and begin thinking about it, you realize you want to express your gratitude to so many people. Then fear settles in, you know the great majority of folks that have helped, encouraged, cajoled, and prodded you, and you realize there are going to be names of folks you forget. In a nutshell, I will attempt to remember all the fine people who have sat through the countless years of excuses and whining whilst I worked away, on what I affectionately refer to, as of late, as 'my book report'. Apologies to those my feeble brain missed.

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ABSTRACT

"The skeletal remains of some other animals, particularly when *fragmentary*, are often difficult to distinguish from human bones and teeth" (White 1991:3, emphasis mine).

Archaeological sites yield evidence that may be culturally modified items such as lithic tools, pottery, beads, buttons, watches, wedding rings, to items in nature classified by Dart (1957) as osteodontokeratic. Osteodontokeratic remains (or bone, tooth, and horn) are osseous human or animal elements that have either been modified tools or strictly osseous tissue itself. Bones of human and non-human origin comprise a significant portion of an assemblage. Deciphering the spatial context of the various forms of evidence is important to anthropologists when reconstructing human behavior. In archaeological sites with bones and fragments of bones, the ability to categorize whole bones and fragments into species is especially important when attempting to determine such parameters as Minimum Number of Individuals – MNI -- or Number of Species Present -- NISP (Davis 1987; White 1991).

One goal is to figure out bone assemblage patterns. Some questions relevant to this endeavor include: Are the bones human or non-human? Under what context are the bones recovered? That is, are the bones part of a culturally modified set (i.e., human and non-human bone tools or burial

practices) or do they result from natural processes (i.e., accidental death and subsequent burial including normal processes of taphonomic factors)? To this end, small elements are recovered on frequent occasion in archaeological contexts. Throughout this study, small osseous fragments are defined as those readily identified macroscopically as bone but without systematic assignment as human or non-human origin.

Many small bone fragments encountered possess no diagnostic features that permit anthropologists to ascertain species. They may, however, possess certain morphology that allow Linnaean assignment by class nomenclature (e.g., mammal versus bird versus reptile). One question then becomes apparent when this problem is encountered: Does a reliable methodology exist to differentiate fragmented human from non-human bone? This is particularly critical in situations where identifying human from non-human bone at recovery scenes where the remains of US military casualties are suspected. Using this research, a method to differentiate species origin of bone fragments will be tested. This study will examine models and methods to easily and readily attempt differentiation of bone fragments and allow them to be assigned into a human versus non-human categorical nomenclature. This research focuses on a select group of large Southeast Asian mammals primarily from The Kingdom of Cambodia, Lao People's Democratic Republic, and Socialist Republic of Vietnam -- or KOC, LPDR,

and SRV respectively. Additionally, mammalian samples from the zooarchaeological collection at the University of Tennessee, as well as one species from a private collection are examined. This study is designed specifically to alleviate situations encountered at the Central Identification Laboratory of the Joint POW/MIA Accounting Command (CIL JPAC) when small, non-diagnostic bone fragments are recovered during excavation of US military casualty sites.

Such goals are lofty. Copious research and many methods, techniques, and procedures have been described throughout the literature (see, for example, Bianco and Ascenzi 1993; Boivin and Meunier 1993; Garland 1993; Grupe and Dreses-Werringloer 1993; Harsanyi 1993; Herrmann 1993; Heuck 1993; Hummel and Schutkowski 1993; Jowsey 1966; Mulhern and Ubelaker 2001; Richman et al. 1979; Ricqles 1993; Stout and Ross 1991; Stout and Teitlebaum 1976; Tersigni 2001; Uytterschaut 1993). Most of this research focuses on utilizing histomorphologic analyses (see edited volume by Grupe and Garland 1993). That is, making bone thin sections and examining the morphology of the inter-cellular matrix under low power light microscopy. The literature is rife with histological comparisons of morphology between human and various animal bones (Harsanyi 1993; Lackey et al. 2001; Mulhern and Ubelaker 2001; Tersigni 2001). From a physical and forensic anthropological standpoint, there is a longstanding literature using human

bone histology to estimate age (see Cho et al. 2002; Eriksen 1991; Hummel and Schutkowski 1993; Jowsey 1960; Kerley 1965; Kerley and Ubelaker 1978; Singh and Gunberg 1970; Stout 1988, 1992; Stout et al. 1994; Streeter et al. 2001). However, histomorphology is only one avenue. Besides examining human versus non-human bone histology, this research focuses specifically on large mammals indigenous to Southeast Asia.

The primary goals are: 1) Examination of bone histomorphology using light microscopy to develop a "user-friendly", reliable, and reproducible method that others can utilize when examining fragmented bone of unknown origin. 2) Produce inter-species comparative micrographs outlining the differential osseous morphologies between species. Other researchers can utilize this guide when examining bone fragments of unknown origin (*vis a vis* Lovejoy et al.'s (1985) auricular surface phase change chart). 3) Finally, creation of an archive of standard reference to aid future identifications of bone fragments of unknown origin.

PREFACE

The opinions expressed in this dissertation are solely those of the author. They are not to be construed as official or as the views of the United States Department of Defense or the Central Identification Laboratory of the Joint POW/MIA Accounting Command.

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CHAPTER 1 INTRODUCTION

Importance of human skeletal identification

Precise analysis and identification of human skeletal tissue is important to many realms of anthropology. From its earliest academic and applied history to modern day physical anthropological studies and forensic anthropological casework, the ability for the scientific community to assign a biological profile to bone is crucial in the identification of missing individuals (Bunch and Shine 2003; Byers 2002; Houck et al. 1996; Mann et al. 2003; Maples and Browning 1994; Owsley et al. 1993; Owsley et al. 1995; Stewart 1979; Ubelaker et al. 1995). Because of this, an intimate working knowledge of skeletal biology is critical. Most knowledge of bones, especially skeletal tissue of human origin, begins in the classroom in an introductory human osteology course. From these beginnings, students learn the importance of differentiating non-bone and human bone from non-human bone (Fazekas and Kosa 1978; Ubelaker 2000). Once these questions are mastered dealing with human bone, students concomitantly begin the task of identifying the skeletal system as it pertains to element, side, and/or portion. Quite early, the value of relatively instantaneous bone recognition becomes realized. That is, one of the first tasks in the understanding of identification and interpretation of osseous tissue is the ability to assign a class origin to the bone in question. So, as many students will recall from their early

introductory osteology courses, one of the first questions pondered during skeletal analysis is: "To what species belongs this bone?" This is an important query, for if one is in the field of forensic anthropology, the ability to quickly scan and recognize human from non-human bone morphology is crucial. If you are looking for human remains in a specific bioarchaeological context (e.g., clandestine burial), it is always prudent to be able to classify the bones that are pertinent to your case and eliminate those discovered within the same context but not essential to the case. For instance, a truck driver pulls over to the side of the road in eastern Tennessee and observes bone in the weeds. To the untrained eye, he sees small bones and lacking the diagnostic skull deduces from their size that they belong to a baby. Law enforcement is contacted, a crime scene is established, with a request made for forensic anthropology to investigate. Upon initial inspection, the forensic anthropologist deduces that they belong to a small mammal, not to a human baby as originally thought based on their size, development, and morphology. This experience is recanted by practicing forensic anthropologists the world over (see Byers 2002; Rhine 1998). From this it is easily recognized that an intimate working knowledge of skeletal tissue is important and crucial.

In a more contemporary example, the abilities of forensic anthropologists to recognize human bone fragments was crucial and critical in the aftermath of the September 11, 2001 mass terrorist disasters (see

Dirkmaat and Miller 2003; Gould and Woodhouse-Beyer 2003; London et al. 2003; Rodriguez 2003; Sledzik et al. 2003; Warren et al. 2003; Wiersema et al. 2003; Zelson Mundorff 2003).

Obviously, the study of human skeletal anatomy has a long history (early Greek and Arabic anatomical studies, early Spanish codices on Conquest; Anderson 1962; Bass 1995; Bennett 1993; Brothwell 1981; Buikstra and Ubelaker 1994; Krogman 1962; Krogman and Iscan 1986; Schwartz 1995; Shipman et al. 1985; Snow 1982; Steele and Bramblett 1988; Stewart 1979; Ubelaker 1989; 1996; White 2000 among others). In addition to studying bone structure, the recognition and analysis of non-human bone also has a long history (Amorosi 1989; Balkwill and Cumbaa 1992; Behrensmeyer et al. 1989; Binford 1981; Brain 1981; Davis 1987, Dart 1957; Gilbert 1990; Lyman 1994; Micozzi 1991, Olsen 1968, 1973, 1979; Reitz and Wing 1999; Schmid 1972). The contribution of these studies provides a better understanding of archaeological concepts such as minimum number of individuals, or MNI (Davis 1987; Grayson 1984; Lyman 1985), number of identifiable species, or NISP (Klein and Cruz-Uribe 1984; Lyman 1994), domestication trends (Morey 1990, 1994; Reitz and Wing 1999), dietary and subsistence patterns (Driskell and Walker 2002; Guilday et al. 1962; Walker 2002; Walker et al. 2001), bone tool modification (Bonnichsen and Sorg 1989),

environments (McMillan and Klippel 1981) and hunting activities (Frison 1991; Stiner 1991).

From physical anthropologists studying human skeletal anatomy to those zooarchaeologists studying non-human vertebrate osteology, it is crucial to recognize the subtle morphological skeletal distinctions that define species. Odontoskeletal tissue is comprised of both organic and inorganic matrices. Regardless of species, during life, bone is not static. The processes of growth and development as well as bone remodeling after maturity is a complex endeavor and ongoing avenue of research (see Carter and Beaupre 2001; Frost 1986; Martin et al. 1998; Scheur and Black 2000; Schwartz 1995; White 1991).

Because of the large percentage of inorganic constituents, bones and teeth survive longer than skin, musculature, fascia, and other soft tissues associated with the body. At death, and during normal decomposition processes, there is a systematic breakdown with disintegration of all tissues (see Bass 1997; Clark et al. 1997; Galloway 1997; Galloway et al. 1989; Gill-King 1997; Love and Marks 2003; Marks et al 2000). Biochemistry accounts for some of the degradation but numerous other taphonomic factors can play in this as well (see edited volumes by Haglund and Sorg 1997, 2002; Lyman 1994). For instance, animal and insect activity, human activity, ambient temperature, environment, weather fluctuation, and soil chemistry all play

significant roles in the decomposition process (Coe 1993; Galloway et al. 1989; Gill-King 1997; Haglund 1997a, 1997b; Haglund et al. 2002; Haskell et al. 1997; Hochrein 2002; Holland et al. 1997; Lindsay 1979; Love 2001; Love and Marks 2003; Marks et al. 2000; Merbs 1997; Micozzi 1991; Perry et al. 1988; Vass et al. 1992). These processes obviously have the ability to radically change the ante- and peri-mortem morphology of a body. During decay, a fully fleshed inanimate being changes to a state of bloating to liquefying until only bone remains (Galloway 1997; Love and Marks 2003; Rhine and Dawson 1997). Given optimal conditions, temperature, and enough time, cortical bone will also delaminate, albeit at a slower rate. In most cases, however, the bone and teeth, as evidence, remains and becomes the record for scientists to analyze.

From human skeletal evidentiary material, analysis usually focuses on estimating a biological profile. A biological profile estimates age, ancestry, sex, stature, and pathology from the remains (see Bass 1995; Fazekas and Kosa 1978; Gilbert and McKern 1973; Gill and Rhine 1990; Krogman and Iscan 1986; Lovejoy et al. 1985; McKern and Stewart 1957; Phenice 1969; Stewart 1979; Trotter and Gleser 1952, 1958). The more complete the remains, the more readily and easily accomplished is this task. When other variables come into play that change the bone morphology (i.e., that cause fragmentation of intact human skeletal tissue), more concentration is

required on the part of the researcher or scientist in order to correctly assign a biological profile to the elements in question. When taphonomic factors alter morphology of bone, narrowing the estimations of the biological profile become more complicated. With all the chances of change to bone the importance of recognizing and identifying skeletal tissue in all conditions is warranted.

Appreciating all the factors that affect survivability of postmortem bone, it is readily observable that a working knowledge of identifying bone from non-bone and human from non-human bone is important. The ability to perform field analysis of bones to estimate an MNI and a biological profile is crucial. However, to complicate matters, the biology or peri/postmortem circumstances of each forensic case is unique. One example of differential bone morphology is the phenomenon of fragmentation. Again, the onus rests on the forensic anthropologist to be able to correctly identify skeletal material, whatever condition, as it pertains to the forensic case at hand.

Bone fragments: their importance in identification

Bone fragments originate routinely from archaeological and forensic sites around the world. In most bioarchaeological contexts, differentiating between human and non-human elements is a valuable phase in analysis, but not necessarily a priority. In other instances however, the ability to assign species designation to fragmented bone is of utmost importance. These

instances are usually related forensic concerns (Bennett and Benedix 1999; Dix et al. 1991; Houck et al. 1996; Mann et al. 2003; Mayne Correia and Beattie 2002; Murad 1997; Owsley et al. 1993, 1995; Stewart 1970; Stout 1986, 2003; Stout and Ross 1991; Ubelaker et al. 1995).

In this same forensic concern lie the missions of the Central Identification Laboratory of the Joint POW/MIA Accounting Command (CIL JPAC), whose goal is to perform humanitarian service by recovering and identifying US military personnel who never returned from duty. The CIL JPAC is called upon to investigate bioarchaeological sites from around the world (Bunch and Shine 2003; Holland et al. 1997; Hoshower 1998; Mann et al. 2003; Moore et al. 2002). These sites are directly linked with activities of the US military, specifically operations in conjunction with affairs concerning military presence. These recovery scenes vary, but some are discovered as isolated burials from ground losses while others are produced from aircraft crashes (e.g., fighter jets, airplane bombers, and helicopters). Some incidents involve multiple casualties while other recovery scenes may be limited to one. Also, some have no attendant material evidence while others contain copious amounts strewn, interlaced, and commingled (e.g., aircraft wreckage). Among aircraft crashes that result in rapid deceleration, disintegration, and conflagration, there is much destruction to the plane, pilot, and crew. The physics of traumatic plane crashes typically render aircraft and crew to

fragmentation and sometimes near complete obliteration. Because of such destructive perimortem events and the ravages of postmortem time, material and artifactual evidence are changed drastically from their living or original appearance.

The significant period of time between loss and recovery is remarkable and, as mentioned, exact a toll on biological and structural remains. While there are many reasons for this, most stem from the fact that these areas where US personnel have been lost remained hostile and inaccessible for long periods of time post incident. Excavations can take place anywhere in a temporal time line initiated immediately after a traumatic mishap up to 140 years after the incident. For example, recent CIL JPAC excavations were undertaken after military helicopter crashes near Kahuku, Hawaii (Adamski and Kakesako 2001) and Quang Binh, Vietnam (Honolulu Star-Bulletin 2001). CIL JPAC also lent valuable assistance to the underwater recovery of some of the US Navy's first sailor casualties from the *USS Monitor*, sunk in 1862 in Cape Hatteras off the coast of North Carolina (Cole 2002). Furthermore, many factors deliver an additional toll on bone quality (Buck and Benedix 2003; Haglund and Sorg 1997, 2002; Hamilton et al. 2000; Mann et al. 2003). Taphonomic factors affecting bioarchaeological sites include, but are not limited to, decomposition, weathering, animal trampling, etc. (Haglund and Sorg 1997; 2002). Non-natural (i.e., cultural) factors also play

a role in affecting bioarchaeological remains from deposition until discovery (see Holland et al. 1997). That is, in some countries, local inhabitants from neighboring areas visit aircraft crash sites, scavenge scrap metal, transport wreckage, and even recover human remains. These scavenging actions affect the spatial/dispersal integrity of the recovery scene as well as the quality of any human remains found.

