5-2019

Disease and De Soto: A Bioarchaeological Approach to the Introduction of Malaria to the Southeast US

Kelly Marie Schaeffer
University of Arkansas, Fayetteville

Disease and De Soto: A Bioarchaeological Approach to the Introduction of Malaria to the Southeast US

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Arts in Anthropology

by

Kelly Schaeffer
Baylor University
Bachelor of Science in Anthropology, 2017

May 2019
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

Jerome Rose, Ph.D.
Thesis Director

George Sabo III, Ph.D.
Committee Member

Ann Early, Ph.D.
Committee Member

Abstract

It is well known through documentation in historical accounts that numerous diseases were introduced to the Americas during the time of Spanish and French exploration. Diseases such as smallpox, measles and yellow fever have been credited in playing a role in the Spanish conquest of the New World through drastic Native American population decline. Many researchers have studied the biological consequences of European contact, some using direct skeletal analyses to study changes in Native American health and disease. However, one major population disease that has not been part of these discussions is malaria. This is mostly due to the current paradigm in epidemiological history that malaria was first introduced by African slaves during the colonial period slave trades. In addition, the skeletal markers of this disease have not been known until recently. However, recent advances in bioarchaeology have developed a method to identify and diagnose malaria in skeletal remains.

This study investigates the possible introduction of malaria to the Central Mississippi Valley (CMV) and Trans-Mississippi South (TMS) regions by the Hernando de Soto expedition. Numerous factors hint at the possibility that the members of the expedition were the first to introduce malaria to the region during their explorations in AD 1541-1542. These factors include endemic malaria in Spain during the age of exploration, the inclusion of numerous African slaves in the crew, the ability of the *Plasmodium* parasite to lay dormant within a hosts' liver for an extended period of time, and various ecological characteristics of the protohistoric CMV/TMS environment. To investigate this possibility, the newly developed bioarchaeological method for identifying malaria in the skeletal record was employed on Native American skeletal data from 113 CMV/TMS sites spanning temporally from 8,000 BC to AD 1920. Results of this study confirm the presence and increase of malaria indicators in the region during the protohistoric

period, which strongly suggests that members of the de Soto expedition could have been the first to introduce malaria to the region. These results will enhance our understanding of the spread of malaria to the New World, and contribute to studies on European contact with indigenous populations.

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

The introduction of novel diseases to the New World by the Europeans is a major research topic in numerous academic fields. Evidence for disease introduction currently stems from historical records, European exploration journals, Native American accounts, paleopathology, and in certain cases DNA evidence from skeletal remains. One disease that has a 10,000 year history in humans and has largely been left out of this discussion is malaria. This is likely due to the current paradigm that malaria was not introduced to the New World until the arrival of African slaves. However, the sheer complexity of the disease could also play a role in its exclusion. Malaria is a disease that requires many conditions and factors working together both in the human and in the environment to allow successful disease transmission. Because of this, it can be challenging to trace the origin and spread of malaria. This thesis works to bring malaria back into the larger discussion of the effects of European contact with the Native Americans.

Because this discussion is immensely broad, I focus on just one geographical region of the New World; the Central Mississippi Valley (CMV) and Trans-Mississippi South (TMS). It will be established throughout the thesis that the ecological and environmental conditions of this region were ripe for the possible transmission of malaria during the age of European exploration. The first European explorers known to traverse this area were Hernando de Soto and his crew in AD 1541-1542. It will also be established that this crew could have possibly been harboring malaria before and during their journey. Thus, my hypothesis is that the Hernando de Soto expedition could have introduced malaria to the region. I will evaluate this hypothesis with skeletal data collected from Native American sites in the CMV/TMS region.

Chapter 2 provides an overview of the epidemiology and etiology of malaria in humans. I begin by discussing the complexities of malarial infection and transmission, including the life cycle of the *Plasmodium* parasite. A discussion of the *Anopheles* disease vector and its habitat is included as well. I introduce the current research paradigms for the origins of *Plasmodium,* specifically those that focus on its introduction to the New World. I then expand on the initial discussion of the pathophysiology and discuss the relationship between malaria and the skeletal lesions attributed to anemia. Lastly, recent research on the skeletal manifestation of malaria is introduced, and I state that this bioarchaeological method will be used in my study of the introduction of malaria to the CMV/TMS.

Chapter 3 provides an overview of indigenous colonization, and European exploration and colonization of the CMV/TMS. I first present a history of the Native Indians of the region, because the skeletal data that is utilized in this study derives from these populations. Cultural, social, and subsistence changes among these populations are recounted from the Archaic to Historic periods. The second part of this chapter provides an account of European explorations in the Americas, specifically focusing on explorers that traveled to the CMV/TMS area. Because it is the focus of this study, the Spanish expedition led by Hernando de Soto is recounted in greater detail. The subsequent French expeditions in the area 130 years later are reviewed as well. The consequences of European contact on Native populations are then discussed, and mainly include disease and population loss. Skeletal studies of European contact are summarized, and focus on the evidence for decreasing health among Native Americans following contact. Lastly, the hypothesis that malaria could have been one of the diseases brought to the CMV/TMS by the Hernando de Soto expedition is explained.

Chapter 4 provides a list of the CMV/TMS sites with available skeletal data that were chosen for this study. A detailed explanation of the bioarchaeological method that is used in this study for diagnosing malaria in skeletal remains by Nicole Smith-Guzmán (2015) is provided. My data collection protocols are detailed here. Also reasoned here is an explanation of my creation of an 'observable population' for each site. Lastly, my methods of data analyses following data collection are explained.

Chapter 5 presents the results of the skeletal data collection and a discussion of some of the caveats or limiting factors encountered throughout the investigation. I present a table of information for the sites that reported positive malaria indicators, including their temporal affiliation and percent of the observable population that had malaria indicators. Results of the statistical analyses are presented here as well. In my discussion, I draw conclusions about my hypothesis based on these results, and discuss the significance of certain sites showing positive malaria indicators. This thesis concludes with a discussion on the implications of the findings and how they can inform future research on the introduction of malaria to the New World.

Chapter 2: Epidemiology and Etiology of Malaria in Humans

2.1 Epidemiology and Pathophysiology of Malaria

Malarial infection of an individual is caused by a parasite belonging to the genus *Plasmodium* and is transmitted through the bite of a female *Anopheles* mosquito (National Institute of Health 2018). There are four species of *Plasmodium* that are known to infect humans: *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax*. Depending on the species and strain, malarial infections can vary in their virulence, symptoms, and duration (Setzer 2010). *P. falciparum* is said to be the most virulent form of malaria, possessing an increased potential to kill its host (Webb 2009). However, *P. vivax* has also been shown to cause major health complications and death in the past, producing the more common form of chronic malaria (Hume 2003). *P. malariae* and *P. ovale* are less virulent and of minor global importance, causing only a very low percentage of total malarial infections. The most common symptoms of malarial infection include intermittent fevers, headaches, chills, sweats, nausea, respiratory distress, delirium, and severe anemia (Masterson 2014). Intermittent fevers are a diagnostic symptom of malaria, because the occurrence of these cyclical patterns, which occur every 48 hours for *P. falciparum*, *P. vivax*, and *P. ovale*, and every 72 hours in *P. malariae*, correlate with the asexual stage of the *Plasmodium* life cycle (NIH 2018; Masterson 2014).

2.1.1 Life Cycle of Plasmodium

Identification of the organism responsible for malaria and its life cycle has relatively recently occurred, within the last 150 years (Sherman 1998). The *Plasmodium* life cycle takes place in both the human and the female *Anopheles* mosquito, requiring habitation in both organisms in order to undergo sexual reproduction (Cormier 2011). The Center for Disease Control (2018) describes the *Plasmodium* life cycle. When a female mosquito blood feasts on an infected human, she ingests the *Plasmodium* gametocytes. Then, sexual reproduction of the gametocytes takes place within the stomach of the mosquito, producing sporozoite offspring. The sporozoites then travel to the salivary glands of the mosquito. When the mosquito then feasts on another human, the sporozoites are transferred into the human's bloodstream. Once in the bloodstream, the sporozoites travel to the liver and infect the liver cells. The sporozoites then mature into schizonts, which then rupture and release daughter cells called merozoites. The merozoites then travel to the bloodstream, infecting the red blood cells. These merozoites then grow and multiply inside the red blood cells, feeding on the available hemoglobin. Merozoites then rupture out of the red blood cell, releasing themselves and toxins into the bloodstream in periodic waves, which correspond with the cyclical fevers distinctive of malaria. This blood cycle continues until the host dies or recovers. In the event that the host recovers, some of the merozoites differentiate into gametocytes. When a mosquito blood feasts this infected human, she will ingest the gametocytes, and thus begin the cycle again. A pictorial representation of this life cycle is presented below in Figure 2.1.

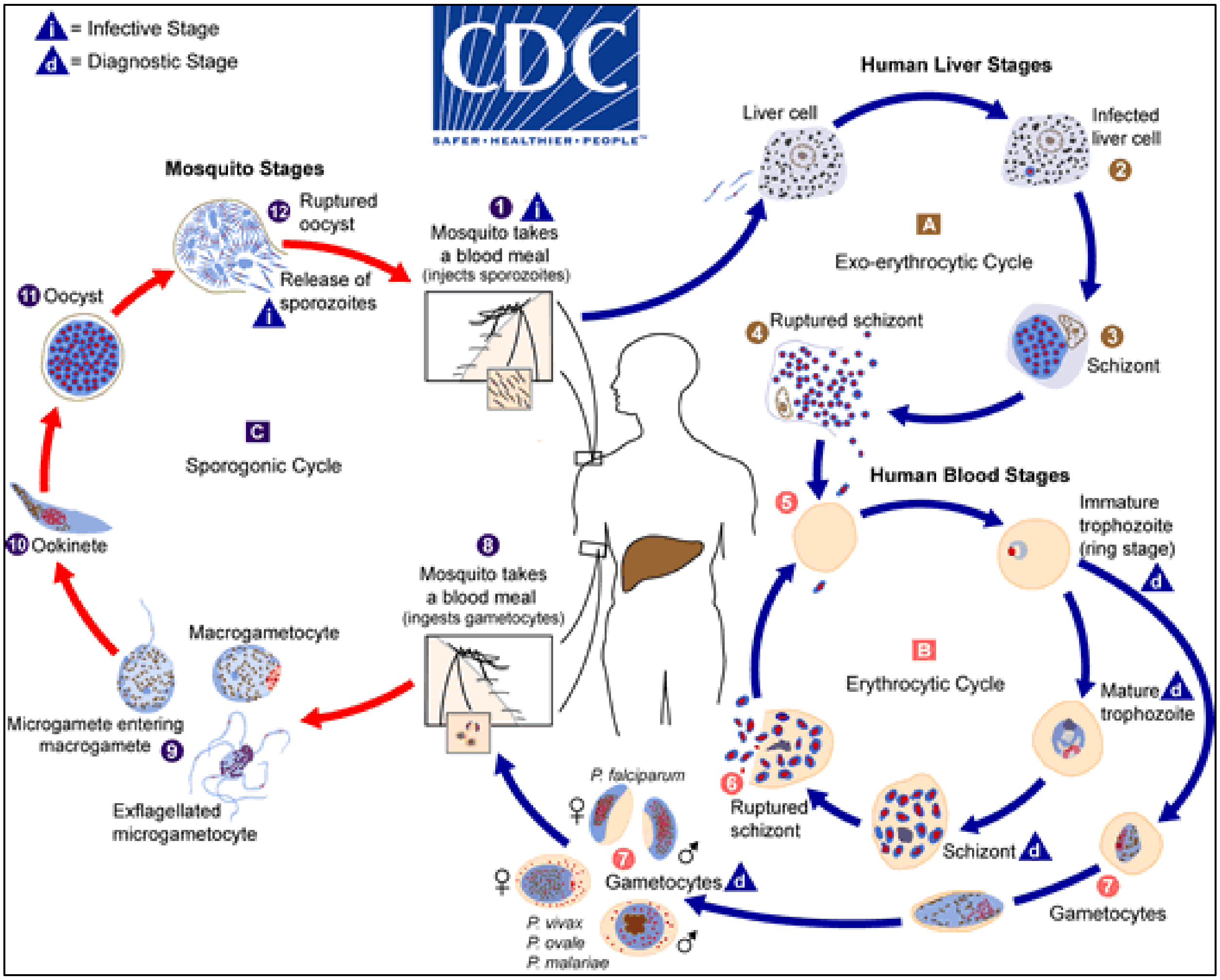

Figure 2.1 Diagram of the life cycle of malaria. Courtesy of the Centers for Disease Control and Prevention (CDC).

There is one important variation, however, in the *Plasmodium* life cycle that is critical to understanding the introduction and transfer of malaria into the New World. That is the presence of a dormant stage called a hypnozoite, which happens between the sporozoite and the merozoite stage (Cormier 2011). In the hypnozoite stage, the parasites can persist within the human liver and if untreated, can eventually invade the bloodstream weeks or even years after initial contraction. This stage seems to involve winter hibernation, where malaria reemerges when the temperature warms and the mosquitoes begin actively biting (Cormier 2011). This dormant stage is unique to *P. vivax* and *P. ovale*, and has not been shown to appear in *P. falciparum* or *P. malariae*. This has implications for the later discussion of how malaria could have traveled to the CMV/TMS region.

2.1.2 Pathophysiology of Malaria

Understanding the pathogenesis of the *Plasmodium* parasite within the human host is critical for the ability to understand and recognize the disease symptoms and manifestations. The clinical manifestation of malaria presents during the blood stage, when the merozoites erupt from the red blood cells and release toxins into the bloodstream (Gilles 1997). This is when the infected individual begins experiencing the classic malarial symptoms previously discussed, the most common including intermittent fevers, headaches, chills, sweats, nausea, respiratory distress, delirium, and severe anemia (Masterson 2014). This is also the stage where recent biomedical research has discovered that free heme, hemozoin, and acid phosphate also get released into the bloodstream (D'Souza et al. 2011; Moreau et al. 2012). An influx of heme and hemozoin into the bloodstream can lead to weakened bone formation and a chemical imbalance that favors bone resorption (Moreau et al. 2012). In addition, acid phosphate is a known osteoclast stimulator, and when released into the bloodstream can also affect rates of bone remodeling and/or bone resorption (D'Souza et al. 2011). According to this research, malarial infection appears to have a negative, resorptive effect on the skeleton. Skeletal evidence of malarial infection and its relationship to skeletal anemia will be discussed below.

2.1.3 Mosquito Vectors and Their Habitat

In order to understand transmission of malarial infection, it is also crucial to understand the habitat of the *Anopheles* mosquito. The *Anopheles* mosquito serves as the vector in disease transmission, first acting as a host to the *Plasmodium* parasite, and then transferring the parasite through the bite of a female mosquito, as previously described. Currently, there are almost 500 species of *Anopheles*, 30-40 of which have proven to be capable vectors for the *Plasmodium* parasite (WHO 2018). *Anopheles* mosquitoes are drawn to moist, wet, and humid areas near water sources, because the females prefer to lay the larvae onto the surface of the water in preparation for hatching (NIH 2018). Therefore, *Anopheles* mosquito larvae are typically found in fresh or salt-water marshes, temporary or small pools of water, mangrove swamps, the edges of streams or rivers, and wet cultivation fields (WHO 2018). The development and eventual hatching of the larvae are dependent on surrounding environmental conditions, especially temperature. The developmental and reproductive timeline of *Anopheles* can also be affected by human-induced changes such as urbanization and deforestation (Afrane et al. 2012).

2.1.4 Geographic Distribution of Malaria

The four species of *Plasmodium* that infect humans have been and continue to be a global concern. However, *P. falciparum* and *P. vivax* have had the greatest impact on human health globally and are therefore most important in this study (Webb 2009). Malaria is most commonly found in tropical and subtropical areas, in which the year-round climate is characterized by warm temperatures and ample rainfall, causing high humidity (CDC 2018). This type of climate is mostly found in equatorial areas and is ideal for the development and reproduction of both the *Plasmodium* parasite and the *Anopheles* mosquito However, while falciparum malaria can only exist in temperature above ~66°F, vivax malaria is able to persist in slightly cooler temperatures, anywhere above ~59°F (Webb 2009). This is why *P. falciparum* is often referred to as "tropical malaria," and is mostly concentrated in Sub-Saharan Africa. On the other hand, *P. vivax* can be found in a wider range of areas, and can arise during the summer months in locations further away from the equator (Cormier 2011; Sherman 1998).

These climatic conditions can be found in the Southeastern United States, and especially in the CMV/TMS region. There are eight to ten months out of the year that the temperature can reach above 59°F, which enables a long breeding season for *Anopheles* mosquitoes, and consequently an extended seasonality for malaria (Faust 1951). In addition, the Mississippi River and its tributaries create a topography that is characterized by broad, low, and flat plains that often hold pools of stagnant water following precipitation or river flooding (Maxcy 1923). This environment is ideal for breeding of *Anopheles* mosquitoes. In fact, it has been established that *Anopheles quadrimaculatus* is a dominant mosquito species in the region. Furthermore, this is one of the species known to carry the *Plasmodium* parasite (Maxcy 1923; Faust 1951). These environmental and climatic factors facilitated the high prevalence of malaria in the region beginning in the 1800s with the arrival of African slaves carrying the *Plasmodium* parasite (Maxcy 1923, Faust 1951). In addition, these factors could have contributed to the possible introduction of malaria to the area by the Hernando de Soto expedition in 1541-1542.

Today, malaria is found to be endemic in numerous countries in Sub-Saharan Africa and South-East Asia (CDC 2018). In addition, while not endemic, high occurrences of malarial infection are also found in Central and South America. Malaria has been eradicated in most areas of the world, and is not a concern for North America, Europe, and most of Asia. In endemic areas, efforts are continuously being undertaken to lessen the severity or eradicate the disease altogether (CDC 2018).

2.2 Origins of *Plasmodium* in Humans

The most recent findings on the origin and evolution of *Plasmodium* come from Dorothy E. Loy and colleagues (2017). Phylogenetic analyses employed by the researchers revealed that both *P. falciparum* and *P. vivax* evolved from parasites infecting African apes (Loy et al. 2017). *P. falciparum* is the result of a parasite cross-species transmission from African gorillas that likely occurred within the past 10,000 years (Loy et al. 2017; Sundararaman et al. 2016). *P. vivax* emerged from an ancestral strain of parasites that infected chimpanzees, gorillas, and humans in Africa, sometime between 100,000-200,000 years ago (Loy et al. 2017; Carter 2003).

2.2.1 Introduction of Plasmodium to the Americas

Recent research from Rodrigues and colleagues (2018) included an analysis of *Plasmodium* mitogenomes in human malarias worldwide. They found that American strains of *P. falciparum* were likely introduced via African slaves in waves over three centuries, beginning in the mid-1500s (Rodrigues et al. 2018). This conclusion for the introduction of *P. falciparum* to the New World seems to be agreed upon and supported by many researchers in many different fields. However, the introduction of *P. vivax* to the Americas still remains unclear and highly debated (Loy et al. 2017). Many researchers believe that *P. vivax* arrived in the New World with the exploration and emigration of Europeans to the Americas. This conclusion is based on evidence that malaria was endemic in many European countries including England and Spain in the times before and during exploration (Dunn 1965; Bruce-Chwatt and Zulueta 1980). However, genetic analyses of current American strains of *P. vivax* show that the species has evolved to be distinct from Asian and African strains, which seems to suggest a presence of thousands of years for *P. vivax* in the New World (Carter 2003). Rodrigues and coworkers' (2018) analysis of *P. vivax* mitogenomes shows that *P. vivax* could have been introduced to the New World through Australasian populations prior to European contact, which would explain the genetic diversity seen in modern American *P. vivax* strains. Carter (2003) and Cormier (2011) argue for the Pre-Columbian presence of malaria in the New World as being the result of the emigration of

Southeast Asian peoples to the Americas via either the Trans-Pacific sea-faring route or the Beringia land-route.

2.2.2 Recorded Malaria Episodes in the Americas

Historically recorded malaria epidemics seem to suggest that the disease was not present in the New World before the arrival of the Europeans. The first cases of malaria in the New World were recorded at the Jamestown, Virginia colony in the early 1600s. Malaria infection increased in prevalence and virulence after the introduction of African slaves to the colony around AD 1620 (Humphreys 2001). This seems to support the hypothesis that the European settlers carried *P. vivax* to the Americas, and *P. falciparum* was later introduced by African slaves. A significant malaria epidemic occurred on the northwest coast of the United States in the 1830s. The summer arrival of a European-American ship at Portland, Oregon harbored a single person that was carrying the *Plasmodium* parasite (Boyd 1999). With Portland's well-established *Anopheles* population, this one infected person initiated a five-year seasonal epidemic of malaria. This epidemic proved to be detrimental to the indigenous populations along the northwest coast that lacked immunity. It has been estimated that roughly 87% of the Native population in the area was lost due to this malaria epidemic (Boyd 1992).

The example from the Northwest Coast demonstrates how malaria is spread and gives us information for uncovering past epidemics. First, we see that malaria can be spread by just one infected person that is traveling to a new area, as long as that area is inhabited by a sizable *Anopheles* population. Second, climate is a large factor in the ability of a malaria epidemic to gain foothold in a population, due to the temperature and environmental limitations of *Anopheles* mosquito habitats. The dynamics of malaria are different than other population diseases that were introduced to the Native Americans such as smallpox. Smallpox and similar diseases are spread easily from person to person, while malaria requires a vector and adequate environmental characteristics. Though complex with many factors involved, the example from the Northwest Coast shows that just one person harboring the *Plasmodium* parasite can cause a seasonal epidemic of malaria. This resulting seasonal epidemic also demonstrates the ability of the *Plasmodium* parasite to lay dormant in the human liver for an extended period of time. The parasite will lay dormant in the liver throughout the cold seasons until adequate environmental and temperature conditions are restored. Then, they can be released into the bloodstream, thus activating the malarial cycle again. Therefore, malaria has the ability to remain in the population as long as there are sufficient environmental characteristics and an adequate *Anopheles* population in place. This is unlike smallpox and other acute diseases which eventually end their reign as the non-immune population decreases.

2.3 The relationship between Malaria and Anemia

As discussed above, the release of free heme, hemozoin, and acid phosphate into the bloodstream, in addition to the red blood cell destruction that results from malarial infection can cause chronic anemia in an infected individual. Chronic anemia has been linked to some skeletal lesions identifiable in skeletal remains; specifically cribra orbitalia (CO) and porotic hyperostosis (PH) (Walker et al. 2009). However the specific cause(s) of these lesions on the skeleton are not always clear, and can typically be attributed to multiple factors, and may not even be related in some cases (Rivera and Lahr 2017). The relationship of anemic skeletal lesions and their causes have been a hot topic in bioarchaeological and paleopathological research during the past 60 years.