Finally, in the processes of archaeological excavation, many unknown items are unearthed. Within the realm of forensic archaeology, a rubric under which CIL JPAC investigations are warranted, the task of separating associated physical evidence from non-associated evidence is an important task (see Dirkmaat and Adovasio 1997; Ubelaker 1997). That is, some material found at a recovery scene is not part of the crash site *per se*. The only connection this material may have with the recovery scene is coincidental. There are biological and non-biological examples of such material. Bone at a recovery scene may or may not represent casualties from the crash itself, but may be remains of animals dead at the site, collateral or later deposits of the traumatic insult that caused the bioarchaeological deposit being investigated.

In other examples, animal remains may be part of the bioarchaeological site. At one site, a military plane crashed with cargo that included the carcasses of water buffalo (Mann et al. 2003; Tyrrell 2001).

Partial loss of the crew occurred and in the aftermath with the recovery scene including a commingled mass of human and water buffalo fragmented bone. In another interesting example, the CIL JPAC excavated an airplane crash site in the LPDR where among the skeletal remains of the crew, canid bones were also discovered. The historic military records revealed that this specific aircrew had a mascot – Snoopy – a "good luck charm" dog who rode with the crew on every mission. In this case, all identities of the crew and their mascot were made during final forensic anthropological analysis.

In other instances, especially when excavating isolated burial sites located near populated areas, various material evidence and "osseous-rubbish" (e.g., discarded animal bones representing dietary refuse) from the local population is usually encountered. Furthermore, bone may be recovered but could represent earlier deposition, at the time of, or after the crash incident. Examples of this phenomenon include finding local unmarked graves from the indigenous population and the possibility of excavating the remains of allies or the enemy. These bones require differentiation from any of those of the crew.

Problems exist where fragmented animal bones are commingled with fragmented human bones. The ability to differentiate between macroscopic human and non-human skeletal material is relatively simple to the trained eye. From a gross morphological standpoint, complete intact bones of human

and non-human origin are clearly diagnostic and typically quite easy to identify. Also there are copious accounts on identifying human from non-human bones (see Bass 1995; Byers 2002; Fazekas and Kosa 1978; Olsen 1968, 1973, 1979; Rhine 1998; Schwartz 1995; Ubelaker 1989; White 2000).

Differentiating between human and non-human bone becomes increasingly difficult as the overall morphology of the bone begins to change. That is, normal bone morphology is static enough that identification is accomplished at gross observation. Upon fragmentation, the ability to identify them by species increases in difficulty. Some studies have described instances of osteological and/or soft tissue confusion in the forensic analysis (see Brothwell 1972; Rhine 1998). For instance Ubelaker (1989) lists a set of animal bones frequently misidentified as human. This list includes black bear, dog, pig, white-tailed deer, and domestic sheep (see also Marks 1995). Ubelaker states that bear paw bone morphology is often confused with the human hand (see also Byers 2002). Brothwell (1972) reports misidentification of non-human osseous tissue as early hominid bone. In another example, a mushroom was mistakenly identified as human soft tissue. In most of these instances, misidentification occurred and the remains in question were actually complete. In those cases where fragmentation occurs and morphology has changed, it is easily seen that more confusion and misidentifications could occur. Studies have been

undertaken in order to find reliable, useful, and easily replicated methods to accomplish such tasks (Harsanyi 1993; Mulhern and Ubelaker 2001).

Differentiating bone from non-bone is usually a straightforward exercise. Bony tissue from all classes of animals possesses diagnostic characters that are typically discernable by gross visual inspection. These characters include but are not limited to the plate-like morphology of cortical bone (i.e., that bony tissue that makes up most diaphyses of long bones), and the visual spongy lattice-like network that is characteristic of trabecular, or cancellous bone. Some specimens can definitely be classified as bone, but assigning these specimens to an individual species cannot be accomplished using gross morphological visual inspection methods. In other situations, non-bone specimens are examined because they first appear to have many familiar qualities seen in bone (Bennett and Benedix 1999; Ubelaker 1998). Examples of such fragmentary material evidence that has fooled or confused many scientists and researchers include: coconut shell, bamboo grass fragments, burned plastic, wood fragments, various flora, and rocks. This is not to mention the confusion in identification of burned/calcined bone given discoloration, fracture, and shrinkage (Bennett and Benedix 1999).

Many of these studies focus on the histological examination of cortical bone thin sections under light microscopy in an attempt to differentiate human versus non-human bone at the cellular structural level (Harsanyi

1993; Mulhern and Ubelaker 2001). Most of this research centers on the differentiation of the histological morphology of compact bone. The theory follows that humans possess a differentiation of cellular structure to that of other animals.

The goals of this project

So why study histology of different mammalian species? Utilizing research that can differentiate human from non-human Southeast Asian mammals is pertinent and has direct relevance to ongoing casework and resolution conducted at the CIL JPAC.

Bioarchaeological humanitarian recovery missions have been conducted in Cambodia, Laos, and Vietnam. In Cambodia and Laos, remains and non-biological material evidence recovered are transported and accessioned directly into the CIL JPAC. In recovery cases from Vietnam, a different protocol is utilized. At the end of each recovery mission, remains and material evidence are transferred to Hanoi, Vietnam and a joint forensic review takes place at a later date. At the joint review US and Vietnamese forensic scientists (i.e., anthropologists and dentists) examine the remains and ascertain the preliminary "human" odontoskeletal attributes of them. In some cases, morphologically distinct non-human bone is recognized and is not required to be returned to the CIL JPAC.

There were 1,816 instances of remains that were recovered or unilaterally turned over to the CIL JPAC where they underwent further analysis. Of this total, 61% of cases proved to be skeletal remains consistent with human origin, 15% cases were diagnostically non-human origin, and 24% cases were unknown origin.

In the instances described above, the need for a method that is easily replicated to allow for deduction of human versus non-human origin is quite important. The explicit mission at the CIL JPAC is to identify and repatriate the remains of missing US military personnel (see www.jpac.pacom.mil). Any additional evidence utilized confirming human or non-human identification is important and relevant. With this in mind, the ability to add more evidence to a case strengthens it. Obviously, being able to differentiate human from non-human origin is important to this goal.

Repatriation in US military history

Repatriation conjures up different thoughts for different groups of people (see Bray 2001). If the anthropological community were engaged in word association, the word "repatriation," may manifest itself as the Native American Graves and Repatriation Act or NAGPRA (Mihesuah 2000; Quigley 2001; Superintendent of Documents 1996, 1999; Trope and Echo-Hawk 2001; Ubelaker 1992). The NAGPRA brings up a range of emotions spanning the continuum of approval to disapproval (Garza and Powell 2001; Mihesuah

2000; Owsley 2000). In short, a majority of American Indian populations agree that ancestors should be returned for re-interment. The NAGPRA legislation states that federally funded museums and academic institutions assess their collection holdings of human remains to ascertain which collections belong back in the ground (Quigley 2001; Trope and Echo-Hawk 2001; Ubelaker 1992). This arena is cause for much speculation and in some instances the identity and ancestry of some ancient skeletal remains is questioned (Green et al 1998; Herrmann et al. n.d.; Ousley et al. 2003; Watkins 2000, 2001). In these instances the morphology of the skeletal material raises questions regarding racial affinity. Recently, two ancient skeletons have been discovered and, of interest, is both have archaeological dates going back 8,000 to 10,000 years. The question then becomes: to whom do these skeletal remains of remarkable antiquity belong? Which living population (or science) is entitled to determine the fate of these individuals (Bray and Killion 1994; Mihesuah 2000; Watkins 2001)?

But repatriation is more than just the scientific community returning American Indian skeletal remains that have been warehoused on shelves of museums and academic institutions for scientific analyses. Webster's New World dictionary defines repatriation: "To restore or return to the country of origin, allegiance, or citizenship" (Neufeldt and Guralink 1988:1137). In this

sense then, repatriation is also a term that can be used to mean the collection and return of America's war dead (Wood and Stanley 1989).

Beginning in the 1840s, a sincere concentrated effort has been expended by the US government to find, recover, and repatriate military service men and women killed in various war conflicts (www.jpac.pacom.mil). With the advent of the Civil War, the US government altered its duty and added more responsibility in this repatriation effort. The responsibility of identification and burial of the dead in registered cemeteries followed. With aid from many in various units throughout the US military, demographic casualty data was collected and sent to the office of the Adjutant General (Steere and Boardman 1957). Some service members were identified and buried in the countries they fell (e.g., Normandy Invasion), while a smaller portion of unaccounted-for service members on these casualty lists, were interred in the countries in which they were killed.

A major policy was created by the US military during the Spanish American War that stated all remains of US service members buried in foreign cemeteries were to be disinterred, repatriated, and reburied in United States soil. World War I (WWI) in Europe caused the military to authorize the Graves Registration Service in the US Army Quartermaster Corps to recover and identify the American war dead (Wood and Stanley 1989; McDermott 2004). At the end of WWI more than 96% of the 79,000 fatalities

were identified (Wood and Stanley 1989). With the onset of World War II, Congress empowered the Secretary of the Army to establish various temporary mortuary/identification laboratories to continue the methods employed to care for the dead (Risch and Kieffer 1955; Stauffer 1956). These Central Identification Points (CIPs), as they were called, were located in France and Belgium. Utilizing the recommendations of Harry Shapiro, the curator of physical anthropology at the New York based American Museum of Natural History, identification procedures were to be "based on techniques of physical anthropology" (Wood and Stanley 1989:1369). At this time, the US military employed academic physical anthropologists and medical anatomists to complete this task (Thompson 1982).

In 1951, several years after World War II these identification laboratories were dissolved. When war broke out in Korea, Congress once again built a temporary central identification laboratory in Kokura, Japan, that was in operation until 1956 (Thompson 1982). With the US escalation (ca. 1965) in the war in Southeast Asia, two US Army mortuaries were established and operating in Saigon, South Vietnam. Their primary goal, as the laboratories before them, was to identify dead US service members. In 1975, when no US military presence in Vietnam, the US Army established the Central Identification Laboratory, Thailand (CIL-THAI).

The CIL-THAI's mission continued to search, recover, and identify US service members killed in the Southeast Asian conflict. After the dissolution of the government in South Vietnam, the Central Identification Laboratory was moved and reestablished in Honolulu, Hawaii. CILHI, as it was now appropriately named, expanded its mission to include the search, recovery, and identification of all unrecovered US service members from past wars. Additional services the CILHI is tasked with includes helping with recent mass disasters (Saul and Saul 1999). In October 2003, the CILHI merged with Joint Task Force – Full Accounting (JTF-FA) to become a military operated unit called the Joint POW/MIA Accounting Command (JPAC).

Historically, battles of the Spanish American War, WWI, WWII, the Korean War, the Vietnam War, the Cold War, and military action during Desert Storm in the Middle East, have incurred the loss of American military service members. In most cases, individuals perishing in foreign lands are retrieved and their remains processed through the various military mortuaries (i.e., Central Identification Laboratory at Schofield Barracks, Hawaii for Pacific Theater WWII action, the Central Identification Points in Strasbourg, France and Neuville-en-Condroz, Belgium for European Theater WWII action, the mortuary at Kokura, Japan for the Korean War, Tan Son Nhat mortuary in Vietnam, Rammstein mortuary in Germany for the Gulf War, and the Dover, Delaware mortuary) and returned back to US soil for

burial. In other cases, suspected casualties are not immediately located and are listed using military status nomenclature: i.e., prisoner of war (POW), missing in action (MIA), killed in action (KIA), body not recovered (BNR), dead, remains not recovered (DRNR), dead, remains recoverable (DRR). In these cases a last known alive location is recorded, but for whatever reason these bodies could not be collected. Sometimes a significant temporal period passes. In such instances, the military convenes and a finding of death date is issued, even though physical evidence (i.e., bodies, skeletal remains), and/or material evidence (i.e., identification tags, wedding rings, watches, etc.) is not retrieved. Subsequently, intensive investigations are undertaken to pinpoint the location of where those missing in action fell. Once preliminary investigations are complete, fully manned specialty teams are sent on bioarchaeological reconnaissance missions worldwide.

In cases the CIL JPAC undertakes, the investigation, recovery, identification, and repatriation of human skeletal remains representing service members has been the standard of conduct. It is in these instances when those listed as MIA are finally recovered and their mortality status changed, positively identified, and repatriated back to the United States that the US Government's obligation to surviving family members comes full circle (Bunch and Shine 2003).

The role of anthropology in the US federal government

Historically, the US federal government has employed anthropologists in many areas. For example, the US Army Corps of Engineers utilizes archaeologists and anthropologists in cultural resource management positions (www.usace.army.mil). The Bureau of Land Management, National Park Service, and the US forest service have all employed anthropologists and archaeologists to continue to monitor and manage public lands ensuring that both known and unknown archaeological sites are protected. The Armed Forces Institute of Pathology (AFIP) employs forensic anthropologists within their medical corps to aid their commitment in pathology consultation and research, as well as aid in the recovery of recent deaths (www.afip.org). The National Museum of Natural History (NMNH) of the Smithsonian Institution (www.nmnh.si.edu/anthro) has traditionally employed cultural anthropologists, ethnologists, physical anthropologists, and archaeologists all providing services for the preservation of America's past. In addition, the physical anthropological section of the NMNH has aided and consulted with the Federal Bureau of Investigation (FBI) in contemporary forensic cases (Ubelaker and Scammel 1992).

Finally, the US federal government and the US military have extensively used anthropologists significantly for over 100 years (www.jpac.pacom.mil). The historical background and symbiotic relationship

between the US military and anthropologists has its root beginnings in the analysis and identification of America's war dead (see repatriation section above). At the forefront of this relationship is the notion of the military instituting an identification process. To this end, laboratories called central identification centers have been utilized at various locales around the globe. Employed by the US government throughout this rich history are physical and forensic anthropologists, as well archaeologists at the various Central Identification Laboratories discussed above.

There has been an important partnership between the US military and physical anthropology, especially forensic anthropology. Evidence of this symbiotic relationship is observed by numerous publications stemming from data collected and utilized to identify America's war dead. Research leading to newer techniques for estimations of the biological profile has its origins in analyzing many US military casualties (i.e., McKern and Stewart 1957; Trotter and Gleser 1952, 1958; Vandervael 1952). These groundbreaking publications were critical for identifications of America's war casualties. They were timely in their origins and continue to be utilized today. Additionally, prior to these publications, other research methodologies in forensic anthropological techniques existed. In the 1930s and 1940s, Krogman (1939, 1943, 1946; and see Byers 2002; Stewart 1979; Ubelaker 2000) authored scientific pamphlets specifically for the FBI. The 1939

publication recognized and outlined the need for an appreciation, better understanding, and repeatable techniques of estimates of age, race, sex, and stature from human skeletal remains. All skeletal biologists recognize the McKern and Stewart reference (1957) that was developed for the US Army Quartermaster Corps. This text dealt with estimations of age in young adult males drawn from casualties of WWII. From this important work, casts of representative age changes in the pubic symphyses has been one critical piece allowing for better age estimation. Anthropomorphic studies at Natick throughout its history have added to the collection of physical anthropological data (http://www.natick.army.mil/about/history.htm). In addition, Trotter and Gleser's (1952, 1958) stature estimation publications grew out of analysis of WWII and Korean War dead.