J.L. Angel was one of the first researchers to suggest malarial infection as being the cause of porotic hyperostosis through his work on Near Eastern archaeological sites (Angel 1966; Angel 1967). He hypothesized that there was a link between the porous lesions (PH) he observed on the crania and the hemolytic anemia that resulted from genetic conditions providing resistance to malaria (i.e. sickle cell anemia and thalassemia) (Angel 1966). The lesions were believed to form as a result of the diploe expansion of the cranium, to allow an increase in red blood cell production in order to compensate for the severe anemia (Zaino 1964). Upon observation of the appearance of these lesions in ancient populations, Angel suggested the presence of falciparum malaria within these populations (Angel 1964; Angel 1966). However, the presence of porotic hyperostosis cannot be attributed to genetic hemolytic anemia at pre-Columbian sites in the Americas, because falciparum malaria was not thought to have existed in the New World prior to European contact (Angel 1966; El-Najjar et al. 1976).

Soon after, theories of the causative agent of porotic hyperostosis shifted toward iron-deficiency anemia. Mahmoud El-Najjar was one of the first researchers to bring attention to the connection between porotic hyperostosis and iron-deficiency anemia, with his work on prehistoric sites in the Southwest US (El-Najjar et al. 1975; El-Najjar et al. 1976). Upon observing higher rates of porotic hyperostosis among these populations, El-Najjar suggested the lack of iron in the diet as being the causative factor, because these populations were largely dependent on maize agriculture (El-Najjar et al. 1976). In addition, nearby populations that were more dependent on meat for subsistence were observed as having a lower frequency of porotic hyperostosis (El Najjar et al. 1976). Thereafter, in investigations of these porotic skeletal lesions, bioarchaeologists started to shift their attention to dietary stress related to agricultural practices, and away from possible presence of malaria in the areas they were studying.

A number of anthropologists have brought attention to the flaws in the iron-deficiency anemia hypothesis. The majority question the attribution of lack of iron in the diet as being the only cause of the cranial lesions, and point toward a multi-factorial etiology that includes other factors such as parasitic and bacterial diseases (Hengen 1971; Lallo et al. 1977; Mensforth et al. 1978; Holland and O'Brien 1997; Walker et al. 2009). An article by Walker and colleagues (2009) offers the main criticism to the iron-deficiency anemia hypothesis. They explained that the bone marrow hypertrophy that produces the porous lesions could not be the result of iron-deficiency anemia, because this type of anemia actually decreases red blood cell production, rather than increasing it. They instead point to hemolytic and megoblastic anemia as the most likely causes in the formation of these porous skeletal lesions. This second type of anemia is seen in individuals possessing genetic disorders that protect against malarial infection (thalassemia and sickle-cell anemia), as well as in individuals actively infected with malaria (Walker et al. 2009).

Another topic of recent debate in paleopathology is the questionable linking of the etiology of porotic hyperostosis and cribra orbitalia. It has long been assumed in the fields of bioarchaeology and paleopathology that these two skeletal lesions have similar etiologies. Because of this, past literature and pathological reports have grouped them together, and often used 'porotic hyperostosis' to indicate the presence of either of these lesions, without specifying 'cribra orbitalia' when the condition was observed on the roof of the eye orbit. Some researchers have suggested they are products of differing levels of severity; specifically that cribra orbitalia is expressed as a preliminary phase to the appearance of porotic hyperostosis on the cranial vault (Stuart-Macadam 1989). Others have suggested different types of anemia producing lesions in either area on the cranium (El-Najjar et al. 1975). More recent research has pointed to the idea that these two phenomena reflect entirely separate conditions (Rothschild 2012; Rivera and Lahr 2017). However, researchers have yet to come to a consensus on the etiology of cribra orbitalia and porotic hyperostosis, but there is evidence for many factors being involved (McIlvaine 2013).

2.3.1 The Skeletal Manifestation of Malaria

In the past, malaria has largely been left out of the discussion and not considered in the diagnosis stage by paleopathologists. This is because many believe that the disease does not present itself on the skeleton, due to its fast acting nature (Nunn and Tapp 2000; Roberts 2000). However, recent research has made strides in proving that this is not necessarily true. Many researchers have provided a link between malarial infection and physical display of skeletal lesions of anemia. Massa and colleagues (2000) tested for immunological evidence of malarial antigens in a sample of ancient Egyptian mummies, and found that out of those that were positive for falciparum malaria, 92% displayed cribra orbitalia and porotic hyperostosis. Nerlich and colleagues (2008) further strengthened this link by finding the coexistence of *P. falciparum* aDNA and skeletal markers of chronic anemia in his sample of ancient Egyptian mummies. Other researchers have taken a more environmental and climatic approach. Gowland and Western (2012) mapped out the distribution of *Anopheles* mosquito populations and concentrated instances of cribra orbitalia across England. They found a positive association between the two, where cribra orbitalia was higher in areas with large populations of *Anopheles* mosquitoes. This also correlated positively with areas with marshy environments, lower altitudes, and higher recording of “fever and ague.” These more recent insights have given support to the hypothesis that malaria does in fact present itself on the skeleton.

The most recent development comes from Nicole Smith-Guzmán's 2015 publication. In her research, she analyzed a modern skeletal sample from Uganda, an area where malaria is known to be holoendemic, and compared the presence and frequency of skeletal lesions to a sample from a malaria-free zone. Five skeletal lesions were identified that occur more frequently in populations that are malaria-endemic; cribra orbitalia, femoral cribra, humeral cribra, vertebral porosity, and periostitis (Smith-Guzmán 2015). With these findings, she also developed a formula for paleopathologists to use when diagnosing malaria in skeletal remains. While this formula will be discussed in greater detail in the Methods and Materials section, essentially an individual is said to be positive for malaria (with 96% accuracy) if they express a specific combination of these lesions.

2.4 Using Skeletal Markers to Explore Malaria in the CMV/TMS

The newly developed method for identifying malaria in skeletal remains by Smith-Guzmán (2015) provides scientists with another avenue for exploring the origin and spread of malaria. This method can greatly assist anthropologists in the study of the appearance and spread of malaria in the New World, specifically in the continental United States. Because of the Native American Graves Protection and Repatriation Act (NAGPRA), archaeologists working in the United States must seek tribal permission to analyze Native American skeletal collections. The method proposed by Smith-Guzmán (2015) is a non-invasive, macroscopic method that requires minimal handling of the bones, and no sample extraction. I imagine that, once this methodology is known to the ancestral Native American communities, research using their skeletal collections will be more likely to get approved.

I will utilize this bioarchaeological method in my investigation of the possible introduction of malaria to the CMV/TMS region by the Hernando de Soto expedition. Numerous bioarchaeological studies on CMV/TMS Native American skeletons, which will be discussed in greater detail later, show increased levels of anemia following European contact. It has been argued that these markers are a result of iron-deficiency anemia brought on by the adaptation to maize agriculture. However, these markers could also be indicative of malaria. This chapter established ecological and epidemiological factors that were in place in the region that could have supported the transmission of malaria in the protohistoric period. The subsequent chapters will present additional lines of evidence that support this hypothesis.

Chapter 3: Exploration and Colonization of the Americas

This chapter first focuses on the cultural and environmental history of the Central Mississippi Valley and Trans-Mississippi South region and its inhabitants. This is important to include because most of the skeletal data that was analyzed for this project comes from these Native populations. Knowledge of the differences in community arrangement, residential location, subsistence patterns, diet and activity, as well as the environment of these populations can influence the way the skeletal data is interpreted. The second part of this chapter presents the history of European exploration in North America, and specifically in the CMV/TMS and surrounding areas. An excellent overview of Native American prehistory in the region is provided by Jeannie M. Whayne and colleagues (2013) in their book *Arkansas: A Narrative History*. Whayne and colleagues (2013) and Milanich (1993) also provide a discussion of European exploration in the region. Biological and cultural effects of European contact are discussed as well, and the usefulness of skeletal data to study the impacts of contact on the Natives of the region is presented.

3.1 Native Indians of the CMV/ TMS

The ancestors of modern Native Americans were Pleistocene Ice Age hunters from Western Europe and Asia who migrated to North America sometime between 17,000-12,000 BC. Warmer conditions during this time caused ice sheet recession along the Bering Strait, revealing a crossable land route from Siberia to Alaska. It is also probable that Paleoindians made it to North America by maritime travel after 14,500 BC. Once in North America, Paleoindians migrated southward in pursuit of hunting megafauna. Paleoindians are first thought appear in the Central Mississippi region around 10,500 BC. For about 2,000 years after their arrival in the southeast, Paleoindians in the area were mainly subsisting on megafauna (mammoths, mastodons, etc.) and therefore were very migratory, often following herds of game. However, around 8,500 BC, Paleoindian subsistence patterns began to shift when the Ice Age megafauna they subsisted on became extinct. They continued and increased their hunting of smaller mammals like deer, elk, and bison, and collected a variety of plant foods, shifting into a hunting and gathering subsistence practice. This change in subsistence patterns resulted in a more sedentary lifestyle, in which they occupied base camps for extended periods of time.

The Archaic period (8,500 to 600 BC) in the LMV was a time of extraordinary change and development. The changes seen in this period are largely the result of climate and environmental change and the Native's response to those changes in efforts to survive in their new environment. Community arrangement in the Archaic was largely characterized by permanent camps surrounded by other specialized sites. This episodically sedentary, not completely permanent, residence lifestyle gave way to population growth and an increase in the number of communities. Population growth increased the need for food, and Natives responded to this need by increasing their use of storable plant foods. In addition, they began to experiment with growing their own plant foods, and established gardens to further increase food supply. This led to the domestication of several indigenous plant species. Increasing complexity of community organization prompted cultural and social expressions, resulting in the formation of regional trade networks.

This population growth and reliance on domesticated plants continued into the Woodland period (600 BC to AD 900). One of the most important advances to come about during this period was the incorporation of maize into the diet around AD 100. The growing importance of maize and other plant-based foods in the diet throughout the Woodland period began to influence settlement and land-use strategies. When considering where to place their villages, local communities began to pay special attention to the location and access of fertile soils with which they could establish productive gardens to cultivate maize and other plant foods. Since the most fertile soils are largely located in alluvial valleys near water bodies, communities began to settle in these areas near the Mississippi and Arkansas Rivers. The Later Woodland period was also characterized by great social and cultural change. An increasing number of communities were influenced by prestige and power structures that emerged as the result of trade networks, ultimately creating hierarchically organized societies. These social and cultural developments gave way to the complex Mississippian cultures that followed.

The Mississippian period (AD 900 to 1600) is characterized by further population growth, the beginning of large-scale agriculture, the emergence of large dense communities, and increasing cultural and social complexity. From AD 1250 and on, Native communities in the area transitioned from a mixed economy to a stronger reliance on agriculture. Maize, beans, and squash made up the majority of their agriculturally produced plant foods, which they also supplemented with meat, fish, birds, turtles, and shellfish. Social stratification and conflicts quickly resulted over the control of agricultural lands and their harvests. Control and distribution of wealth created a settlement landscape comprised of hierarchically arranged towns, in which the large towns that were able to efficiently manage their wealth and resources emerged as leaders over the smaller communities. Trade networks were also apparent during this time period, and occupational specialization within the communities developed to contribute to trade resources. Increasing social and cultural complexity led to cultural differentiation among the communities in the LMV and the appearance of diverse Native tribes like the Caddo, Natchez, and Tunica Indians. In addition, the arrival of the Quapaw Indians to the area in the Late Mississippian period added to the culturally diverse landscape of the CMV/TMS.