This trend continues at the CIL JPAC today. Modern academic research has been aided and greatly influenced by the recovery and analysis of America's war casualties. For instance, research has focused in various academic realms such as new forensic techniques in the use of ground penetrating radar to locate buried remains (Buck 2003; Miller 1996), the study of anatomical pathologies and skeletal anomalies (Tyrrell and Benedix 2004); forensic taphonomy (Holland et al. 1997); the role of forensic anthropological techniques within the military (Adams and Maves 2002; Hoshower 1998; Mann et al. 2003; Webster 1998); research on identification

tags (i.e., “dog tags”) and the proliferation of their prevalence and manufacture whether genuine or not in modern day Vietnam (Mann et al. 2002); the study of archaeological strategy and analyses of recovered crew-related material (Moore et al. 2002); commingled remains (Byrd and Adams 2003); and the examination of morphological skeletal data in Southeast Asia (Rankin and Moore 2004).

Justification of this research

The goal of this research is examination of known specimens at the gross morphological/macroscopic level and the microscopic level so that when non-diagnostic portions of fragmented bones are recovered, the CIL JPAC forensic anthropologists are aided to more easily identify them. The whole point is that there are key fauna that are routinely encountered in bioarchaeological recovery of MIA remains. Sometimes they are in such a fragmented state that they may be mistaken for fragmented human cortical long bone.

Some of the options the CIL JPAC has at its disposal to aid in skeletal analysis include skeletal exemplars (i.e., a study reference collection of human and non-human bone); osteological data bases (e.g., FORDISC, see Ousley and Jantz 1996; OdontoSearch, see Adams et al. 2003, see also Adams 2002); a scanning electron microscope; biological profile type-specimen casts, and mitochondrial DNA (mtDNA) sampling procedures. MtDNA analysis

begins when bony samples are cut and sent to CIL JPAC's laboratory counterpoint, the Armed Forces DNA Identification Laboratory (or AFDIL) in Rockville, Maryland. In some instances, to aid in the identification process, circumstantial evidence is utilized to strengthen cases. MtDNA is one avenue that provides additional circumstantial evidence. MtDNA is used in approximately 25% of the total accessions the CIL JPAC has analyzed in the past ten years (Damann personal communication). Statistically, the CIL JPAC has cut approximately 3,871 samples and sent them to AFDIL for analysis. Of that total, 3,026 samples have undergone processing by the AFDIL. The number of samples does not represent a total number of individuals. That is, some cases require more than one sample to be cut and analyzed. For example, in some cases, samples are taken from multiple elements, this is especially true in commingled incidents. The average number of samples per case is slightly above eight. The success rate for sequencing, on average, is 71% (n = 2292). 21% (n = 660) of those cases produced inconclusive results, in 6% (n = 195) of the cases no sequence has been obtained, and 2% (n=59) of the samples were not used based poor condition. While the numbers are positive that mtDNA samples will be produce successful sequences, there are certain limitations to this procedure, the primary one being time cost-effectiveness. That is, producing samples and analyzing them requires a time commitment. So while skeletal cases can

be written, sometimes there is a time lapse waiting for the AFDIL results before the final identification report can be completed. In addition, some recovered remains possess taphonomic characters that preclude them as candidates for sampling as their condition, based on previous results, leads analysts to presume they will not sequence, or the sequence will return as inconclusive. With this in mind, any additional research techniques that can be employed, are done routinely to assist in the identification process.

To date, there are approximately 1,878 missing in action military personnel from the Vietnam War. The chances that some of these remains have become commingled with non-human bone in the subsequent years is not a rare occurrence. Therefore, the designation of cortical bone fragments into species origin is critical. And, there are still parents, siblings, children, and other family members actively interested in establishing closure (Bunch and Shine 2003; Desher 2003; see also, www.pow-miafamilies.org).

In the CIL JPAC's line of work, the motivation is to recover and positively identify missing persons from various past war conflicts. Those directly affected by the work done are the families of those still missing. There are instances of "bone trading" occurring in Southeast Asia. This phenomenon is when the local indigenous people have inherited the misconception that the US Government pays large sums of money as "reward" for the remains of missing servicemen (Holland et al. 1997; Mann et

al. 2003). Because of this notion, the experiences of forensic anthropologists at the CIL JPAC includes examining a plethora of skeletal, non-organic material, and photographic evidence that indigenous Southeast Asians claim are the remains or personal effects of those MIA. For instance, a bone trader discovers that an excavation is taking place and visits a recovery scene. He states he is in possession of a small amount of human remains or an identification tag (e.g., "dog tag"). A blurry photograph is usually produced showing skeletal remains along with personal effects that appear to be US military issue and of the Vietnam War era. The bone trader will state that the remainder of the skeleton is back in their village, but as a gesture of good will, has a portion of the skeleton to prove that their story is true. When the bone sample is produced, the portion usually consists of a small non-diagnostic cortical bone fragment. While recognized as bone, assigning or estimating a biological profile is not possible. This is frustrating and occurs with regular frequency. In other situations, bone samples are produced but are morphologically distinct so that species origin can be assigned. These examples demonstrate that the ability to substantiate claims from local people asserting they possess remains of missing service members is a difficult task. Of course, all claims are fully investigated and all leads are exhausted in the endless quest to find and repatriate MIAs. The stated mission of CIL JPAC is: "To conduct global investigation, recovery, and

identification operations to achieve the fullest possible accounting of those missing as a result of service to our nation" (www.jpac.pacom.mil).

To this end, this research will histologically evaluate the cortical bone anatomy of five Southeast Asian mammals and two North American mammals to establish possible genus/species designation. A visual guide will present the histological subtleties and nuances that characterize these non-human mammals from human.

Southeast Asian mammals

Throughout Southeast Asia, various mammalian species live and thrive (Francis 2001; National Research Council 1983; Van Peenen et al. 1969). Some mammals are domesticated and others feral. Some are indigenous to the region while others are introduced. Because of the abundance of such a varied and numerous fauna, it is not surprising that skeletal remains of many different mammals may be inadvertently recovered during bioarchaeological excavations performed by the CIL JPAC. Of course, depending upon excavation location, the mammalian fauna may vary. Obviously, certain rural areas support more varied and abundant species than urban settings. However, in the 30 years since resolution of the inter-governmental conflict, many rural areas may have become closer to urban dwellings. For Cambodia, Laos, and Vietnam the most common local

mammals include cow, horse, goat, deer, pig, dog, cat, monkey, water buffalo, and porcupine (Francis 2001).

A working knowledge and general understanding of animals encountered in specific regions is important. It aids in identification of zooarchaeological osseous elements and fragments recovered. In some instances though, commingled assemblages of fragmented human bone and fragmented non-human remains causes problematical concerns during forensic analysis at the CIL JPAC.

CHAPTER 2 LITERATURE REVIEW

The history of studying bone and understanding the microstructural, biochemical/physiological, and mechanical complexities surrounding it is vast. Historically, such a study is part of the broader development of human anatomy as a parallel endeavor to the practice of medicine (see Persaud 1997). The earliest anatomical studies of human skeletal structure paved the way for understanding, studying, and undertaking research in the many levels of bone biology. With the advent of microscopy, studying histological skeletal anatomy at the cellular level became possible (see Martin et al. 1998; Persaud 1997; Schultz 1997a, 1997b; Stout 1982). The histomorphological study of bone in anthropology has a young history (see Bergman et al 1996; Berman 2003; Junqueira et al. 1971).

Discerning histological differences in bone structure within and between species is a complex endeavor. Early zoological studies have focused on the simple structural differences. Quekett (1849) utilized the microscope to document and compare differences in bone between four classes of animals, namely, mammals, birds, reptiles, and fishes. Kolliker (1857) examined microscopic differences in fish skeletal morphology. While rudimentary, these early studies paved the way in histological examination of bone. In the 1950s, Enlow and Brown (1956a, b, and c) examined histomorphology of non-human animals specifically examining and discussing characteristic, distinct

non-human histomorphological structures, primarily the presence of plexiform bone. Singh and colleagues (1974) reported on examinations of sections of ribs, tibiae, and femora comparing humans, lab rodents, and various animals from the Bronx zoo. In 1993, Harsanyi continued this process in his examination of archaeological non-human histological bone morphology.

Qualifying and quantifying a histological perspective of bone has many applications. For instance, from a clinical standpoint, histological sections of bone augment our understanding of the effects of immobilization, i.e., disuse atrophy, in patients affected by neuro-muscular diseases or trauma that impede or prevent walking (Stout 1982). Additionally, histomorphological examinations have aided in diagnosing skeletal diseases of antiquity (Weinstein et al. 1981). Everything from the anthropological usefulness of palaeohistopathological research in diseases of antiquity (Bell and Piper 2000) to the clinical value of cortical bone remodeling rates in post-menopausal women (Riggs and Melton 1988) have been explored. Bone histomorphology has been employed in bioarchaeological studies. The diverse taphonomic nature of archaeological bone assemblages, leads many researchers to examine changes in the histomorphology of archaeological bone (Garland 1987, 1993; see also edited volume by Grupe and Garland 1993).

From a forensic perspective, histomorphometry relates primarily to age estimation (see Ahlqvist and Damsten 1969; Bouvier and Ubelaker 1977; Cho et al. 2001, Cool et al. 1995; Currey 1964; Eriksen 1991, Kerley 1965, Kerley and Ubelaker 1978, Robling and Stout 2000; Samson and Branigan 1987; Singh and Gunberg 1970, Stout 1992, Stout and Paine 1992, Stout and Stanley 1991, Stout et al. 1994, Streeter et al 2001). One of the most important publications on age estimation from bone histology is Kerley's (1965). This method uses thin sections cut from femur diaphyses to identify osteon formation morphology with quantification of the osteons to estimate age. Kerley and Ubelaker (1978) revisited Kerley's original method to reassess its applicability and to improve the original regression equations. They found that the age estimates garnered from analysis of disrupted osteons in the fibula produced the most accurate aging estimation results.

Finally, the identification of bone fragments as human is another important endeavor. Most of these studies involve forensic situations where anthropology can help solve homicide investigations. For instance, Dix et al. (1991), Stout and Ross (1991), and Stout (2003) discuss circumstances where a murder was believed to have taken place but no body was recovered. Among the circumstantial evidence recovered were blood spatter, shotgun pellets, glass, and small bone fragments. In another example, Tersigni (2001) examined human and large canid remains histologically and biochemically to

further differential between human and large dog bone. This research stemmed from a forensic case involving the analysis of a small number of bone fragments depicted on television's *New Detectives* (www.discoverychannel.com).

CHAPTER 3 BONE

The human skeleton contains over 200 bones that are different morphologically. These bones can be termed long, flat, short, irregular, or sesamoid (Bass 1995; Steele and Bramblett 1988; White 2000). Bone, with its unique properties, serves many functions. For instance, it protects the internal organs, it gives form and rigidity, it supports musculature (providing areas for origin and insertion of muscles), it produces red blood cells (i.e., hematopoeisis); and it helps with calcium metabolism (Carter and Beaupre 2001; Martin et al. 1998; Scheur and Black 2000). With so many functions, understanding bone on all levels is central to those working with it on a daily basis. Bones and the human skeleton are studied on several different levels, from gross morphology to microstructure. There are six specific skeletal system functions: support, protection, movement, mineral storage, production and storage of blood cell-producing cells, and storage of energy (Alberts et al. 1998; Gray 1992; Tortora 1991). The skeleton protects internal organs, that is, the skull protects the brain and the rib cage protects viscera like the heart, lungs, stomach and liver. Hematopoesis and calcium metabolism are essential for life. Bones aid cellular function by storing the minerals essential for the cell's survival. Due to the variety of function that bones perform, it is intuitive that various bones will have different structural components based on function. Bone provides the supportive structure to

which soft tissue of the body attaches. As a support system, bone is a remarkably hard substance yet is flexible enough to withstand a significant amount of compressive, tensile, and shearing stress (Martin et al. 1998). For this reason, bone is not static but instead a living tissue that can adapt in form to the mechanical and biomechanical stresses placed upon it (Carter and Beaupre 2001).

Much of this adaptation takes place at the molecular level. At this level, bone is comprised of both organic and inorganic components. As such, this unique union of compounds makes bony tissue into a composite material with both strength and flexibility (Carter and Beaupre 2001; Martin et al. 1998; Schwartz 1995). Simplistically, most of the organic component of bone is made up of a collagen matrix. This material forms "flexible, slightly elastic fibers in bone" (White 1991:19). These fibers and their actions allow bones to resist fracture when under stress. Additionally, collagen is the most widespread protein found throughout the body. Because of its abundance there is continued flexibility over time. The inorganic material or the mineral component in osseous tissue is termed hydroxyapatite with a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ (Martin et al. 1998; Tuross 2003). Although there are numerous biochemical variations with substitutions for other apatites (see Hillson 1996). The base inorganic mineral ingredient is a

form of calcium phosphate ($CaPO_4$). This material provides the framework for strength to bone.

Both the inorganic and organic matrices work in conjunction with one another to produce and then maintain bone in all the aspects of mechanical and biological strain. Over time, these stressors cause bone to remodel its structure in order to supply the organic and inorganic needs of the functioning human body. This is a continuous process that occurs over the lifetime of the tissue.

Bone formation (ossification)

For purposes of this study, the bony tissue described herein applies to mammalian bone. Mammalian bone comes in two forms, mature and immature. Immature bone develops *in utero* during prenatal life, and because of bone's ability to grow and develop, immature osseous material is replaced throughout life via osteogenesis with mature bone. Immature bone does appear in the adult skeleton but only in times of trauma (e.g., at areas of fracture healing, see Galloway et al. 1999, Sauer 1998) and pathology (e.g., osseous tumors, see Ortner and Putschar 1985; Dorfman and Czerniak 1998).

The stages of pre-osseous development occurs via a process called chondrogenesis, this is the cartilaginous model from which bone arises (Scheur and Black 2000). Chondrogenesis begins when mesenchymal cells (the embryonic connective tissue) travel to areas of future osteogenic activity.

The mesenchyme differentiates into osteogenic cells to begin the production of bone in localized ossification centers (Hall 1988). Throughout growth and development, this cartilage template is slowly replaced by bone.

There are two types of bone replacement: endochondral and intramembraneous. The method for replacement is based upon future function. Endochondral bone forms from a cartilaginous model and is characteristic of long bones of the appendicular skeleton (Scheur and Black 2000; Schwartz 1995). Intramembraneous bone is formed by a matrix of woven fibers that mineralize. These latter bones are neurocranial elements. Osseous formation for these types occur in two types of mature bone: cortical and trabecular.