The Protohistoric period (AD 1492 to 1686) was characterized by increasing encounters with European explorers. After these encounters, Native American presence in the region began to diminish. Many Natives succumbed to diseases and/or violence that resulted from contact with the Europeans. Little is known about the cultural landscape of the CMV/TMS in the 130 years between de Soto's expedition (AD 1541-1543) and the French explorers' arrival in 1673. However, it has been established that the French explorers observed a profoundly different landscape upon their arrival than what had been described by de Soto. The French did not observe nearly as many individuals in the area, suggesting significant population loss following the de Soto expedition (Kelton 2007). However, Europeans may not be wholly to blame. Climate reconstructions for Arkansas show an onset of drought conditions that appeared in the mid to late sixteenth century (Stahle 1985). This likely led to social and cultural stresses associated with the decline of available foods and agricultural productivity. A combination of these factors resulted in population decline or migration out of the area during the protohistoric period.

Though little is known about the cultural landscape of during this period, it is apparent that in addition to population decline, population relocation and reorganization was also taking place. The de Soto expedition observed many highly populated areas mainly along the Mississippi, while the French observed smaller, more dispersed settlements concentrated further away from the Mississippi (Burnett and Murray 1992). Because of this relocation and reorganization, a few societies in the region were able to persist into the Historic period. The most notable of these include the historic Caddo in East Texas, Natchez communities in Mississippi, and the Quapaw in the Arkansas River Valley who probably migrated to the region after 1600. But, even if communities persisted, they ultimately lost the social and cultural complexity that was found among their Pre-Columbian ancestors.

3.2 European Exploration in the Americas and the CMV/ TMS

3.2.1 Early Explorations

The first known European explorer to set foot in North America was Leif Erikson. The Viking explorer from Iceland landed on an island off the coast of Canada called Newfoundland in ca. 1000 AD (Crosby 1986). In the following years, more Norsemen traveled to Newfoundland in hopes of establishing a settlement there; however, the settlement did not persist. Nearly 500 years later is the next documentation of European contact in the New World, when Christopher Columbus arrived in the West Indies in 1492. Over the following ten years, Columbus completed four voyages, visiting the islands of Cuba, Hispaniola, and Jamaica, as well as Venezuela, and several Central American countries. After Columbus' expeditions, the Spanish increased excursions to the New World and subsequently built their empire in the Caribbean and Central America, marking the beginning of the Spanish Conquest. Most notably, the arrival of Hernán Cortés and his men to Mexico in 1519 brought on devastating effects for the indigenous populations. In a matter of two years, the Spanish had taken control of Mexico but had also destroyed the Aztec capital of Tenochtitlan, killing hundreds of thousands of indigenous people via both violence and transmission of diseases (Crosby 1986).

The first European explorer to reach the continental United States was Juan Ponce de León in 1513 (Milanich 1993). He landed on the east coast of Florida somewhere near present-day San Augustine, and later explored up and down the east coast. In the years following, numerous other Spaniards sought to explore the coasts of La Florida. In 1526, Lucas Vásquez de

Ayllón attempted to establish the first settlement on the Atlantic coast of La Florida, but was unsuccessful. The first inland expedition of Florida began in 1528 and was led by Panfilo de Narváez (Milanich 1993). After arriving on the coast near present-day Tampa Bay, the men traveled north into the mainland, and eventually to the territory of the Apalachee Indians in northwest Florida. They stayed here for about twenty-five days before heading west to Aute, at the mouth of St. Marks River. The expedition went awry here, as many men fell ill. Attempts to finish the journey to New Spain (Mexico) were by boat, but quickly failed. One survivor of the crew, Cabeza de Vaca, was held captive by Native Americans in Texas for nearly eight years. He later escaped and made his way back to Spain in 1538. He told tales of the wondrous land of La Florida, which further convinced Spanish explorer Hernando de Soto to make his already planned journey to La Florida later that year (Milanich 1993).

3.2.2 The Hernando de Soto Expedition

To make his journey to the New World, Hernando de Soto compiled a group of 600-700 people, which was variable in its composition. The ship crew contained numerous Spaniards, some Portuguese, at least a dozen African slaves and servants, and included both sexes of adults and children (Avellaneda 1997). The crew set out for the New World in April 1538. They later arrived in Santiago, Cuba and made their way to Havana to take Spanish control of the island and finalize plans for the La Florida expedition. While in Cuba, de Soto acquired roughly 220 horses and hundreds of pigs, which he later carried on to Florida (Avellaneda 1997). In addition, it is suspected that as many as 150 men were drawn from the island and joined the expedition (Ramenofsky and Galloway 1997).

In May 1539, the crew left for Florida, and landed at present day Tampa Bay on May 25th. Here, the crew unloaded and established a camp, staying for about six weeks. During the late summer of 1539, the crew along with captive Natives started to journey northward and west to present day Tallahassee, FL. They stayed here through the winter, and in the spring of 1540 they headed northeast across Georgia to the Carolinas. After failing to discover the mythical land of Chicora, they headed northwest across the Appalachian mountains to Tennessee, and then turned south, proceeding down into Alabama. They reached present-day Mobile and came in contact with a town in the chiefdom of Tuscaluza. Here they fought and won a hard battle with the Native peoples. After, the crew began to head northwest to northern Mississippi, and spent the 1540-1541 winter season here. That spring, they headed out northwest and finally reached the Mississippi River in May 1541. Here, they entered the Native province of Quizquiz, and stayed here for about a month in order to prepare to cross the river.

Soon after crossing west over the river on rafts, de Soto and his crew entered the Native province of Casqui. One of the noteworthy occurrences at Casqui that is described in a few narratives was the construction and rising of a wooden cross atop the mound where the chief's house was located. After its erection, a Catholic mass ceremony was performed. These actions were performed after the Natives had asked for help from the Europeans (and their gods) in alleviating the extended drought they had been experiencing. The crew soon departed and subsequently spent the third year of the journey exploring the state of Arkansas. They traveled up and down the Arkansas River in search of non-existent mineral wealth, coming into contact with numerous Native American villages. A year later, they had returned to the banks of the Mississippi River, where de Soto had fallen ill with a fever. He later died in late May of 1542, and Luís de Moscoso took over as the leader of the expedition. Moscoso led the crew southward into Texas in hopes of reaching New Spain (Mexico). Along the way, they also came into contact with many Native villages in East Texas. But, the journey proved to be futile, and the crew turned around and headed back to their previous camp along the Mississippi River. Over the following six months, the crew constructed boats with which they could travel down the Mississippi River to the Gulf of Mexico and subsequently on to Mexico. They set out down the river in late June and reached the Gulf twenty days later. Two and half months later, the survivors reached a settlement near present-day Tampico, Mexico, and thus ended their incredible journey. Figure 3.1 shows Charles Hudson's illustration of the de Soto expedition route.

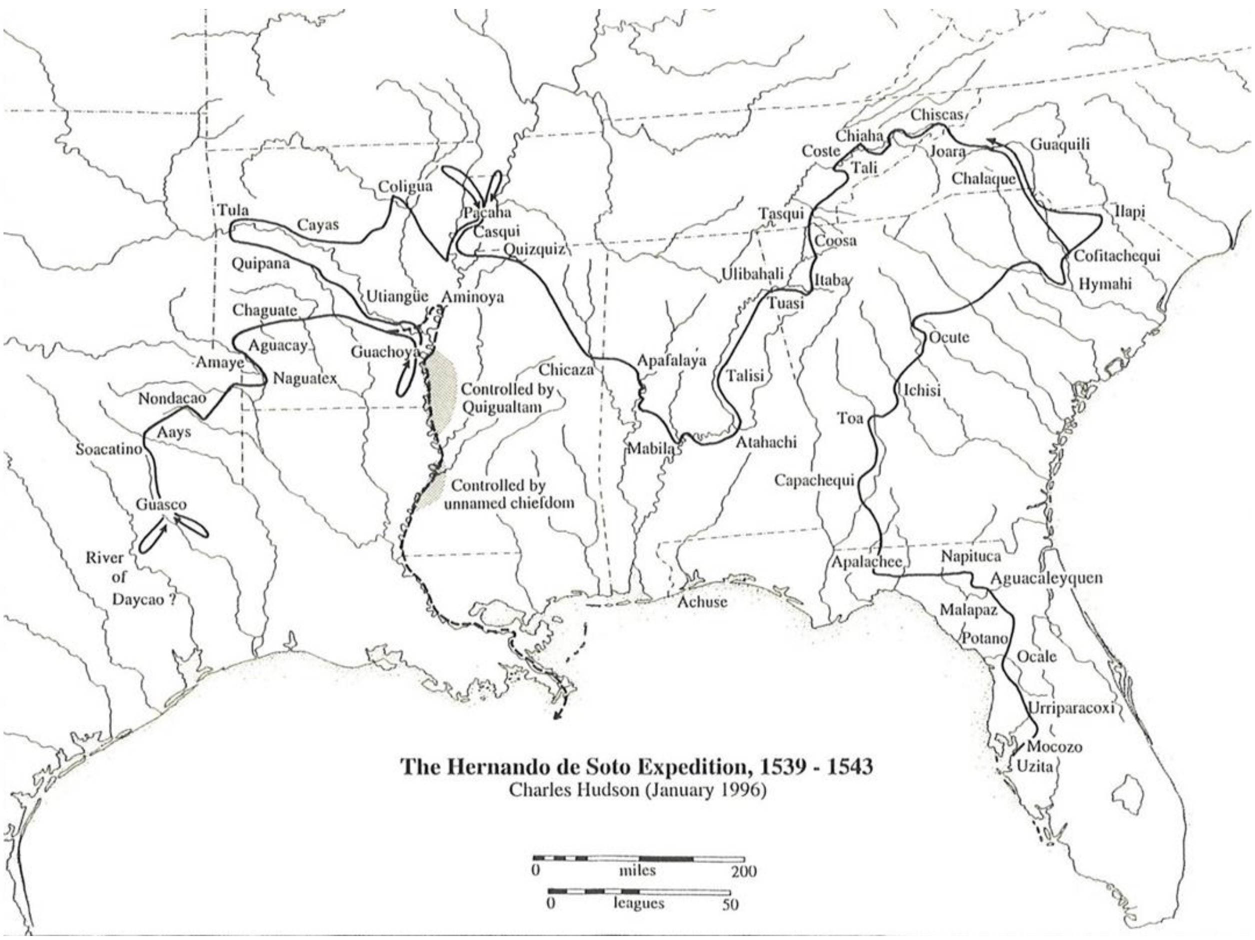

Figure 3.1 Illustration of the Hernando de Soto expedition route by Charles Hudson, Hudson (1996).

3.2.3 The Connection of the De Soto Route to Protohistoric CMV/TMS Archaeological Sites

It has been suggested that some of the archaeological sites in the CMV/TMS represent locations of specific interaction between the Spanish explorers and the Natives that are reported in accounts of the de Soto expedition. The Parkin site in particular is thought to have been the capital of the Casqui province, where de Soto and his crew stayed for a period of time (Morse 1981). The Casqui people also joined de Soto's crew here to invade the neighboring province of Pacaha. The Pacaha province is thought to have encompassed several Nodena phase sites, most notable being the Bradley site (Ann Early, personal communication, 2019). Recent excavations at the Parkin site have uncovered a wooden post base that some archaeologists believe to be the cross erected at Casqui by Hernando de Soto. Radiocarbon dates on the post returned a temporal range of AD 1445 to 1650, which firmly places the site in the protohistoric period (Mitchem et al. 2016). Historians and archaeologists have also mentioned specific sites that de Soto and his crew were likely to have traversed along their route (Hudson, Smith and DePratter 1990; Hudson 1997; Young and Hoffman 1993). These sites are of specific interest to this research question because close contact between Europeans and Natives leads to a higher likelihood of disease transmission (See Figure 3.1 for site locations).

While there is a focus on the Hernando de Soto expedition for this project, it is worth mentioning that there were other possible avenues for the transmission of malaria to the Native Americans of the region. As mentioned above, there were other European explorers in the Southeast before and during the time of the de Soto expedition that had considerable contact with Native populations. These Native populations also had contact and trade relations with Natives in the CMV/TMS (Mathers et al. 2013). These factors do present the possibility of malaria transmission through these explorations. However, the focus on the de Soto expedition is warranted by the epidemiological knowledge of the crew composition, the environmental characteristics of the study area, and the location of the sites included in this study. In addition, de Soto and his crew spent a long period (over a year) just in the state of Arkansas. This extended length of stay increases the chance of malaria transmission from the crew to the Natives.

3.2.4 French Explorations in the CMV/TMS

In 1673, the French began to explore the American Southeast, with Jesuit missionary Jacques Marquette and his companion Louis Jolliet reaching the convergence of the Arkansas and Mississippi Rivers. The explorers arrived at a Quapaw village just north of the convergence, where they were greeted with a welcoming ceremony and feast. During their brief stay in this village, the Quapaws warned them of traveling any further south down the Mississippi, because this area was inhabited by many enemy tribes. Marquette and Jolliet decided to then return north. Nine years later, in 1682, a larger group of French explorers led by Sieur de La Salle arrived in the area. As they had done with the previous explorers, the Quapaw put on a celebration to welcome the guests. Shortly after French arrival, an alliance was formed between the Quapaw and the French. The French took possession of the territory and called it La Louisiane. After this, La Salle and his crew visited three other Quapaw villages located along the Arkansas River. With the help of Quapaw guides, the crew eventually made their way down the Mississippi to the mouth of the river, and proclaimed the area for the French.

After a return to France, La Salle set out to make his way back to the land he had claimed for the French at the mouth of the Mississippi. The expedition initially sailed past the mouth, and landed near present-day Victoria, TX in February of 1685. Storm encounters in the Atlantic and multiple ship crashes upon landing left La Salle with no ships and barely any resources left to undertake his expeditions. Shortly after the rough landing, he departed on three separate overland routes in search for the area. In the second expedition toward the northeast, La Salle fell ill with a fever and spent a month at a Caddo village to recover. On the third expedition, he was killed by his own companions who disagreed with the decisions he had made along the way. After this, some of the expedition members took refuge in numerous Caddoan villages, but some continued their north-eastward journey. With the help of Caddo guides, the explorers made their way back to Arkansas country, where they were led to the Quapaw village of Osotuoy. There, they were relieved to discover a French trading post operated by Frenchmen Jean Couture and Delaunay. They learned that the post had been established by Henry de Tonti, who had revisited the Quapaw villages during his trek down the Mississippi in search for La Salle's settlement.

Between AD 1686 and the early 1800s, this post allowed Natives to have sustained contact with the Europeans. Over this time the Post moved locations a few times, but it mainly stayed along the Mississippi or Arkansas River, within the borders of present-day Arkansas and Desha counties. The Post served as a trade hub and communal meeting area for the French and the Quapaw. Items traded here often included animal meat and skins, liquor, and various European metal goods like knives. The Post played a significant role in the amicable relationship established between the Europeans and Native Americans that characterized the LMV area at least until 1810.

3.3 European Colonization of the CMV/TMS

The CMV and TMS region came under the control of the United States in 1803 through the Louisiana Purchase. This purchase essentially marked the beginning of American governmental authority over the region. Over the following years, numerous American explorers traveled to the area to explore the newly purchased land, bringing with them crews of soldiers, and African-American servants and slaves. Migration and settlement of European Americans to the area increased through the following period. This period was also characterized by a "middle-ground" relationship between European Americans and the Natives. They often partook in trade relations, hunting excursions, and some even formed alliances. This relationship would culminate with the introduction of tens of thousands of European American settlers to the area between 1810 and 1820. With the settlers' interest in becoming part of the larger market economy, they resented the Native's presence on valuable agricultural land, and sought to displace them. In short time, the American government would demand cessation of Native land and the removal of numerous Native groups (Indian Removal Act of 1830). The Act initiated the movement and relocation of the members of these nations westward into Oklahoma territory, often called the "Trail of Tears." With the removal of the Natives, political and economic development in the area further developed. Agricultural enterprises arose mainly through cotton plantations. The arrival of African slaves to the area to work on these plantations became a common practice. The fertile soils along the Mississippi and Arkansas rivers resulted in ample agricultural production of many plants. The economic successes of the CMV/TMS region eventually led to the separation and rise as the separate states of Arkansas, Louisiana, and Missouri beginning in 1836. The remaining part of the historic period was essentially characterized by more political and economic growth, massive increase in the European American population, and further displacement of the Natives.

3.4 Consequences of Contact: Disease and Population Loss

It is well known that upon European contact in the New World, diseases were introduced that devastated the Native American population. Alfred Crosby (1972) discusses the effects of European contact in *The Columbian Exchange: Biological and Cultural Consequences of 1492*. Diseases that have been discussed as being introduced upon European arrival in the New World include smallpox, measles, yellow fever, chicken pox, typhoid fever, and influenza (Boyd 1999, Crosby 1972). These are all diseases that are easily spread in large and dense populations, and are characterized by rapid onset and short duration (Ramenofsky et al. 2003). Smallpox was especially virulent and easily spread among the Native Americans, and therefore played a large role in their suffering and population decline. In particular, smallpox was a large factor in the devastation of the Aztec capital city of Tenochtitlan in AD 1519-1522 (Crosby 1972). Diseases that have been discussed as being brought to the Southeast and LMV area by the Hernando de Soto expedition are smallpox, typhoid fever, and possibly malaria (Ramenofsky and Galloway 1997).

Many historians write about Pre-Columbian America as a ‘disease-free paradise’ or a ‘virgin-soil’ land, indicating that no diseases were present prior to the arrival of the Europeans (Dobyns 1983). However, this is simply not true. It has been suggested and supported by evidence that diseases such as tuberculosis, nonvenereal syphilis, hepatitis, and various intestinal parasitic diseases were present among the Native Americans prior to European contact (Boyd 1999). Roberts and Buikstra (2003) discuss evidence of Pre-Columbian tuberculosis at numerous North American sites in Alabama, Arizona, Arkansas, Illinois, Kentucky, New Mexico, South Carolina, and Tennessee. In Arkansas, the Parkin site, which dates to the Late Prehistoric/Early Protohistoric Period, shows evidence of tuberculosis infection (Murray 1985; Roberts and

Buikstra 2003). In addition, as will be discussed below, skeletal evidence on Pre-Columbian Native Americans suggests that they were not in the best of health. Many populations displayed signs of disease, vitamin or nutritional deficiencies (El-Najjar et al. 1976; Verano and Ubelaker 1992; Larsen 1994; Larsen and Milner 1994; Rose 1999; Steckel and Rose 2002; Ramenofsky et al. 2003).

The transfer of disease played a major role in the catastrophic population loss suffered by the Native American communities (Dobyns 1983). Specifically in the CMV/TMS region, significant population loss between Hernando de Soto's expedition and Marquette and Jolliet's expedition was recorded in European expedition records (Burnett and Murray 1993; Dye and Cox 1990; Kelton 2007; Ramenofsky 1987). Archaeologists have shown interest in the study of Southeastern Native American population loss using archaeological records and analyses (Ramenofsky 1987; Burnett and Murray 1993). Some researchers suggest that Native American population loss may not have been that severe, or rather that populations moved to new locations between the de Soto expedition and the French expeditions (Ramenofsky 1987; Kelton 2007). Disease certainly could have been a factor in the population relocation and reorganization observed between the two expeditions, where we see populations moving away from the Mississippi River and dispersing into smaller settlements (Burnett and Murray 1992). However, other researchers hold their position that some populations were completely devastated, and that is why they were not present when the French arrived in the area roughly 130 years after de Soto (Dobyns 1983). Though a consensus does not seem to have been reached concerning the extent and severity of Native American population loss, there is no doubt that disease and violence resulting from contact with the Europeans contributed to significant population loss as well as relocation and reorganization among these communities.

The introduction of slaves to colonial America and specifically the CMV/TMS region brought more diseases that were endemic in Africa; the most notable of these being malaria. By 1850, malaria had become established in the majority of settlements across the United States (Bradley 1966). In addition, because of the climate and environment, the southeastern states and the CMV/TMS region were highly malarial (Bradley 1966). The southeast continued to be plagued by malaria well into the 20th century. The agricultural practices prominent in the southeast during this time further increased the prevalence and transmission of malaria. Rice agriculture creates swampy lands with numerous pools of standing water in the fields, which are ideal environments for *Anopheles* mosquitoes (Bradley 1966). Near the turn of the 20th century, scientists began to understand the factors causing malarial infection, and started to undertake efforts to eradicate the disease. These efforts included land drainage, killing of mosquito populations, and later included the use of the pesticide DDT. The disease was officially eradicated in the United States by the 1950s, but few isolated incidences continued to appear resulting mainly from travel (Bradley 1966).

3.5 Skeletal Studies of European Contact

Many anthropologists have studied the biological consequences of contact through analyzing changes in Native American health using skeletal data. Two early edited volumes discussing the health impacts of European contact using skeletal analyses are: *Disease and Demography in the Americas* edited by John Verano and Douglas Ubelaker (1992) and *In the Wake of Contact: Biological Response to Conquest* edited by Clark Larsen and George Milner (1994). Selections included in both of these volumes analyze skeletal evidence for declining health among the Native Americans after contact. They find increases in the frequency of declining health indicators (periostitis, porotic hyperostosis, cribra orbitalia, linear enamel hypoplasias, degenerative joint disease, reduced height, etc.) among Native American populations beginning shortly after the arrival of the Europeans. More recent syntheses and studies seem to be in agreement with findings of increased indicators of decreasing health among Native Americans after contact (Larsen et al. 2001; Steckel and Rose 2002; Hogue 2007; Offenbecker and Case 2016).

However, what these studies show are general signs of deteriorating health among the Native Americans that are non-specific in nature and could have been caused from many factors acting together (disease, dietary deficiencies, malnutrition, higher stress levels, warfare, etc.). In the CMV/TMS region especially, the decrease in the quality of the diet from the transition to maize agriculture before the arrival of the Europeans could have contributed to the decline in overall health, and made the Native Americans more susceptible to disease (Armelegos and Cohen 1984; Larsen 1995; Walker et al. 2009). Because of this and the fast acting nature of diseases introduced by the Europeans, it is rare that researchers are able to study the introduction of a specific disease using the skeletal record. However, introduction of a method for identifying malaria in the bioarchaeological record (Smith-Guzmán 2015) gives researchers a chance to study the timing of the introduction of this disease into the New World.

3.6 The Case for Malaria

It has been established in chapters two and three that it is highly possible that the Hernando de Soto expedition could have brought malaria to the CMV/TMS. First, it has been confirmed that malaria was endemic in many European countries including Spain at the time of exploration (Dunn 1965; Bruce-Chwatt and Zulueta 1980). Next, the composition of de Soto's crew including at least a dozen African slaves further increases the likelihood of his crew harboring the *Plasmodium* parasite (Avellaneda 1997). The ability of the *Plasmodium* parasite to lay dormant in a human host's liver for an extended period of time would have allowed it to survive within the crew members during the ship's journey to the New World (Cormier 2011). The brief stay in Cuba and the acquisition of horses, pigs, and Cuban Natives by the de Soto expedition likely increased the pathogen load on the ships (Avellaneda 1997; Ramenofsky and Galloway 1997). It is also evident that the environment of the region at the time of exploration was ripe for the transmission of malaria. The mild, subtropical climate could have been able to sustain *Anopheles quadrimaculatus* populations for eight to ten months out of the year. In addition, de Soto's exploration records often mentioned the swampy areas the crew had to traverse on their journeys as well their encounters with swarms of pests (Hudson 1997; Kelton 2007). These ecological epidemiological factors suggest that malaria could have been one of the diseases brought to the CMV/TMS by the Hernando de Soto expedition in the protohistoric era, before the first documented epidemics occurred with the arrival of African slaves in the early 1800s.