Though comprised of identical material, cortical (or compact), and trabecular (or cancellous or spongy) have characteristic gross and microscopic structure (Figure 3-1). Cortical bone tissue is dense and forms the rigid outer structure of all bone. Internally, cortical bone contains the following structures: Haversian canals (or osteons), Volkmann's canals, and resorption cavities (Martin et al. 1998; McGowan 1999; Ten Cate 1998). Haversian canals are organized roughly longitudinal to the axis of long bones and contain nerves and capillaries. Volkmann's canals are not as large compared to Haversian canals, run in a transverse manner, serving as connections between Haversian canals. Similarly, blood vessels are contained within

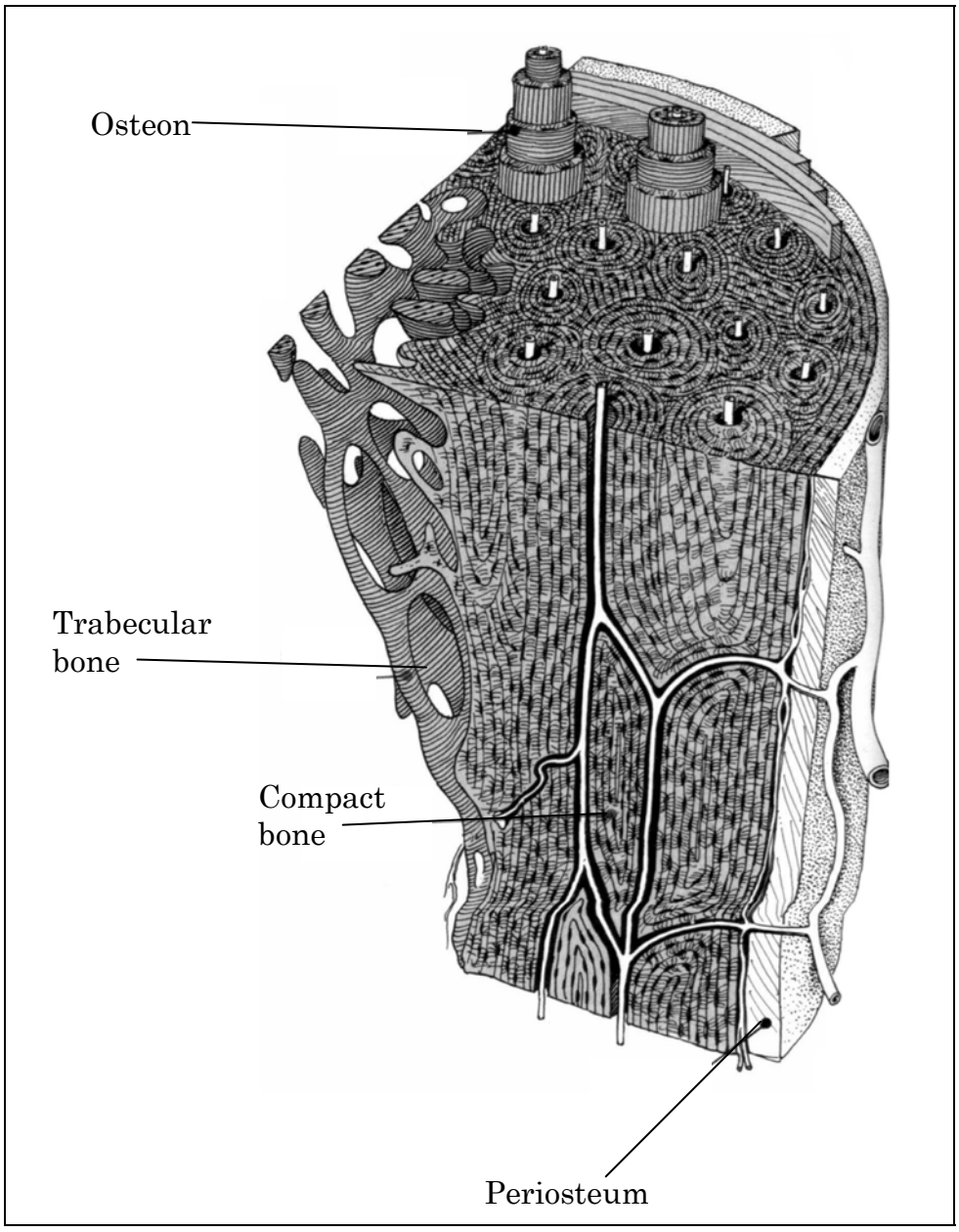


Figure 3-1. Diagrammatic representation of the microstructural components of a human long bone (modified from Dox et al. 1993).

Volkmann's canals (Martin et al. 1998). Resorption cavities are produced by osteoclasts during the primary remodeling stages. Resorption cavities are non -permanent spaces that aptly demonstrate the re-structuring ability of bone. Cortical bone can also be broken down and differentiated by being comprised of either primary or secondary bone (Carter and Beaupre 2001). Primary bone is laid down and formed first on existing bone surfaces (e.g., on the periosteal surface) during normal growth and can be two different layers. These two regions of primary bone are termed circumferential lamellar bone and plexiform bone. Circumferential lamellar bone has lamellae lined parallel to the axis of the bone surface. Within its structure, blood vessels are present and form primary Haversian canals. Plexiform bone is a mixture of woven bone and lamellar bone and is described below. Secondary bone is the bone laid down immediately after the resorption of existing bone.

Trabecular, or cancellous bone is lattice-like or honeycomb in morphology, is located internal to the dense, compact casing and contains marrow to augment red blood cell production (Bergman et al. 1996; Ross et al. 1989). Trabecular bone is primarily found within the epiphyseal and metaphyseal regions of the growing lone bone and the epiphyseal areas of the mature long bones. Also, within the centra, or bodies, of the vertebra, and between the compact layers of membraneous bone (or diplöe). That is, within the proximal and distal regions of long bones, cancellous bone resides. This

differential structure can be observed when the heads of the humerus and femur are dissected. An outer cortex of thin cortical bone is noted, while underneath it, the honeycombed trabecular bone is observed. This duality permits bones to be light while maintaining overall strength and allowing for specialized bone function.

The two types of bone work in a symbiotic relationship with one another. Compact bone withstands mechanical stress while functioning as a protective coating for cancellous bone. This protection is important to allow the less-dense cancellous bone to continue its major purpose of producing red blood cells, e.g., hematopoeisis and fat production in the marrow.

When trabecular and compact bone are viewed at a smaller scale, it is readily apparent that they may contain two types of bone tissue (Martin et al. 1998). These tissue types are known as woven and lamellar bone. Woven bone appears as developing bone and forms quickly, is poorly organized, and not very strong (Scheur and Black 2000). Contrasting woven bone, lamellar bone is highly organized, forms slowly, and is observed in most all mature compact bone of the adult (Martin et al. 1998; Scheur and Black 2000).

As discussed earlier, compact and cancellous bone are living tissues that are modified throughout life based on the stresses they encounter. The very nature of bone is one of a dynamic porous organization (Martin 1998). That is, the porosity will change based on normal mechanical, biomechanical,

or pathological stresses placed upon it. Bone will adapt to these stresses on both gross and microscopic levels. As originally described by Wolffe (1868), this phenomenon, termed Wolffe's Law, states that bones will modify to the stresses and strains that are placed upon them. The law may be summarized as: Form follows function. That is, the morphology of bone will be positively or negatively modified based on overall use and function.

This modification is routinely observed in the micromorphology of the bone. Examining bone microstructure allows insight into the crucial evidence in understanding the mechanical, biological, and pathological stressors affecting the skeletal system and the bony response to these stressors. To this end, the microstructure of bone is outlined below.

Bone histology (Cellular level of osseous tissue)

At the cellular level, bone is a byproduct of three types of cells: osteoblasts, osteoclasts, and osteocytes (Dorfman and Czerniak 1998; Junqueira et al. 1971; Scheur and Black 2000). Bone growth is termed osteogenesis and during this process, cells called osteoblasts differentiate from osteogenic precursor stem cells and appear. Osteoblasts produce bone. They are mononucleated and they secrete osteoid, a super-saturated matrix containing collagen proteins, water, noncollagenous proteins, and proteoglycans that are laid down. This osteoid eventually mineralizes, or

hardens, and becomes mature bone (Dorfman and Czerniak 1998; Martin et al. 1998).

However, osteogenesis is not only a onetime event. Bone continually changes and remodels throughout life. In the beginning of osteogenesis, bone modeling occurs. Throughout life then, bone remodeling occurs.

During the process where remodeling (e.g., throughout the entire growth and development maturity period, at times of pathological insults, during traumatic insults, etc.) bone tissue sometimes needs to be removed, or remodeled. The cells responsible for these actions are called osteoclasts. Osteoclasts are known as resorber cells (Martin et al. 1998) and are large multinucleated cells with ruffled borders that erode through bone tissue via a method of demineralization using acids and then in turn dissolving the collagen with an enzymatic action.

Mature bone material is maintained by cells called osteocytes. Osteocytes are former osteoblasts that have become encased by the matrix they secrete during osteoblastic activity. These enclosed osteocytes then are converted to maintenance functioning cells and maintain serum calcium levels amongst other functions. Osteocytes are housed in areas called lacunae (Figure 3-2). Once there, they communicate with both osteocytes and osteoblasts through tunnels called canaliculi (Martin et al. 1998).

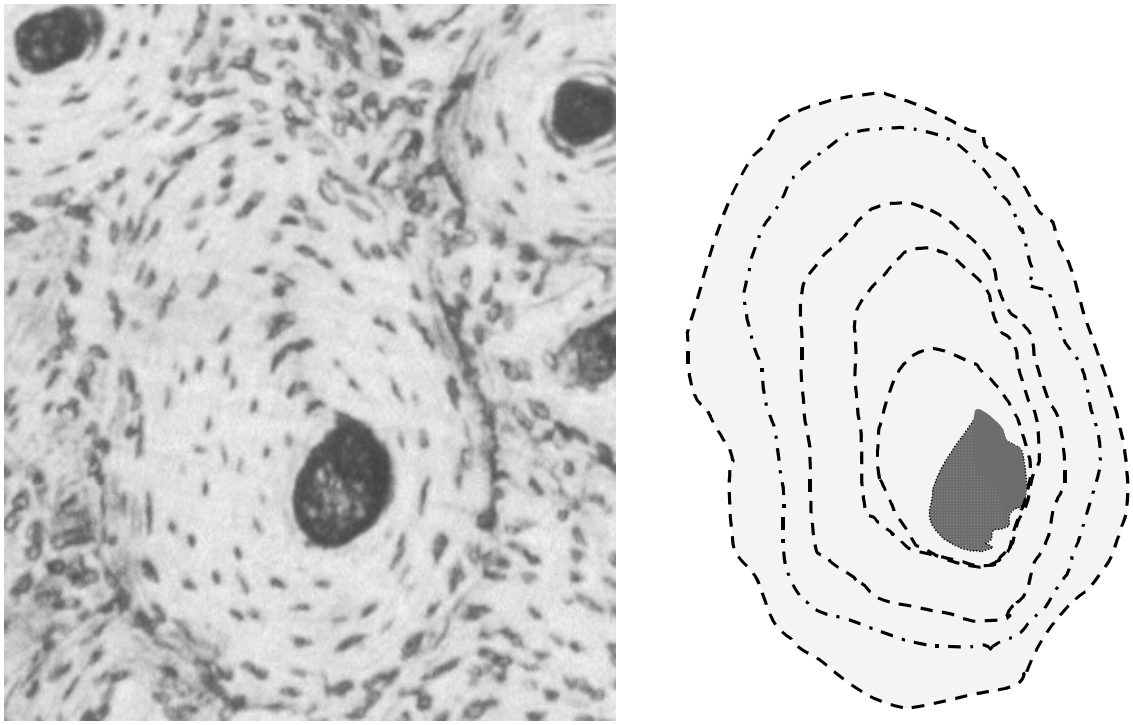


Figure 3-2. Micrograph of human cortical bone (10x) showing osteon and Haversian canal, small black dots are lacunae. Drawing at right is representation of an osteon, darkened area is Haversian canal.

Differences in human versus non-human mammalian bone

Besides gross morphological distinctions, there are subtle differences in both the gross morphology as well as histological levels of human and non-human bone. In non-humans, bone formation called plexiform bone is observed (Figure 3-3). Plexiform bone is characterized by its "brick wall" appearance (Martin et al. 1998; see also "Results"). This is because plexiform bone is a conglomeration of woven and lamellar bone and right angles to each other. The rate of bone formation in increased because trabeculae form and the gaps are filled in.

Figure 3-3. A thin section of goat (*Capra hircus)* bone taken at 5x showing the plexiform structure with its "brick wall" appearance.

CHAPTER 4 MATERIALS AND METHODS

Sample

This research focuses solely on mammal non-human bones from a representative suite of domesticated and feral mammals from Laos, Vietnam, and Cambodia (Figure 4-1). However, future research will include fish, bird, and reptiles from the same biogeographic zone. Human bone samples are from various North American populations.

The non-human mammalian samples consist of 24 long bones that were purchased in various urban markets in Laos, Vietnam, and Cambodia or collected in the field of various areas of Southeast Asia (see Table 4.1; Figures 4-1 through 4-9, and Figures A-1 through A-6). The dog and deer specimen are from the zooarchaeological collection at the University of Tennessee Knoxville and one rhesus macaque was provided by Murray Marks from a captive research colony at the Louisiana State University in Baton Rouge.

The non-human bone from Southeast Asia was purchased fully fleshed so processing was accomplished by removing all soft tissue (Figures 4-10 through 4-12).

Processing the bones was accomplished by boiling them in water. This occurred in various field settings in Southeast Asia with some samples on scaffolding in the field for insects to consume adherent soft tissue.

Table 4-1. Osseous samples.

Common name/Genus species	Bone	Provenience
Water buffalo *Bubalus bubalus*	Femur, Tibia, Radius, Ulna	Siem Pang District, Stung Treng Province, KOC
Cow *Bos* sp.	Femur, Tibia, Radius, Ulna	Stung Treng market, KOC
Pig *Sus scrofa*	Femur, Tibia, Fibula	Stung Treng market, KOC
Pig *Sus scrofa*	Femora (right and left)	Ho Chi Minh Market, SRV
Goat *Capra hircus*	Femur, Tibia, Metatarsal	Svay Rieng market, KOC
Rhesus macaque *Macaca mulatta*	Femur	Vicinity of Xepon, LPDR
Rhesus macaque *Macaca mulatta*	Femur	Marks, personal collection (captive primate collection at Louisiana State University)
Dog *Canis familiaris*	Humerus, Tibia, Fibula	University of Tennessee, Knoxville, zooarchaeological collection
Deer *Odocoileus virginianus*	Femur, Tibia, Radius	University of Tennessee, Knoxville, zooarchaeological collection