To investigate this possibility, this study follows the likes of Verano and Ubelaker (1992) and Larsen and Milner (1994) in taking a bioarchaeological approach to disease introduction. It will utilize skeletal data from Native American populations in the CMV/TMS. The newly published method for identifying malaria in the bioarchaeological record discussed earlier (Smith-Guzmán 2015) will be used in conjunction with ecological and epidemiological factors to evaluate the hypothesis that the Hernando de Soto expedition could have introduced malaria to the region.

Chapter 4: Materials and Methods

4.1 Materials

Excavation records and burial forms from excavations and bioarchaeological analyses on numerous southeastern US sites were used in this study. These records are currently housed at the Arkansas Archaeological Survey (AAS), the University of Arkansas Museum, and the Osteology Lab at the University of Arkansas. The archaeological sites span the six states of Arkansas, Louisiana, Mississippi, Missouri, Oklahoma, and Texas. Together, the sites represent many cultural traditions (Caddo, Quapaw, etc.) and stretch over a long period of time, from Archaic to Historic periods. Additional information about the sites, or more in depth paleopathological analyses were pulled from the existing literature. Also, a review of the literature was performed to collect information from additional southeastern sites that did not have records at the AAS. A list of the sites surveyed and their temporal affiliation are presented in Table 4.1.

With my research question in mind, I was mostly interested in looking at sites that were located along the Hernando de Soto expedition route in the Central Mississippi Valley (CMV) and Trans-Mississippi South (TMS) (Figure 3.1), where the Native people would have had direct or close contact with de Soto and his explorers. In addition, sites that were specifically mentioned as locations of contact between de Soto's explorers and the natives in many historical records including Charles Hudson's 1998 book, *Knights of Spain, Warriors of the Sun*, and Young and Hoffman's 1993 edited volume *Hernando De Soto West of the Mississippi, 1541-1543*, were of high interest. Therefore, sites in Arkansas, Northeastern Mississippi, Louisiana, and East Texas were of particular importance to this research question.

Research on the ecological and epidemiological factors that contribute to the transmission of malaria (presented in chapter 2) was explored to evaluate my hypothesis. Information was gathered pertaining to the ecology and environment of the CMV/TMS that could have contributed to large *Anopheles* mosquito populations. Historical and ethnographic resources were utilized as well. Historical accounts of the de Soto expedition were used to verify the composition of the members of de Soto expedition. These records also mention illnesses, sicknesses, and symptoms experienced by both the explorers and the Native Americans during their journeys. They also contain information about the terrain and environment they had to traverse during their travels. These sources were used to gain insight into the likely ecological and epidemiological factors that were in place in the protohistoric CMV/TMS that could have been productive for the transmission of malaria.

4.2 Methods

4.2.1 Data Collection

While looking through the archaeological records, I looked for any recordings of the five lesions indicative of malaria (cribra orbitalia, femoral cribra, humeral cribra, spinal porosity, and periostitis), described by Nicole Smith-Guzmán (2015). In addition, I looked for any note of pathologies described by the observer that were highly likely to be descriptive of any of the five lesions. For example, the observer may not have written down “cribra orbitalia”, but they wrote down “diploe expansion in eye orbits”, therefore I would record that the individual expressed cribra orbitalia. In noting these lesions observed on the skeletons, I diagnosed an individual with malaria if their lesion combination satisfied the equation formulated and presented by Smith-Guzmán (2015).

Figure 4.1. Pictorial representation of the outcome algorithm described by Smith-Guzmán (2015). Lesions on the top row from left to right are: cribra orbitalia, humeral cribra, and femoral cribra; bottom row: spinal porosity and periostitis. Example photographs are from the Galloway Osteological Collection, Uganda. Created by Nicole Smith-Guzmán.

$$Ci = 1 \text{ if } \{(\text{CO or HC or FC}=1) \text{ AND } (\text{SP or P} =10\}; \text{ else } Ci = 0$$

In this algorithm, *Ci* represents the case number/individual/skeleton number. A value of "1" given to *Ci* denotes a positive diagnosis of malaria. Skeletal lesions are also scored in this way, where a score of "1" given to a lesion denotes presence on the skeleton, and a score of "0" denotes absence of lesion. Lesions are abbreviated as CO=cribra orbitalia, HC=humeral cribra, FC=femoral cribra, SP=spinal porosity, and P=periostitis (Smith-Guzmán 2015). Only the presence or absence of the lesion(s) on the skeleton was recorded, because the severity of these lesions was not the focus for this study. In addition, severity of the lesions could not be discerned in most cases, due to the lack of specific notation of severity in the records. Demographic information including sex and age for individuals diagnosed with malaria was collected as well, when reported.

The archaeological records analyzed indicated a wide degree of skeletal preservation in addition to varying degrees of completeness of skeletons. Even though in some cases it was very limiting, an ‘observable population’ was created for each site from the original burial population. The ‘observable population’ consisted of those skeletons that had the five skeletal elements needed for the study of malarial infection: frontal bone w/orbit, humeral head/neck, femoral head/neck, tibia or other mostly complete long bone, and some complete vertebral bodies.

4.2.2 Data Analysis

A total of 243 sites were reviewed at the AAS, the UA Museum, the UA Osteology Lab, and through the literature. However, in some cases, pathological analyses were not performed for the skeletal assemblages recovered, or had poor skeletal preservation, or did not have a temporal association. These sites were not included in analyses. Therefore, the remaining site count was 113. These remaining sites were grouped into three time periods: Prehistoric (8000 BC-AD 1492), Protohistoric (AD 1492-1686), and Historic (AD 1686-1920). Malarial percentages for each site were calculated by dividing the total positive malarial individuals by the total observable population for that site. The malarial population was further broken down demographically, into percent female and percent subadult, since these individuals are shown to be the most vulnerable to malarial infection.

Statistical analyses were conducted to see if there were significant differences in malaria rates among the three time periods. A non-parametric ANOVA (Kruskal-Wallis) was performed with post-hoc tests for pair-wise comparisons. All statistical analyses were performed in SPSS with α set at .05.

Table 4.1. List of sites included in this study. Site code indicates state and county/parish. 3= AR, 16= LA, 22= MS, 23= MO, 34= OK, 41= TX. Following initials indicate the county/parish where the site is located.

Site	Name	Source
Prehistoric (8000 BC-AD 1492)		
3BR40	Saline Sand and Gravel	AAS files
3CA3	Bangs Slough	AAS files
3CA265	Little Mud Lake	AAS files
3CG218	Burris 2	AAS files
3CH14	Powel Canal	AAS files, Blaeuer and Rose 1982
3CH49	McArthur	AAS files
3CL418	Hardman	AAS files, Burnett 1990
3CN117	Alexander	AAS files
3CT50	Mudhole	AAS files
3CT98	Broughham Lake	AAS files
3CW11	Beaver Bond	AAS files
3CW34	McClure	AAS files
3DR2	Taylor Mound	AAS files
3GA1	Adair	AAS files
3HE54	Hood	AAS files
3HE63	Ferguson 1	AAS files
3HE70	Purtle	AAS files
3HE92	Martin Farm	AAS files
3HS1	Cooper	AAS files
3HS19	Middle Meadow	AAS files
3HS28	Jones Mill	AAS files
3LE29	Carnes	AAS files
3LN42	Toltec	AAS files
3LN119	Bill Carr	AAS files
3LO226	Wild Violet	Porter 2016
3LR49	Old Martin Place	AAS files

Table 4.1 (Cont.)

Site	**Name**	**Source**
Prehistoric (8000 BC – AD 1492)		
3LR50	Bowman Place	AAS files
3LW106	John Wilson	AAS files
3MI1	Haley Place	AAS files
3NW637	Beech Creek Shelter	AAS files
3PH11	Helena Mounds	AAS files, Giles et al. 2010
3PO52	Hyneman 1	AAS files
3PO52	Hyneman 2	AAS files
3PO82	Bay Village	AAS files
3UN23		AAS files
16AV2	Greenhouse	AAS files
16IB3	Morton Shell Mound	AAS files
16IV4	Bayou Sorrel	AAS files
16IV128	St. Gabriel	AAS files
16JE37	Coquile	AAS files
16LA3	Crooks	AAS files
16MA18	Mnt Nero 1	AAS files
16MA18	Mnt Nero 2	AAS files
16OR1-5	Little Woods	AAS files
16OR6	Big Oak Island	AAS files
16RR1	Gahagan Mound	AAS files
16RR4	Hanna	AAS files
16SA48	Coral Snake Mound	AAS files
16SM17	Lafayette Mound	AAS files, Ford et al. 1945
16ST1	Tchefuncte	AAS files
16VM102	Copell Place	AAS files
22LO530	Shell Bluff	AAS files
22QU525	Shady Grove	AAS files, Davis 2015
22YZ557	Lake George	AAS files, Listi 2013
23BY9001	Montgomery Farm	AAS files
23PM42	Kersley	AAS files
23SH10	Owls Bend	AAS files
41CE19	George Davis	AAS files
41COL0		AAS files
41DT1	Monton Miller	AAS files
41DT4	Tick 1	AAS files
41DT124	Cooper-Pool 1	AAS files

Table 4.1 (Cont.)

Site	Name	Source
Prehistoric (8000 BC – AD 1492)		
41HP102	Arnold	AAS files
41HP105	Cox	AAS files
41RW1	Lower Rockwall	AAS files
41RW2	Upper Rockwall	AAS files
41RR11	Haldeman	AAS files
41SA101	Jonas Short 1	AAS files
41UR10	Harron	AAS files
Protohistoric (AD 1492-1686)		
3AS152	Gordon	AAS files
3CL24	Saline-Bayou	AAS files
3CL63	Moore Mound	AAS files
3CL195	Copeland Ridge	AAS files
3CS29	Parkin	AAS files, Murray 1998
3CT9	Wapanocca	AAS files
3CT30	Belle Meade	AAS files
3DR214	Ables Creek	AAS files
3HS15	Denham Mound	AAS files
3HS60	Hedges	AAS files
3LA83	Spirit Lake	AAS files
3LA97	Cedar Grove Caddo	AAS files, Rose 1984
3LE11	Clay Hill	AAS files
3MO61	Walnut Ridge	AAS files
3MS4	Upper Nodena	AAS files
3MS20	Zebree	AAS files
3MS71	Smith	AAS files
3OU128	Albritton Bottom	AAS files
3PO6	Hazel	UA Osteo files, Zinke 1975
3PU306	Goldsmith-Oliver II	AAS files
3UN13	Boytts Field	AAS files
16LF17	Bowie 2	AAS files
16OU17	Myatt's Landing	AAS files
16SA37	Salt Lick	AAS files
23PM5	Campbell	AAS files, Holland 1991
34MC215	Roden	AAS files, Rose et al. 1981
34CK44	Smullins	AAS files

Table 4.1 (Cont.)