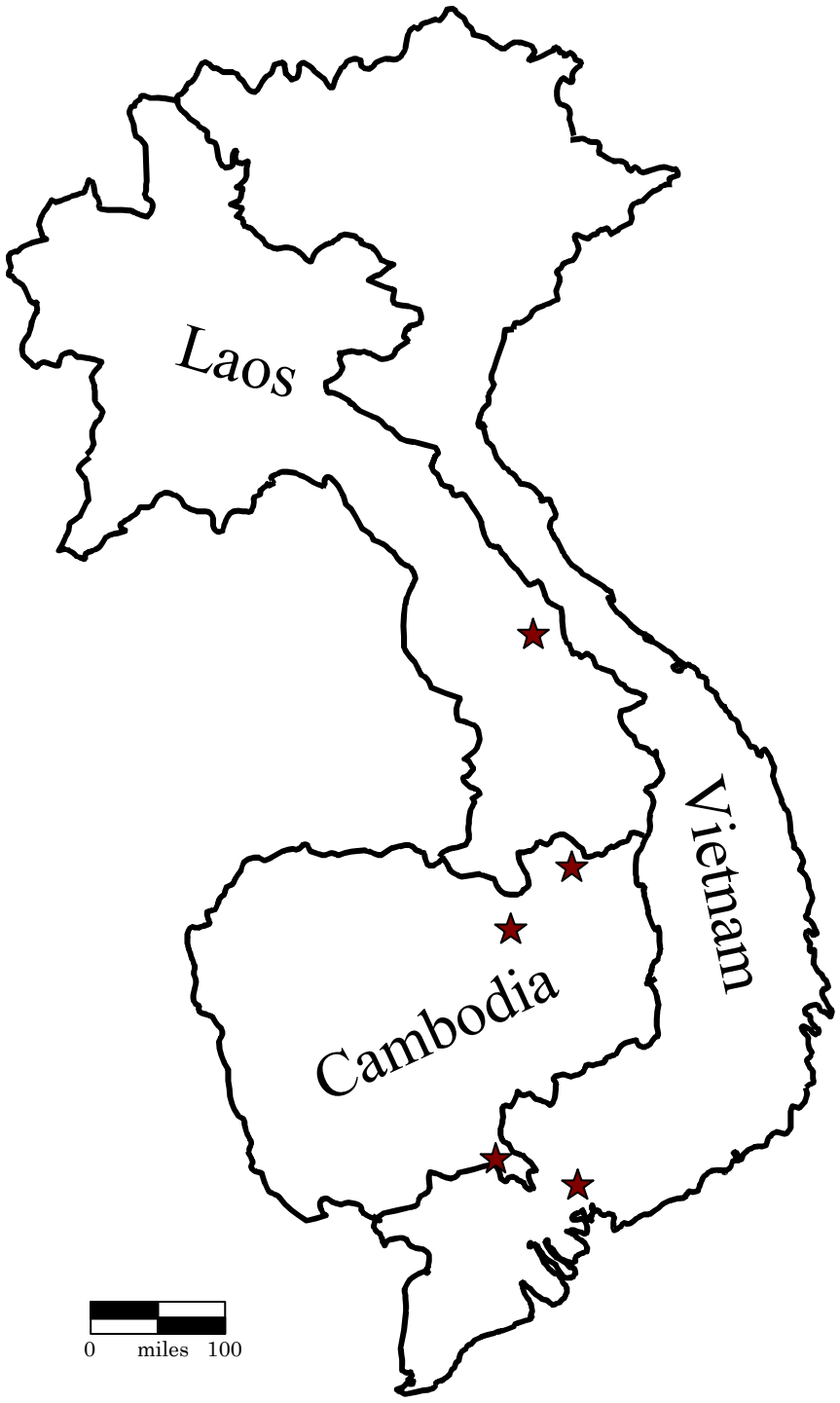


Figure 4-1. Map of Cambodia, Laos, and Vietnam. Red stars depict geographical areas where samples were obtained.

Figure 4-2. Open market for local merchants in Stung Treng, KOC where pig (*Sus scrofa*) and cow (*Bos* sp.) bones were purchased.

Figure 4-3. Merchant stall in Stung Treng, KOC open market where cow (*Bos* sp.) bones were purchased.

Figure 4-4. Merchant stall in Stung Treng, KOC open market where pig (*Sus scrofa*) bones were purchased.

Figure 4-5. Aerial photograph of geographic area near Xepon, LPDR where one monkey (*Macaca* sp.) femur was procured.

Figure 4-6. Aerial photograph of geographic area near Siem Pang, KOC where water buffalo (*Bubalus bubalus*) sample was procured.

Figure 4-7. Bovids (*Bos* sp.) on the streets of Stung Treng, KOC.

Figure 4-8. Water buffalo (*Bubalus bubalus*) in Siem Pang, KOC.

Figure 4-9. Rhesus macaque (*Macaca mulatta)* in Kampong Cham, KOC.

Figure 4-10. Removing soft tissue from water buffalo, photo 1.

Figure 4-11. Removing soft tissue from water buffalo, photo 2.

Figure 4-12. Local Cambodian women processing water buffalo bones in Siem Pang District, KOC.

Materials and methods

Bone samples were sectioned from complete long bones using a Dremel® Multi*pro* variable speed rotary tool (model number 395 type 5) with a Dremel® cut-off wheel (# 409 sand discs). The ventral portion of each midshaft was removed to procure a small sample (Figure 4-13). The average weight of each sample was 35 grams. Once procured, samples were placed into an irregular chuck and cut in a transverse section using a Buehler® IsoMet Low Speed saw (Figure 4-14) at an average thickness of 0.2 millimeters. Examination of the thin sections revealed interesting, yet not unknown, results. Thin sections were examined at 5x, 10x, 20x, and 40x magnifications.

Figure 4-13. Dremel® tool with sanding disc used to remove cortical bone sample.

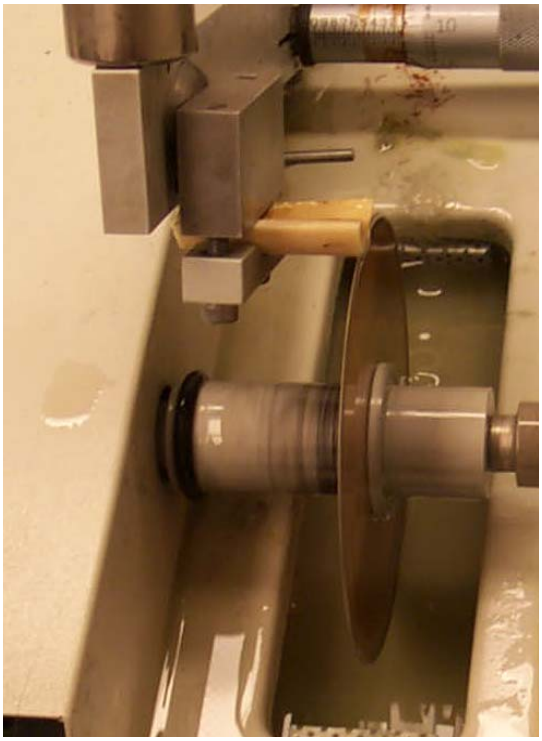


Figure 4-14. Buehler® Isomet™ low speed saw used to make thin sections of cortical bone samples.

The production of thin sections follows a modified protocol of the method that Marks and coworkers (1996) created for dental thin sections and incorporates variations of the methodological procedures outlined by Frost (1958) and Maat et al. (2001). The materials needed in the undertaking of this research are twofold: 1) to produce the thin sections of bone for light microscopy, a Buehler® IsoMet Low Speed oil-cooled petroglyphic thin section saw. A Buehler® Diamond Wafering Blade (Series 15HC Diamond No. 11-4244, measuring 10.2cm x 0.3mm). The blade was lubricated with Buehler® Isocut fluid, and 2) a Leica® DMRX light microscope and ImagePro® Express computer software version 4.5.1.3.

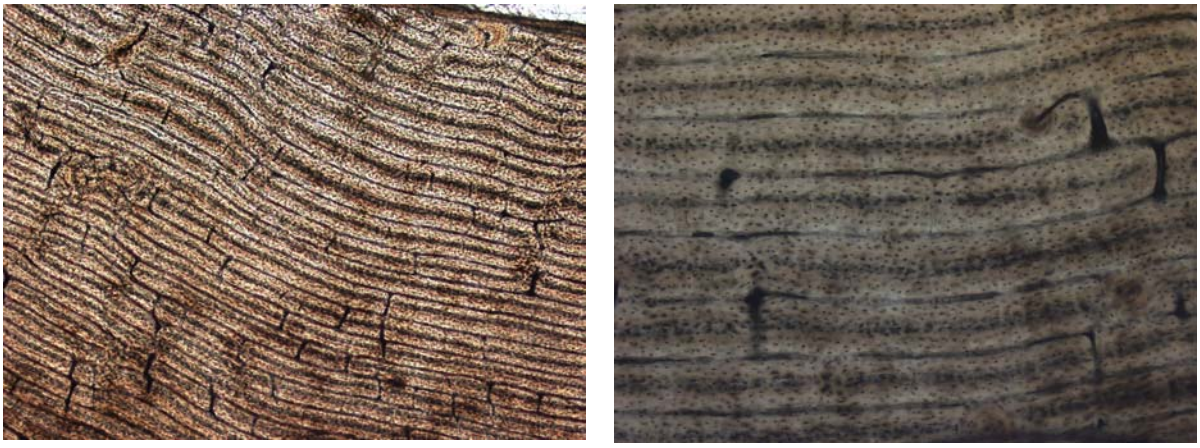
Microscope slides (Buehler® petrographic slides No. 40-8000, measuring 27x46mm), were used to mount sections on slides. After bone thin section samples were produced, they were examined using light microscopy utilizing the procedures of Harsanyi (1993), Rogers (1996), Stout (1988, 1992), and Tersigni (2001, 2002).

CHAPTER 5 RESULTS

Plexiform bone morphology

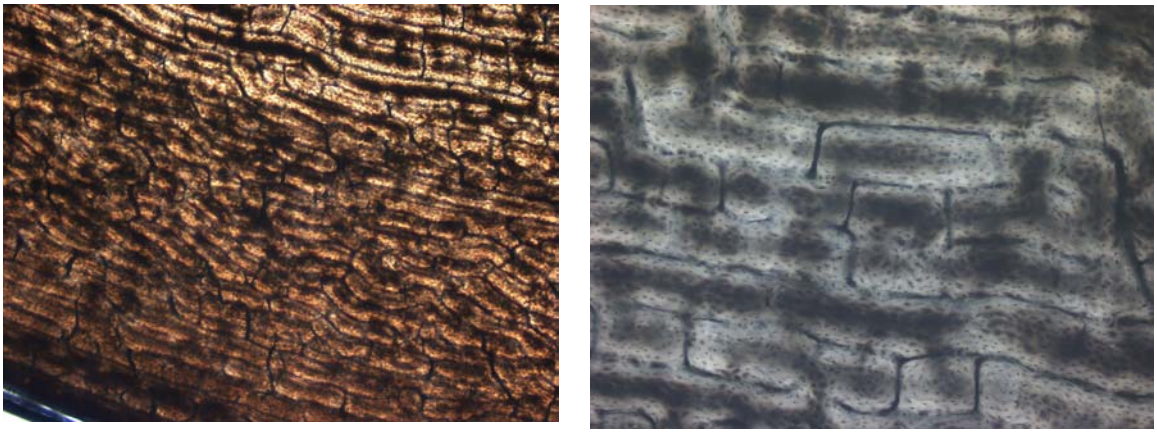
The primary distinguishing histological hallmark in non-human mammals is plexiform bone. This tissue contour is well illustrated in figures 5-1 and 5-2.

Revealed in the micrographs below (Figure 5-1) are the obvious structural nuances characteristic in many non-human mammals, namely the brick wall stacking of plexiform bone described by Martin et al. (1998). Characteristic to goat is this morphology layered throughout the entire thickness of the cortex from periosteum to endosteum with a trace/hint amount of Haversian contour. Detection of this diagnostic feature immediately relegates the sample a non-human category.



a. **b.**

Figure 5-1. Micrograph of goat (*Capra hircus*) tibia at 5x (a) and 10x (b). Note the lamellar brick wall appearance.



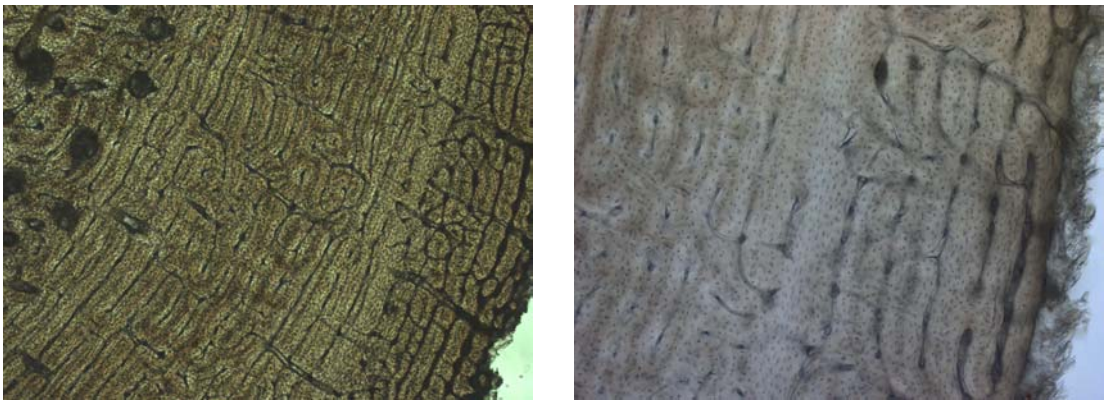
a. b.

Figure 5-2. Micrograph of deer (*Odocoileus virginianus*) tibia at 5x (a) and 10x (b) showing characteristic plexiform bone morphology.

Similarly, deer reveals plexiform bone structure and the brick wall appearance permeates the entire cortical layer from periosteum to endosteum (see Figure 5-2). Some osteons are present, but again, the presence of plexiform bone yields a non-human origin to this sample.

Using the Leica® DMRX light microscope, average measurements were taken of individual layers of plexiform bone for goat and deer at 5x magnification. The individual average size of plexiform bone layers for goat is 119.0069 microns. The individual average size of plexiform bone layers for deer is 99.0049 microns (see Table 5-1). Examination of the micrographs of the pig tibia again reveal the presence of copious plexiform bone (Figure 5-3). Average measurements were taken of individual layers of plexiform bone for the pig tibia at 5x magnification. The individual average size of plexiform bone layers for pig is 118.4666 microns (see Table 5-2).

Table 5-1. Average width of plexiform layers for deer and goat.			
Deer tib5xa	Measurement microns	**Goat tib5xb**	Measurement microns
1	148.4004	1	169.1746
2	117.0688	2	155.5526
3	140.0108	3	141.955
4	124.5028	4	158.084
5	118.2737	5	108.9562
6	103.0719	6	107.3834
7	122.5131	7	114.7859
8	105.1599	8	113.0435
9	99.24098	9	108.9562
10	77.41084	10	99.33617
11	126.7598	11	111.1038
12	64.19488	12	163.3765
13	95.35527	13	78.38155
14	82.03462	14	83.6321
15	78.38155	15	147.1211
16	83.97047	16	138.1077
17	101.78	17	97.22035
18	59.13683	18	97.22035
19	111.7823	19	89.52722
20	114.1253	20	97.22035
21	124.5028		
Total	**2079.103**		**2380.139**
Mean	**99.0049**		**119.0069**

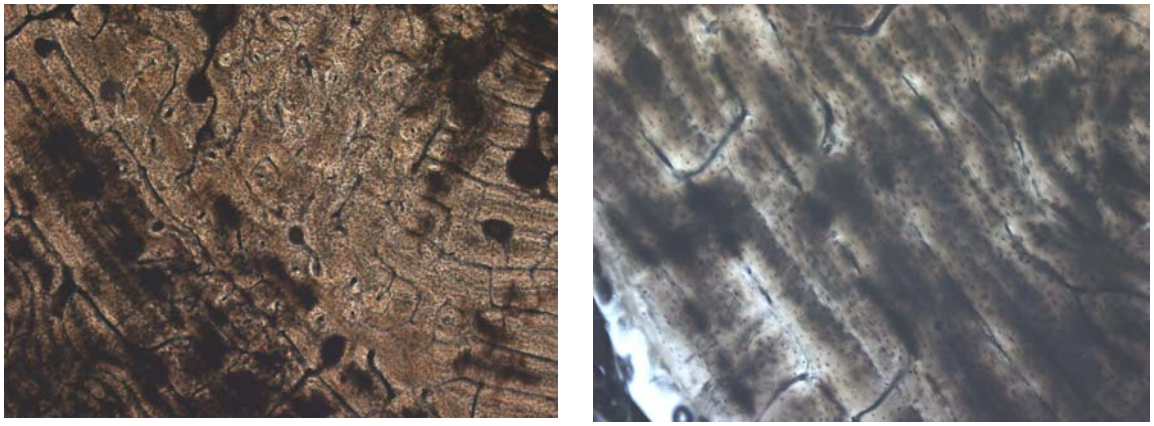


a. **b.**

Figure 5-3. Micrograph of pig (*Sus scrofa*) tibia at 5x (a) and 10x (b) showing characteristic plexiform bone morphology.