Site	**Name**	**Source**
Protohistoric (AD 1492-1686)		
41BW3	Hatchell	AAS files
41BW4	Mitchell	AAS files
41HS74		AAS files
41RR_	Clark	AAS files
41RR16	Kaufman-Williams	AAS files, Loveland et al. 1985
41RR41	Bentsen-Clark	AAS files
Historic (AD 1686-1920)		
3LA97	Cedar Grove Historic	AAS files, Rose 1989
16BO236	McLelland	AAS files, Kelley et al. 1995
16BO237	Joe Clark	AAS files, Kelley et al. 1996
16OR92	St. Peters Secret Cemetery	AAS files
16SA17		AAS files
41BW2	Moores	AAS files
41CE12	Jim Allen	AAS files
41DT37	Tick Historic	AAS files
41DT80	Cooper-Pool Historic	AAS files
41DT104	Tucker Cemetery	AAS files
41SA101	Jonas Short Historic	AAS files

Chapter 5: Results and Discussion

5.1 Results

Presence of malaria indicators were reported on a total of 47 individuals at 15 sites spanning all time periods included in this study. Reports of malaria indicators make up 13.27% of total sites surveyed and 3.45% of the total observable individuals. Table 5.1 presents the number and percentage of sites showing presence of malaria indicators for each time period, with ten reported in the protohistoric period (10, 30.30%). Figure 5.1 depicts percentages reported in Table 5.1. Table 5.2 presents the number and percentage of individuals that had presence of malaria indicators by time period, with the most individuals reported in the protohistoric period. Table 5.4 presents a condensed summary of results and Table 5.5 presents complete results, with demographic breakdown of the malarial populations.

Table 5.1 Results showing the number and percentage of total sites that reported malaria indicators by time period.

Time Period	Total Sites	Sites w/ malaria	%
Prehistoric	69	3	4.35
Protohistoric	33	10	30.30
Historic	11	2	18.18
Total	113	15	13.27

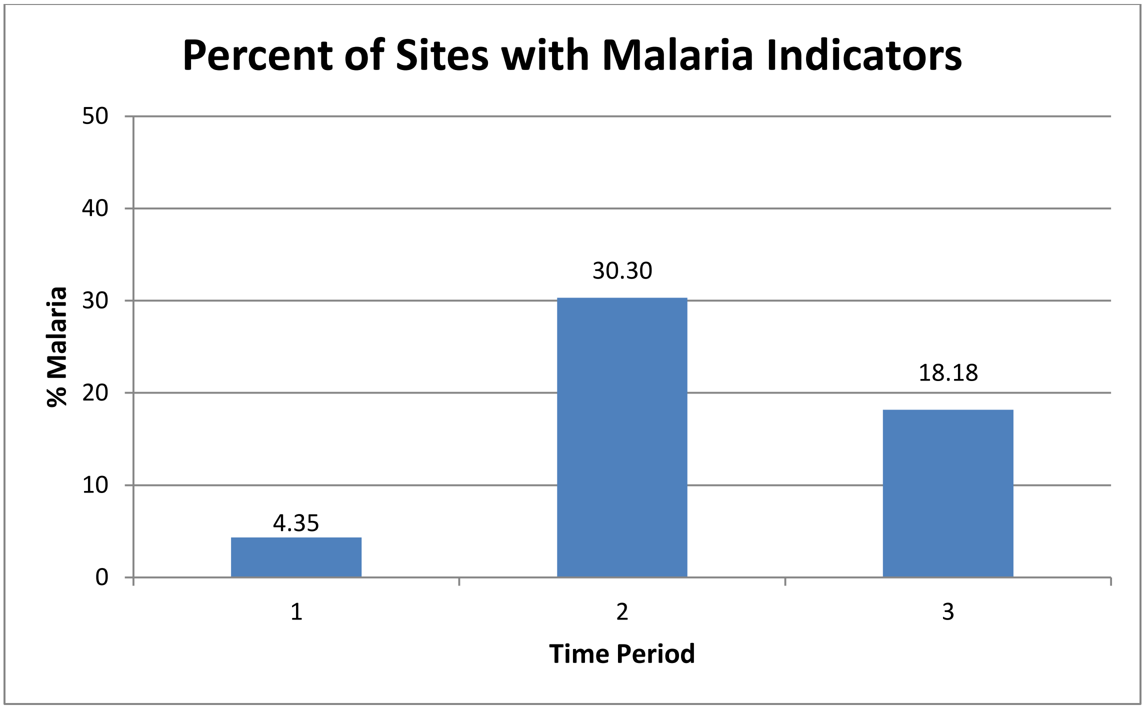

Figure 5.1. Bar graph showing the percentage of surveyed sites that reported malaria indicators by time period. 1= prehistoric, 2= protohistoric, 3= historic

Table 5.2 Results showing the number and percentage of individuals that had malaria indicators by time period.

Time Period	**Total Inds.**	**Inds. w/ malaria**	**%**
Prehistoric	761	5	0.66
Protohistoric	396	27	6.82
Historic	177	14	7.91
Total	1334	46	3.45

A Kruskal-Wallis test showed that there was a statistically significant difference among the average frequencies of malaria indicators per time period, H (2) = 13.132, p = 0.001. Post-hoc tests for pairwise comparisons showed a statistically significant difference between the average frequency of malaria indicators between the prehistoric and protohistoric periods, H (2) = -14.726, p = 0.001. There were no significant differences between the prehistoric/historic and protohistoric/historic periods. Results of the post-hoc tests for pairwise comparisons can be found in Table 5.1.

Table 5.3. Results of the Kruskal-Wallis post-hoc tests for pairwise comparisons.

Sample Comparison	**Test Stat (H)**	**Std. Error**	**Adj. Significance**
Prehistoric/Historic	-7.136	6.107	.728
Prehistoric/Protohistoric	-14.726	4.089	.001*
Protohistoric/Historic	7.589	6.574	.745

Table 5.4. Condensed results for the sites that reported presence of malaria indicators. %Total was calculated by dividing the observed individuals (Obs. Ind.) by the total observable population (N). The last two columns show demographic breakdown of the malarial population at each site; F=female, M=Male, SA=subadult (15 or younger).

Site	**Name**		**Cases Malaria**				
Prehistoric (8000 BC - AD 1492)		**N**	**Obs. Ind.**	**%Total**	**%F**	**%M**	**%SA**
3CH14	Powel Canal	4	2	50.0	0.0	100.0	0.0
3LE29	Carnes	2	2	100.0	100.0	0.0	0.0
22LO530	Shell Bluff	19	1	5.3	0.0	0.0	0.0
Protohistoric (AD 1492 - 1686)							
3CS29	Parkin	15	6	40.0	33.3	66.7	0.0
3CT9	Wapanocca	16	3	18.8	66.7	33.3	0.0
3HS60	Hedges	1	1	100.0	0.0	100.0	0.0
3MS20	Zebree	10	1	10.0	100.0	0.0	0.0
3MS71	Smith	1	1	100.0	0.0	100.0	0.0
3PO6	Hazel	13	6	46.2	33.3	33.3	33.3
34MC215	Roden	15	1	6.7	0.0	0.0	100.0
41BW3	Hatchell	14	2	14.3	0.0	100.0	0.0
41RR_	Clark	2	1	50.0	100.0	0.0	0.0
41RR16	Kaufman-Williams	19	5	26.3	20.0	60.0	20.0
Historic (AD 1686 - 1920)							
3LA97	Cedar Grove Historic	89	13	12.4	7.7	15.4	76.9
41DT80	Cooper-Pool Historic	2	1	50.0	100.0	0.0	0.0

Table 5.5 Complete results for the sites surveyed. %Total was calculated by dividing the observed individuals (Obs. Ind.) by the total observable population (N). The last two columns show demographic breakdown of the malarial population at each site; F=female, M=Male, SA=subadult (15 or younger).

Site	Name	N	Cases Malaria				
Prehistoric (8000 BC-AD 1492)			**Obs. Ind.**	**%Total**	**%F**	**%M**	**%SA**
3BR40	Saline Sand & Gravel	1	0	0.0	-	-	-
3CA3	Bangs Slough	1	0	0.0	-	-	-
3CA265	Little Mud Lake	1	0	0.0	-	-	-
3CG218	Burris 2	1	0	0.0	-	-	-
3CH14	Powel Canal	4	2	50.0	0.0	100.0	0.0
3CH49	McArthur	5	0	0.0	-	-	-
3CL418	Hardman	8	0	0.0	-	-	-
3CN117	Alexander	2	0	0.0	-	-	-
3CT50	Mudhole	3	0	0.0	-	-	-
3CT98	Broughham Lake	2	0	0.0	-	-	-
3CW11	Beaver Bond	9	0	0.0	-	-	-
3CW34	McClure	1	0	0.0	-	-	-
3DR2	Taylor Mound	2	0	0.0	-	-	-
3GA1	Adair	12	0	0.0	-	-	-
3HE54	Hood	2	0	0.0	-	-	-
3HE63	Ferguson 1	1	0	0.0	-	-	-
3HE70	Purtle	1	0	0.0	-	-	-
3HE92	Martin Farm	3	0	0.0	-	-	-
3HS1	Cooper	6	0	0.0	-	-	-
3HS19	Middle Meadow	2	0	0.0	-	-	-
3HS28	Jones Mill	4	0	0.0	-	-	-
3LE29	Carnes	2	2	100.0	100.	0.0	0.0
3LN42	Toltec	2	0	0.0	-	-	-
3LN119	Bill Carr	2	0	0.0	-	-	-
3LO226	Wild Violet	6	0	0.0	-	-	-
3LR49	Old Martin Place	2	0	0.0	-	-	-
3LR50	Bowman Place	9	0	0.0	-	-	-
3LW106	John Wilson	19	0	0.0	-	-	-
3MI1	Haley Place	13	0	0.0	-	-	-
3NW637	Beech Creek Shelter	1	0	0.0	-	-	-
3PH11	Helena Mounds	19	0	0.0	-	-	-
3PO52	Hyneman 1	2	0	0.0	-	-	-
3PO52	Hyneman 2	1	0	0.0	-	-	-
3PO82	Bay Village	2	0	0.0	-	-	-
3UN23		1	0	0.0	-	-	-

Table 5.5 (Cont.)

Site	Name	N	Cases Malaria				
Prehistoric (8000 BC-AD 1492)			**Obs. Ind.**	**%Total**	**%F**	**%M**	**%SA**
16AV2	Greenhouse	10	0	0.0	-	-	-
16IB3	Morton Shell Mound	24	0	0.0	-	-	-
16IV4	Bayou Sorrel	19	0	0.0	-	-	-
16IV128	St. Gabriel	16	0	0.0	-	-	-
16JE37	Coquile	2	0	0.0	-	-	-
16LA3	Crooks	4	0	0.0	-	-	-
16MA18	Mnt Nero 1	40	0	0.0	-	-	-
16MA18	Mnt Nero 2	46	0	0.0	-	-	-
16OR1-5	Little Woods	30	0	0.0	-	-	-
16OR6	Big Oak Island	25	0	0.0	-	-	-
16RR1	Gahagan Mound	15	0	0.0	-	-	-
16RR4	Hanna	6	0	0.0	-	-	-
16SA48	Coral Snake Mound	2	0	0.0	-	-	-
16SM17	Lafayette Mound	20	0	0.0	-	-	-
16ST1	Tchefuncte	43	0	0.0	-	-	-
16VM102	Copell Place	55	0	0.0	-	-	-
22LO530	Shell Bluff	19	1	5.3	0.0	0.0	0.0
22QU525	Shady Grove	22	0	0.0	-	-	-
22YZ557	Lake George	50	0	0.0	-	-	-
23BY9001	Montgomery Farm	26	0	0.0	-	-	-
23SH10	Owls Bend	3	0	0.0	-	-	-
41CE19	George Davis	19	0	0.0	-	-	-
41COL0		13	0	0.0	-	-	-
41DT1	Monton Miller	6	0	0.0	-	-	-
41DT4	Tick Prehistoric	4	0	0.0	-	-	-
41DT124	Cooper-Pool Prehistoric	4	0	0.0	-	-	-
41HP102	Arnold	13	0	0.0	-	-	-
41HP105	Cox	3	0	0.0	-	-	-
41RW1	Lower Rockwall	5	0	0.0	-	-	-
41RW2	Upper Rockwall	11	0	0.0	-	-	-
41RR11	Haldeman	51	0	0.0	-	-	-
41SA101	Jonas Short Prehistoric	2	0	0.0	-	-	-
41UR10	Harron	1	0	0.0	-	-	-

Table 5.5 (Cont.)