Table 5-2. Average width of plexiform layers for pig, cow, and water buffalo.

Pig tib 5xa	Measurement microns	**Cow tib 5x**	Measurement microns	**Water buffalo 5x**	Measurement microns
1	132.7334	1	200.0473	1	184.8721
2	115.7698	2	317.4211	2	171.3397
3	130.2898	3	283.5436	3	147.1211
4	111.1038	4	169.5652	4	155.674
5	126.3865	5	241.177	5	151.4267
6	120.8039	6	163.2028	6	153.7189
7	137.4903	7	126.087	7	141.955
8	124.5787	8	182.6604	8	135.2726
9	95.35527	9	198.482	9	136.1779
10	155.8561	10	221.9096	10	133.3727
11	120.5689	11	207.4237	11	163.3765
12	118.6726	12	229.1185	12	177.0372
13	126.7598			13	153.7189
14	140.2132			14	169.1746
15	158.2633			15	160.3399
16	122.7443			16	110.8483
17	70.24128			17	159.9858
18	68.05424				
19	95.35527				
20	98.09143				
Total	**2369.332**		**2540.6382**		**2605.412**
Mean	**118.4666**		**211.71985**		**153.2595**

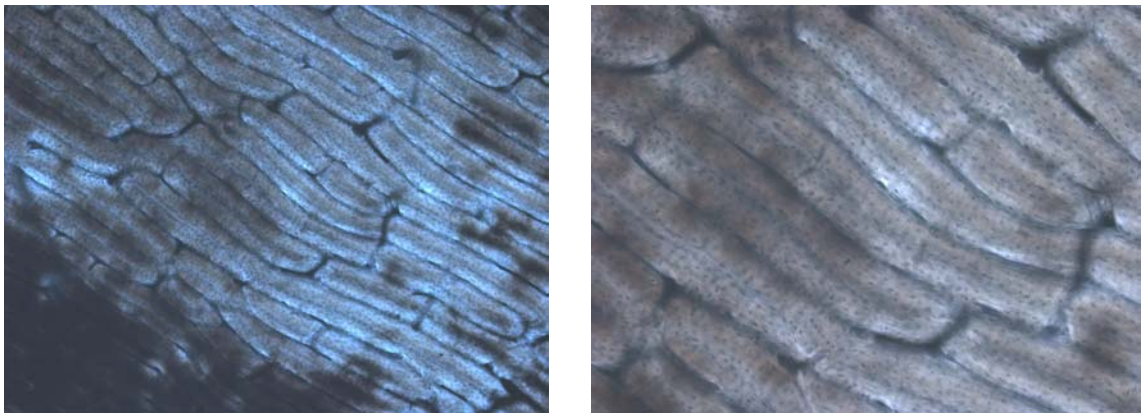


a. **b.**

Figure 5-4. Micrograph of cow (*Bos* sp.) tibia at 5x (a) and 10x (b).

Likewise, examination of the micrographs of the plexiform bone in the cow tibia at 5x magnification allowed for measurement of the width of individual sections of plexiform bone (Figure 5-4). The mean size of plexiform bone layers in the cow is 211.7199 microns (Table 5-2). The micrographs of the water buffalo (Figure 5-5) again, revealed the presence of plexiform bone. Interestingly, areas of Haversian systems are observed as well (see Haversian section below). For water buffalo tibia, measurement of the individual layers of plexiform bone yielded an average size of 153.2595 microns at 5x magnification (Table 5-2).

The criteria for measuring plexiform bone layer width for the above samples began with their examination under 5x magnification. Using ImagePro® Express version 4.5.1.3 measurement software, the individual layers were measured and tallied. Only those layers visible in the field of view were measured. Hence, those samples with larger plexifom band widths



a. **b.**

Figure 5-5. Micrograph of water buffalo (*Bubalus bubalus*) tibia at 5x (a) and 10x (b).

did not have as many measurements (e.g., cow) as those with smaller plexiform layer widths (e.g., pig, deer, and goat).

Statistical analysis of the mean sizes of plexiform width were undertaken using a Mann-Whitney *U* test to compare means of plexiform size among four mammals. This statistical test is nonparametric and is equivalent to the Student's t-test. This test was used instead of a T-test because of the limited dataset, which may account for (or contribute to) the statistically significant differences in the sample variances. The results are presented in the following tables (see Tables 5-3 through 5-8).

The SPSS (1999) statistical computer software package was used to perform analyses. These tables (5-3 through 5-8) demonstrate the statistical analyses of all possible combinations of mammals versus plexiform bone widths. Within the Mann-Whitney *U* test, the "exact significance" section of the table was used to determine whether or not the differences

Table 5-3. Mann-Whitney Test Pig vs. Cow.

Ranks				
	VAR2	**N**	**Mean Rank**	**Sum of Ranks**
VAR1	**PIG**	20	10.90	218.00
	COW	12	25.83	310.00
	Total	32		

Test Statistics(b)	
	VAR1
Mann-Whitney *U*	8.000
Wilcoxon W	218.000
Z	-4.360
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	**.000**(a)
a Not corrected for ties.	
b Grouping Variable: VAR2.	

Table 5-4. Mann-Whitney Test Pig vs. Buffalo.

Ranks				
	VAR2	**N**	**Mean Rank**	**Sum of Ranks**
VAR1	**PIG**	20	12.45	249.00
	Water Buf	17	26.71	454.00
	Total	37		

Test Statistics(b)	
	VAR1
Mann-Whitney *U*	39.000
Wilcoxon W	249.000
Z	-3.993
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	**.000**(a)
a Not corrected for ties.	
b Grouping Variable: VAR2	

Table 5-5. Mann-Whitney Test Pig vs. Goat.

Ranks				
	VAR2	**N**	**Mean Rank**	**Sum of Ranks**
VAR1	**PIG**	20	21.23	424.50
	GOAT	20	19.77	395.50
	Total	40		

Test Statistics(b)	
	VAR1
Mann-Whitney *U*	185.500
Wilcoxon W	395.500
Z	-.392
Asymp. Sig. (2-tailed)	.695
Exact Sig. [2*(1-tailed Sig.)]	**.698**(a)
a Not corrected for ties.	
b Grouping Variable: VAR2	

Table 5-6. Mann-Whitney Test Cow vs. Buffalo.

Ranks

	VAR2	N	Mean Rank	Sum of Ranks
VAR1	COW	12	21.42	257.00
	BUFF	17	10.47	178.00
	Total	29		

Test Statistics[b]

	VAR1
Mann-Whitney *U*	25.000
Wilcoxon W	178.000
Z	-3.410
Asymp. Sig. (2-tailed)	.001
Exact Sig. [2*(1-tailed Sig.)]	**.000**[a]

a Not corrected for ties.

b Grouping Variable: VAR2

Table 5-7. Mann-Whitney Test Cow vs. Goat.

Ranks				
	VAR2	**N**	**Mean Rank**	**Sum of Ranks**
VAR1	**COW**	12	25.75	309.00
	GOAT	20	10.95	219.00
	Total	32		

Test Statistics([b])	
	VAR1
Mann-Whitney *U*	9.000
Wilcoxon W	219.000
Z	-4.323
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	**.000**([a])
a Not corrected for ties.	
b Grouping Variable: VAR2	

Table 5-8. Mann-Whitney Test Buffalo vs Goat.

Ranks				
	VAR2	**N**	**Mean Rank**	**Sum of Ranks**
VAR1	**Buff.**	17	25.35	431.00
	GOAT	20	13.60	272.00
	Total	37		

Test Statistics([b])	
	VAR1
Mann-Whitney *U*	62.000
Wilcoxon W	272.000
Z	-3.293
Asymp. Sig. (2-tailed)	.001
Exact Sig. [2*(1-tailed Sig.)]	**.001**([a])
a Not corrected for ties.	
b Grouping Variable: VAR2	

measured in plexiform widths were significant. This is because the exact significance is defined as:

> The significance level based on the exact distribution of a test statistic. When the data set is small, sparse, contains many ties, is unbalanced, or is poorly distributed, it is preferable to calculate the significance level based on the exact distribution (SPSS, v. 10.0, 1999).

In table 5-3, the difference in plexiform bone widths between pig and cow is significant (i.e., exact significance = .000). Which means there are definite differences between the measured widths of pig plexiform bone and those of cow plexiform bone. This significance is observed likewise in tables 5-4 (i.e., pig versus water buffalo), 5-6 (i.e., cow versus water buffalo), 5-7 (i.e., cow versus goat), and 5-8 (i.e., water buffalo versus goat). In table 5-5 (pig versus goat), the exact significance value is .698, meaning there is no significant difference in the widths of either pig or goat.

Along with the Mann-Whitney *U* test, a box plot (i.e., box and whiskers) graph was produced showing the distribution of plexiform bone widths (Figure 5-6). The box plot graph shows the distribution of plexiform bone widths as they were recorded for pig, cow, water buffalo, and goat.

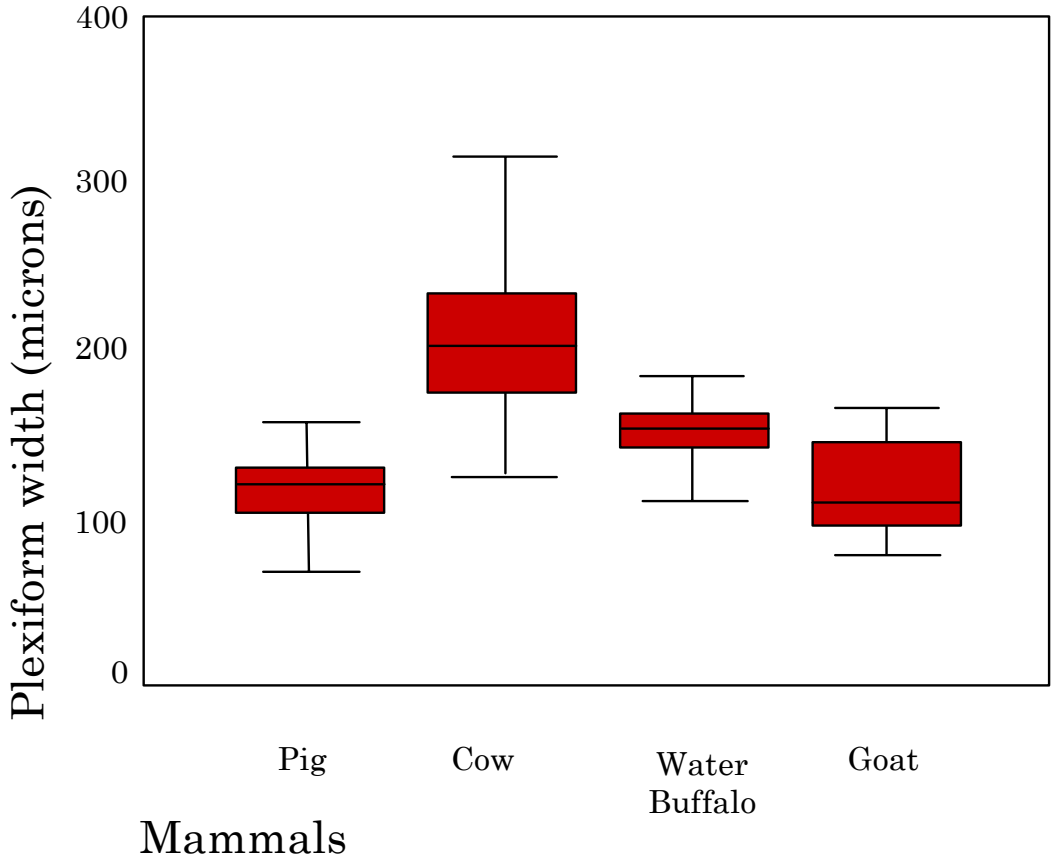


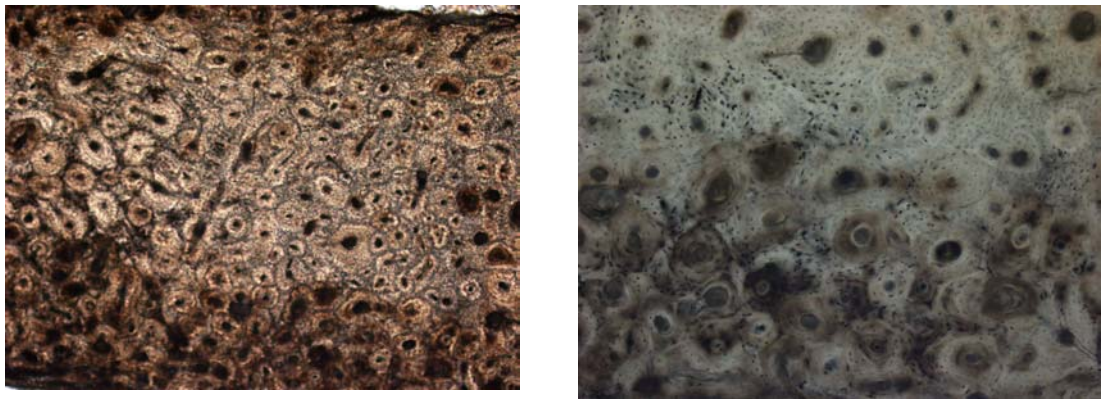
Figure 5-6. Box plot graph of plexiform width among four Southeast Asian mammals.

Haversian system and plexiform morphological considerations

Haversian system morphology makes up mature, or lamellar, adult human bone (Scheur and Black 2000). In some instances, Haversian systems are readily apparent in non-human mammalian bone. This is characterized by osteon morphology observed in human bone histological specimens. Within this research, most non-human bone samples presented several types of morphologies: complete plexiform bone, mixed plexiform and Haversian morphology, or solid Haversian system morphology (i.e., dog and monkey samples).

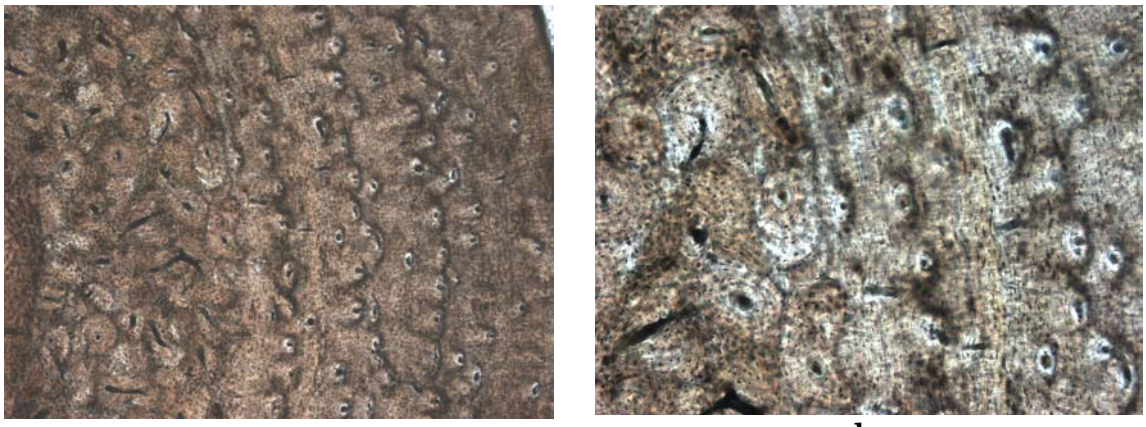
Because some non-human mammals possess Haversian histological morphology, research exists into differentiating between species origin (Lackey 2001; Tersigni 2001; Whitman 2004). In this research, thin sections of dog, monkey, water buffalo, and human Haversian system morphologies were examined (Figures 5-7 through 5-10).

In the case of water buffalo, both Haversian systems and plexiform bone morphologies were observed in the same thin section sample. Observation of plexiform bone immediately yielded non-human origin, but it is included here because of the shear number of osteons observed in portions of the cortical bone not showing plexiform bone layering. The histological morphology of human bone primarily differs from non-human bone in that it does not contain plexiform bone (see Figure 5-10).



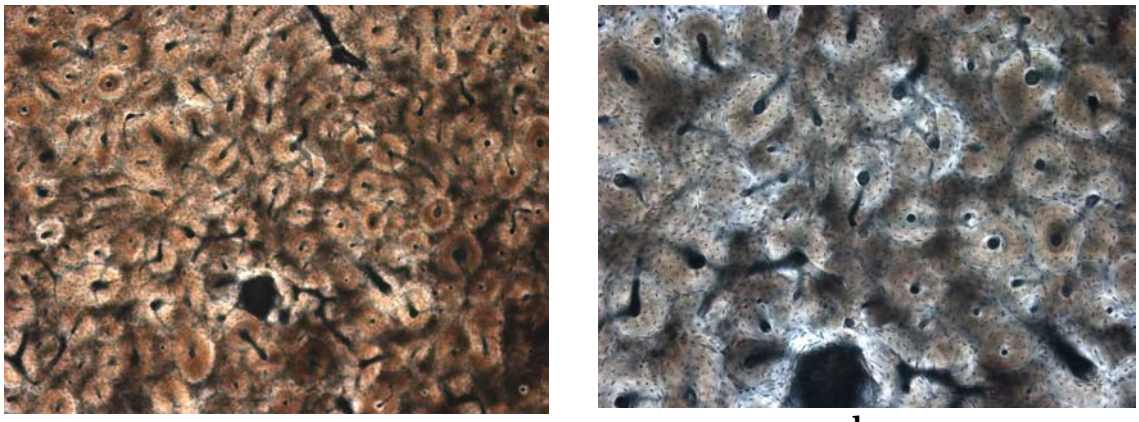
a. **b.**

Figure 5-7. Micrograph of dog (*Canis familiaris*) tibia/fibula at 5x (a) and 10x (b).



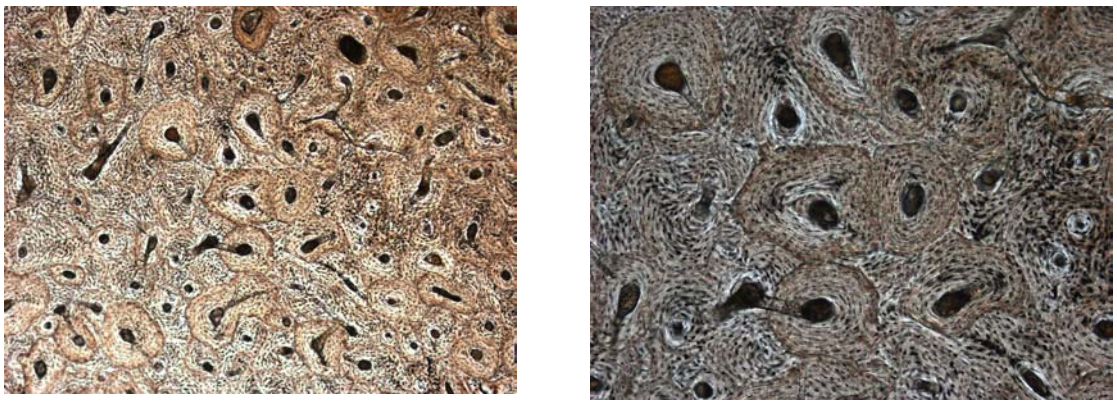
a. **b.**

Figure 5-8. Micrograph of rhesus monkey (*Macaca mulatta*) femur at 5x (a) and 10x (b).



a. **b.**

Figure 5-9. Micrograph of water buffalo (*Bubalus bubalus*) femur at 5x (a) and 10x (b).



a. **b.**

Figure 5-10. Micrograph of human (*Homo sapiens*) femur at 5x (a) and 10x (b).

Human histological morphology includes Haversian systems (i.e., osteons and lacunae) which are located throughout human bone samples from periosteum to endosteum.

Measurements of Haversian canal size in the known human samples shows that on average, human osteons are larger than any of the non-human mammalian samples in this research (Table 5-9.). Mann Whitney *U* statistical analyses were performed on the measurements of Haversian canals in these four species and the results follow in Tables 5-10 through 5-15. The criteria for selection of Haversian canals, included choosing only those canals in the field of view, and only those which were complete. That is, Haversian canals that included a visible Volkmann's canal attached, were not included.

Analyses of the Mann Whitney *U* tests show that there are significant differences in Haversian canal size between humans and the three non-human samples, i.e., dog, monkey, and water buffalo (Tables 5-10, 5-13, and 5-14). Within the non-human sample, Tables 5-11 and 5-12 show significance in dog compared with monkey as well as dog versus water buffalo. There are no significant Haversian canal size differences between monkey and water buffalo (Table 5-15).

A box plot graph (Figure 5-11) of the Haversian canal sizes for dog, human, monkey, and human illustrates the preliminary findings in

Table 5-9. Haversian canal average sizes.							
Dog tib/fib 20x	Meas. microns	**Human fem 10x**	Meas. microns	**Monkey fem 10x**	Meas.microns	**Water buffalo tib 20x**	Meas.micron
1	26.16203	1	53.76512	1	30.18685	1	36.31463
2	27.2242	2	46.01795	2	29.44041	2	37.91163
3	17.11518	3	62.91966	3	18.49351	3	27.75801
4	19.77972	4	82.70551	4	19.51056	4	32.47026
5	18.21998	5	64.97113	5	27.25039	5	23.09606
6	22.11923	6	106.4794	6	32.5396	6	26.75437
7	14.94662	7	84.52651	7	23.36259	7	17.24786
8	14.9847	8	57.51225	8	23.95665	8	16.57385
9	42.20121	9	71.42857	9	26.06406	9	20.50823
10	36.33424	10	78.4016	10	21.75298	10	17.37952
11	19.7581	11	80.87247	11	17.45077	11	17.37952
12	19.81571	12	59.11905	12	11.03684	12	18.24342
13	11.7559	13	86.49888	13	30.08969	13	29.00301
14	13.60948	14	81.24816	14	34.53042	14	30.57186
15	12.90005	15	52.12812	15	31.84835	15	24.76316
16	18.86541	16	84.30451	16	37.80141	16	23.70491
17	17.03173	17	54.11255	17	28.22166		
18	14.42269	18	47.61905	18	34.78389		
19	23.70491	19	58.281	19	21.64502		
20	25.36009	20	75.29233	20	23.80952		
				21	26.01908		
				22	17.11189		
Total	**416.3112**		**1388.20382**		**566.9061**		**399.6803**
Mean	**20.81556**		**69.410191**		**28.34531**		**24.98002**

Table 5-10. Mann-Whitney Test Dog vs. Human.

Ranks				
	VAR00002	**N**	**Mean Rank**	**Sum of Ranks**
VAR00001	**Dog**	20	10.50	210.00
	Human	20	30.50	610.00
	Total	40		

Test Statistics(b)	
	VAR00001
Mann-Whitney *U*	.000
Wilcoxon W	210.000
Z	-5.410
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	**.000** (a)
a Not corrected for ties.	
b Grouping Variable: VAR00002	

Table 5-11. Mann-Whitney Test Dog vs. Monkey.

Ranks

	VAR00002	N	Mean Rank	Sum of Ranks
VAR00001	Dog	20	16.65	333.00
	Monkey	22	25.91	570.00
	Total	42		

Test Statistics(a)

	VAR00001
Mann-Whitney U	123.000
Wilcoxon W	333.000
Z	-2.443
Asymp. Sig. (2-tailed)	.015

a Grouping Variable: VAR00002

Table 5-12. Mann-Whitney Test Dog vs. Buffalo.

Ranks				
	VAR00002	**N**	**Mean Rank**	**Sum of Ranks**
VAR00001	**Dog**	20	15.43	308.50
	Buffalo	16	22.34	357.50
	Total	36		

Test Statistics(b)	
	VAR00001
Mann-Whitney U	98.500
Wilcoxon W	308.500
Z	-1.958
Asymp. Sig. (2-tailed)	.050
Exact Sig. [2*(1-tailed Sig.)]	**.049**(a)
a Not corrected for ties.	
b Grouping Variable: VAR00002	

Table 5-13. Mann-Whitney Test Human vs. Monkey.

Ranks

	VAR00002	N	Mean Rank	Sum of Ranks
VAR00001	Human	20	32.50	650.00
	Monkey	22	11.50	253.00
	Total	42		

Test Statistics(a)

	VAR00001
Mann-Whitney U	.000
Wilcoxon W	253.000
Z	-5.541
Asymp. Sig. (2-tailed)	**.000**

a Grouping Variable: VAR00002

Table 5-14. Mann-Whitney Test Human vs. Buffalo.

Ranks				
	VAR00002	**N**	**Mean Rank**	**Sum of Ranks**
VAR00001	**Human**	20	26.50	530.00
	Buffalo	16	8.50	136.00
	Total	36		

Test Statistics(b)	
	VAR00001
Mann-Whitney U	.000
Wilcoxon W	136.000
Z	-5.094
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	**.000**(a)
a Not corrected for ties.	
b Grouping Variable: VAR00002	

Table 5-15. Mann-Whitney Test Monkey vs. Buffalo.

Ranks

	VAR00002	N	Mean Rank	Sum of Ranks
VAR00001	Monkey	22	20.36	448.00
	Buffalo	16	18.31	293.00
	Total	38		

Test Statistics(b)

	VAR00001
Mann-Whitney U	157.000
Wilcoxon W	293.000
Z	-.562
Asymp. Sig. (2-tailed)	.574
Exact Sig. [2*(1-tailed Sig.)]	**.589**(a)

a Not corrected for ties.

b Grouping Variable: VAR00002

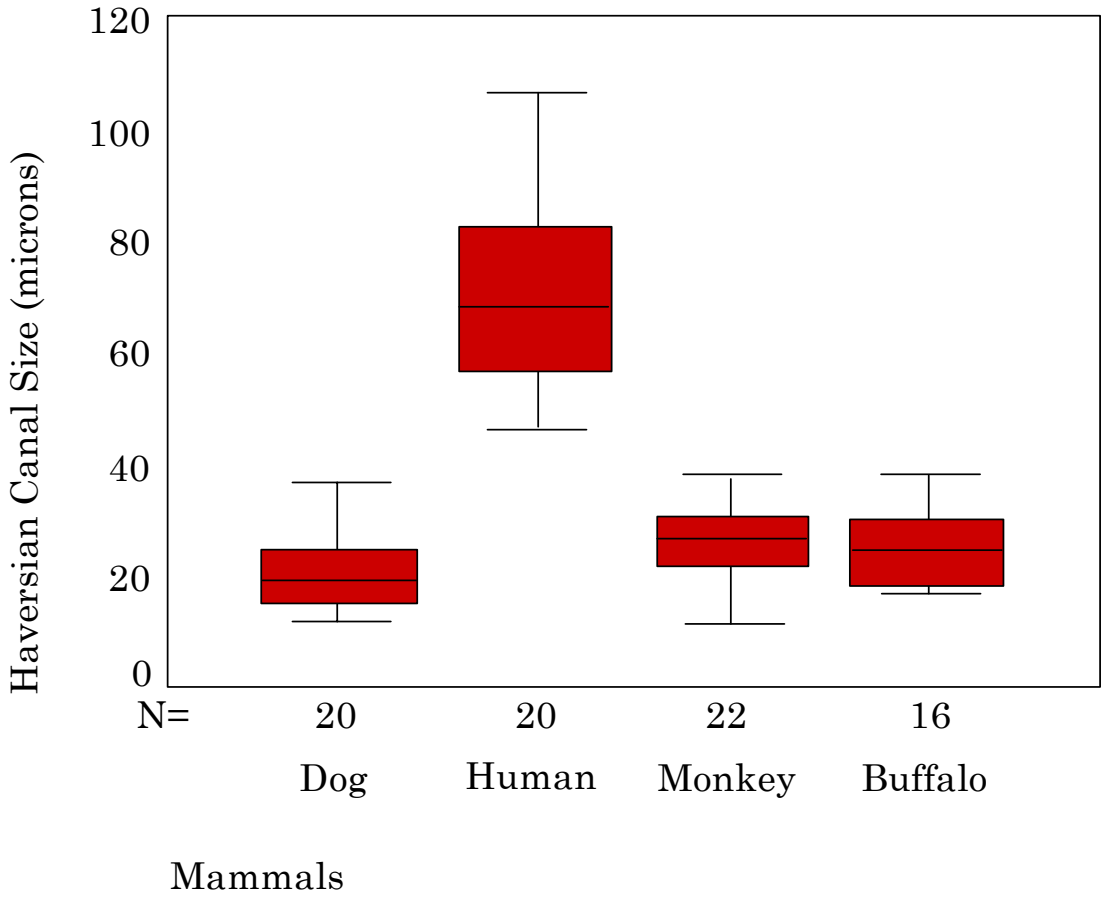


Figure 5-11. Box plot graph of Haversian canal size among dog, human, monkey, and buffalo samples.

size differences of humans compared with the non-human sample. From this graph, human Haversian canal size is set apart from the three non-human samples that were analyzed. These are interesting results and may aid in the analysis of future cases of thin section samples from unknown species.

CHAPTER 6 CONCLUSIONS

Discussion

This study histomorphometrically examines mammalian cortical bone. The emphasis is comparing the structure of large mammals from Southeast Asia to that of human bone morphology. In the course of this study, long bones from seven non-human mammals (i.e., cow, deer, dog, goat, monkey, pig, and water buffalo) and one human were thin sectioned and examined under light microscopy. The morphology of both plexiform and Haversian bone were analyzed. Measurements were taken of the size differences in plexiform bone width and these results were analyzed in a Mann Whitney *U* test to compare mean size differences. The same statistical analysis was used to compare Haversian canal size differences between human, monkey, dog, and water buffalo.

Applicability for CIL JPAC

The research entailed herein arose out of the need at the CIL JPAC to develop a user friendly method to distinguish human from non-human bone. As detailed in the opening chapter, the CIL JPAC receives or recovers copious amounts of bone fragments either through unilateral turnover or during bioarchaeological mission excavations in Southeast Asia. In a majority of the cases, these fragments are morphologically recognized as osseous tissue at the gross macroscopic level. Most are fragmentary portions consistent

with cortical long bones. In some cases, an adequate amount of personal effects (i.e., rings, watches, identification tags, etc.) are recovered in conjunction with the bony fragments. These non-osseous pieces of material evidence positively correlate recovery scenes to incident losses and unaccounted-for individuals, but no morphological skeletal landmarks appear. Thus, by virtue of circumstance, the incident is identified, but without the ability to designate a species origin to the nondescript bone fragments, a problem exists.

Utilizing thin sections of cortical bone and examining them under the light microscope will help remedy some of these issues. Foremost in this study, thin sectioning bone samples and examining their microstructure allows the identification of non-human mammalian bone. This is accomplished by observing plexiform bone. While this does not tell the exact type of non-human species being examined, it immediately answers the question, "Is this bone fragment human or non-human"? From this, anthropologists at the CIL JPAC are able to designate the analyzed fragments and write them up as a non-human bone report: an important distinction in the report writing phase of this humanitarian mission.

The applicability for examining thin sections of fragmentary cortical bone at the CIL JPAC from this research is helpful. In cases where species origin is unknown, the ability to examine the histological morphology of a

bone fragment is warranted. Test cases were examined at the CIL JPAC using fragmentary osseous tissue recovered in the field setting. Material (i.e., osseous) was known, but species origin was not. Thin sections were taken from several cases and examined under a microscope. In one case, the presence of plexiform bone yield instantaneous results: non-human origin.

Problematical are the instances where the histological morphology of the thin sections demonstrate Haversian systems, seen readily in humans and some non-humans (see Chapter 5 above). In these instances, measurements of Haversian systems may allow analysts to draw conclusions of the bone fragment in question. That is, average size of Haversian systems can lead to being able to state that bone fragments are more consistent with human or non-human origin.

Direction of future research

Of course, this research takes into account only a small amount of mammals that are usually encountered during bioarchaeological excavations at CIL JPAC recovery scenes throughout Southeast Asia. Beyond mammals, there are a host of many other classes of animals whose remains or fragmentary remains may be recovered in the archaeological processes of excavations. Future research needs to delve into additional mammals as well as other classes of animals. Other classes of animals include birds, reptiles, fish, and amphibians. Beyond bone, interesting postulations into dental

remains exists as well. For instance in those circumstances where fractured dentitions are recovered (e.g., enamel fragments), is there a way to differentiate human virgin teeth from non-human teeth at the histological level?

From the preliminary findings in this research, questions surrounding origin of some fragmentary remains will get answered. Further research into measurements of Haversian canal morphology is required.

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APPENDIX

Table A-1. Osseous samples gross measurements.

Common name/Genus species	Element	Maximum specimen length
Water buffalo *Bubalus bubalus*	Femur, Tibia♠, Radius/Ulna	310 mm, 315 mm, 313 mm
Cow *Bos* sp.	Femur, Tibia, Radius, Ulna	298 mm, 293 mm, 315 mm
Pig *Sus scrofa*	Femur*, Tibia*, fibula*	145 mm, 122 mm, 109 mm
Pig *Sus scrofa*	Femor	Femur = 155mm
Goat *Capra hircus*	Femur, Tibia, metapodial	159 mm, 185 mm, 98 mm
Rhesus macaque *Macaca mulatta*	Right Femur	208 mm
Rhesus macaque *Macaca mulatta*	Left Femur*	92 mm
Dog *Canis familiaris*	Humerus, tibia, fibula	173 mm, 196 mm
Deer *Odocoileus virginianus*	Femur (right), tibia (right), radius (right)	255 mm, 294 mm, 215 mm
*minus epiphyses; ♠ minus distal epiphysis		

Table A-2. Thin sectioned specimen numbers.			
Species	Element	Sample number	Provenience
Water buffalo	Radius	1	KOC
	Tibia	2	KOC
	Femur	3	KOC
Goat	Left Metapodial	4	KOC
	Left Femur	5	KOC
	Left Tibia	6	KOC
Monkey	Right Femur	7	LPDR
Dog	Humerus	8	UTK
	Tibia/Fibula	9	UTK
Cow	Right Radius	10	KOC
	Right Tibia	11	KOC
	Right Femur	12	KOC
Pig	Fibula	13	KOC
	Left Femur	14	KOC
	Left Tibia	15	KOC
Pig	Left Femur	16	SRV
Deer	Right Femur	17	UTK
	Right Radius	18	UTK
	Right Tibia	19	UTK
Monkey	Left Femur	20	UTK

Figure A-1: Monkey femur from LPDR.

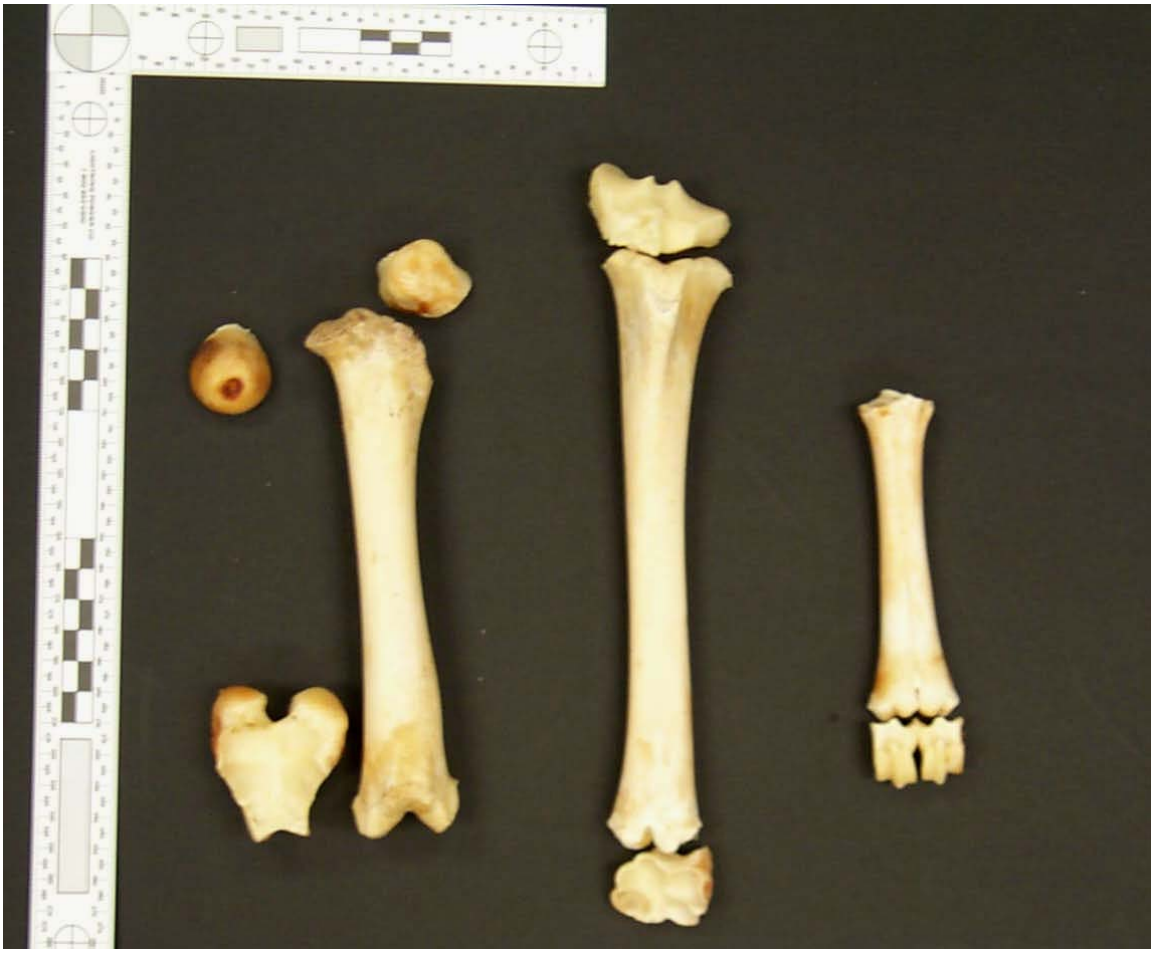

Figure A-2. Goat femur, tibia, and metapodial from KOC.

Figure A-3. Dog humerus and tibia from UTK Zooarchaeological collection.

Figure A-4. Pig femur, fibula, and tibia from KOC.

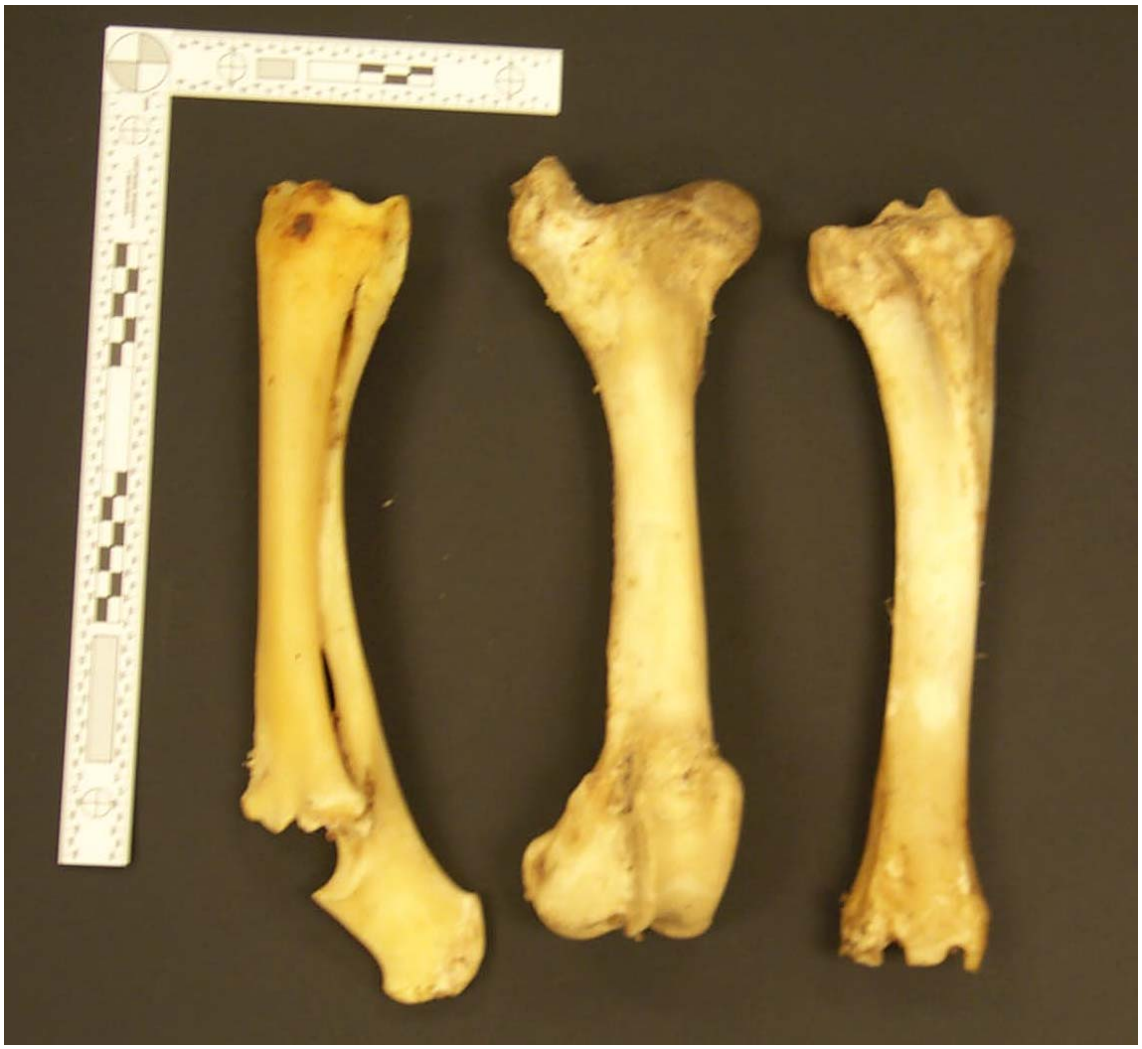

Figure A-5. Cow radius, ulna, femur, and tibia from KOC.

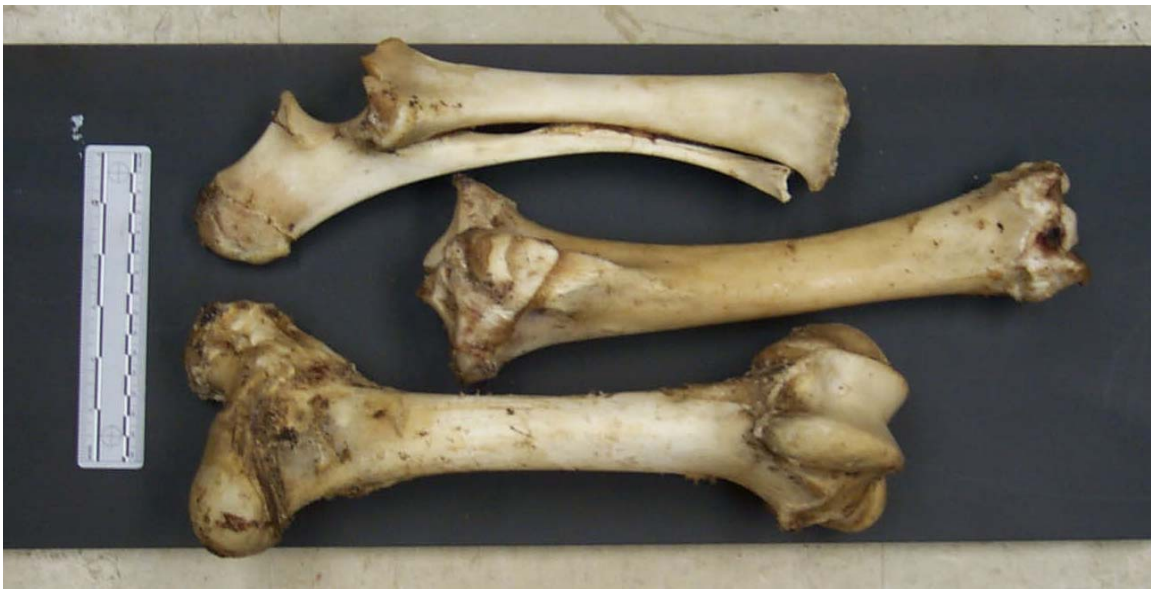

Figure A-6. Water buffalo femur, tibia, radius, and ulna from KOC.

VITA

Derek Christiaan Benedix was born in Baguio City, Benguet Province, Republic of the Philippines on 20 February 1970. In the years that followed he and his family moved, and moved a lot. In fact, they joined the circus. Not really, but after moving from the Philippines to Idaho to California to Idaho to California to Idaho to Tennessee, Derek was tired of moving.

Derek graduated from Filer High School (Idaho) in 1988. In 1992, he graduated from the University of California, Santa Cruz with a Bachelor of Arts degree in Anthropology. In the spring of 1998, he graduated from the University of Tennessee, Knoxville with a Master of Arts degree in Physical Anthropology. He immediately began his pursuit of a doctorate in Physical Anthropology and by the Fall of 2000, finished all of the requirements save for his dissertation. In January 2001, Derek did something he thought not possible, he moved….to Honolulu, Hawaii and began working as a forensic anthropologist for the Central Identification Laboratory Joint POW/MIA Accounting Command located at Hickam Air Force Base, Hawaii. Experiences at the CIL JPAC have led him on many adventures in Thailand, Cambodia, Laos, Vietnam as well as in Papua New Guinea and Australia.