Site	Name	N	Cases Malaria				
Protohistoric (AD 1492-1686)			**Obs. Ind.**	**%Total**	**%F**	**%M**	**%SA**
3AS152	Gordon	10	0	0.0	-	-	-
3CL24	Saline-Bayou	7	0	0.0	-	-	-
3CL63	Moore Mound	1	0	0.0	-	-	-
3CL195	Copeland Ridge	33	0	0.0	-	-	-
3CS29	Parkin	15	6	40.0	33.3	66.7	0.0
3CT9	Wapanocca	16	3	18.8	66.7	33.3	0.0
3CT30	Belle Meade	7	0	0.0	-	-	-
3DR214	Ables Creek	67	0	0.0	-	-	-
3HS15	Denham Mound	2	0	0.0	-	-	-
3HS60	Hedges	1	1	100.0	0.0	100.0	0.0
3LA83	Spirit Lake	1	0	0.0	-	-	-
3LA97	Cedar Grove Caddo	7	0	0.0	-	-	-
3LE11	Clay Hill	3	0	0.0	-	-	-
3MO61	Walnut Ridge	2	0	0.0	-	-	-
3MS4	Upper Nodena	7	0	0.0	-	-	-
3MS20	Zebree	10	1	10.0	100.0	0.0	0.0
3MS71	Smith	1	1	100.0	0.0	100.0	0.0
3OU128	Albritton Bottom	17	0	0.0	-	-	-
3PO6	Hazel	13	6	46.2	33.3	33.3	33.3
3PU306	Goldsmith-Oliver II	3	0	0.0	-	-	-
3UN13	Boytts Field	10	0	0.0	-	-	-
16LF17	Bowie Historic	3	0	0.0	-	-	-
16OU17	Myatt's Landing	38	0	0.0	-	-	-
16SA37	Salt Lick	8	0	0.0	-	-	-
23PM5	Campbell	6	0	0.0	-	-	-
34MC215	Roden	15	1	6.7	0.0	0.0	100.0
34CK44	Smullins	9	0	0.0	-	-	-
41BW3	Hatchell	14	2	14.3	0.0	100.0	0.0
41BW4	Mitchell	14	0	0.0	-	-	-
41HS74		3	0	0.0	-	-	-
41RR_	Clark	2	1	50.0	100.0	0.0	0.0
41RR16	Kaufman-Williams	19	5	26.3	20.0	60.0	20.0
41RR41	Bentsen-Clark	32	0	0.0	-	-	-

Table 5.5 (Cont.)

Site	Name	N	Cases Malaria				
Historic (AD 1686-1920)			**Obs. Ind.**	**%Total**	**%F**	**%M**	**%SA**
3LA97	Cedar Grove Historic	89	13	12.4	7.7	15.3	76.9
16BO236	McLelland	7	0	0.0	-	-	-
16BO237	Joe Clark	1	0	0.0	-	-	-
16OR92	St. Peters Secret Cemetery	29	0	0.0	-	-	-
16SA17		10	0	0.0	-	-	-
41BW2	Moores	12	0	0.0	-	-	-
41CE12	Jim Allen	19	0	0.0	-	-	-
41DT37	Tick Historic	2	0	0.0	-	-	-
41DT80	Cooper-Pool Historic	2	1	50.0	100.0	0.0	0.0
41DT104	Tucker Cemetery	4	0	0.0	-	-	-
41SA101	Jonas Short Historic	2	0	0.0	-	-	-

5.2 Discussion

The factors discussed in chapters two and three hinted at the possibility that the Hernando de Soto expedition could have introduced malaria to the CMV/TMS region during their travels. These factors included endemic malaria in Spain during the age of European exploration, the inclusion of at least a dozen African slaves in the expedition crew, the ability of the *Plasmodium* parasite to lay dormant in the liver for an extended period of time, the stop and exchange on the island of Cuba, and the humid and swampy environment of the protohistoric CMV/TMS that was highly likely to attract *Anopheles* mosquitoes. The analyses of the skeletal data from Native American skeletons uncovered the presence of 15 sites with malaria indicators, with 10 of those being protohistoric sites. In addition, the protohistoric period had a higher percentage of sites with malaria indicators (30.30%) than the other two periods. A higher number (27) and percent

(6.82%) of individuals from the protohistoric period showed malaria indicators compared to the prehistoric.

These results demonstrate that there was an increase in the prevalence and frequency of malaria indicators from the prehistoric to the protohistoric period. The Kruskal-Wallis test revealed a significant difference in the average frequencies of malaria indicators between the prehistoric and protohistoric periods, which supports these observations. These results strongly suggest that malaria could have been one of the diseases introduced to the Native Americans of the CMV/TMS region by the Hernando de Soto expedition. Furthermore, compared to other malaria sites, malaria indicators were found in higher frequency (40%) at the Parkin site, which was a site of direct contact between the members of the de Soto expedition and the Natives.

5.2.1 Quality of Data

The highest frequency (46.15%) of malaria indicators was found at the protohistoric Hazel site (3PO6). This is significant because the Hazel skeletons were analyzed after Nicole Smith-Guzmán's (2015) publication of a method for diagnosing malaria in skeletal remains. Thus, these skeletons, as well as the prehistoric Wild Violet skeletons (3LO226), were evaluated with knowledge of malarial indicators, and are of higher significance. The fact that malaria indicators were not reported at Wild Violet (3LO226), but were reported at Hazel (3PO6), supports current hypotheses that malaria was not present in the CMV/TMS region before the arrival of the Europeans, and further supports the hypothesis that the de Soto expedition could have introduced malaria to the LMV.

5.2.2 Iron-deficiency Anemia vs. Malaria

As previously discussed in chapter two, the long-held paradigm in the field of bioarchaeology is that porous skeletal lesions are caused by iron-deficiency anemia. Iron-deficiency anemia results from a diet lacking in iron, such as a diet high in maize. Most of the skeletal data used in this study comes from Native populations that practiced maize agriculture, at least those after AD 1000. Because these porous lesions, especially cribra orbitalia and porotic hyperostosis, were reported on numerous individuals throughout the records, it is possible that some of them were suffering from iron-deficiency anemia due to their diet. However, recent research has suggested that these lesions are likely caused by multiple factors and may not even be related in some cases. In addition, it has been speculated that iron-deficiency anemia cannot produce these lesions, and researchers instead credit hemolytic anemia as the main factor triggering the formation of these lesions (Walker 2009). Hemolytic anemia is seen in individuals with malaria infection. Furthermore, the topic of interest for this study was the specific combination of these lesions (cribra orbitalia, femoral cribra, humeral cribra, periostitis, and vertebral porosity), not the presence of one single lesion. Smith-Guzmán's (2015) algorithm for diagnosing malaria in the skeletal record demonstrates that it is a combination of these porous and inflammatory lesions that is indicative of malarial infection.

5.2.3 Limitations of this Study

This study was limited by many sites that reported the presence of cribra orbitalia and porotic hyperostosis together, where the observers combined both conditions into the name porotic hyperostosis. This stems from the previous assumptions that porotic hyperostosis and cribra orbitalia are of the same etiology (iron-deficiency anemia). However, recent research sheds light on the possible dissociation of these two conditions. This limited the study because there were numerous individuals across all time periods that were recorded as displaying porotic hyperostosis, and many cases in combination with other skeletal lesions indicative of malaria. If cribra orbitalia had been reported separately from porotic hyperostosis throughout the archaeological records, the results might have included more individuals and more sites with positive malaria indicators. Nevertheless, the data that was retrieved still strongly supports the hypothesis.

Chapter 6: Conclusion

The newly developed bioarchaeological method for diagnosing malaria in the skeletal record provides a rich opportunity to contribute to the broader understanding of the introduction and spread of malaria among human populations. Specifically, it provides a non-invasive, macroscopic method that requires minimal handling of skeletal material and no sample extraction. This will greatly benefit bioarchaeologists working in the United States who must seek tribal permission to analyze Native American skeletal collections and must comply with NAGPRA standards when conducting their analyses. When analyzed in tandem with ecological and epidemiological factors, bioarchaeological analysis of malarial infection can inform current and future research on the introduction of malaria to the New World.

The results of this study suggest that malaria could have been introduced to the Central Mississippi Valley and Tran-Mississippi South region by the Hernando de Soto expedition. This paper established that ecological and epidemiological factors were at play in the CMV/TMS region and among the members of the de Soto expedition that could have contributed to the transmission of malaria from the Europeans to the Natives. Analyses of the Native American skeletal material confirmed the presence of malaria indicators from 10 protohistoric CMV/TMS sites. In addition, the data show an increase in prevalence and frequency of malaria indicators from the prehistoric to protohistoric period. These results are strengthened by the fact that a high frequency of malaria indicators was reported for the Parkin site skeletons, which was a site of direct contact between the Natives and the Hernando de Soto expedition. The results are further strengthened by the high frequency of malaria indicators reported at the protohistoric Hazel site, which was analyzed after knowledge of the skeletal manifestation of malaria was developed.

This study changes our understanding of the spread of malaria to the New World. There is currently no solid evidence of the presence of malaria in the CMV/TMS before the introduction of African slaves to the area in the 1800s. However, this study suggests the presence of malaria in the region during the period of European exploration. Furthermore, it suggests that malaria could have been one of the diseases introduced to the Natives of the area by the Hernando de Soto expedition. These results and the methods used to obtain them can inform future research on the introduction and spread of malaria in the New World.

References

Afrane, Y. A., A. K. Githeko, and G.Y an. 2012. The Ecology of Anopheles Mosquitoes Under Climate Change: Case Studies from the Effects of Environmental Changes in East Africa Highlands. *Annual NY Academy of Sciences*, 1249:204–210.

Angel J. Lawrence. 1964. The Reaction Area of the Femoral Neck. *Clinical Orthopedics and Related Research* 32:130–142.

Angel, J. Lawrence. 1966. Porotic Hyperostosis, Anemias, Malarias, and Marshes in the Prehistoric Eastern Mediterranean. *Science* 153:760–763.

Angel, J. Lawrence. 1967. Porotic Hyperostosis or Osteoporosis Symmetrica. *Diseases in Antiquity* 378-389.

Armelagos, George J., and Mark Nathan Cohen, eds. 1984. *Paleopathology at the Origins of Agriculture*. Academic Press, Orlando.

Avellaneda, Ignacio. 1997. Hernando de Soto and His Florida Fantasy. In *The Hernando De Soto Expedition : History, Historiography, and "Discovery" in the Southeast,* edited by P.K. Galloway. University of Nebraska Press, Lincoln, pp. 207-218.

Boyd, Robert. 1992. Population Decline from Two Epidemics on the Northwest Coast. In: *Disease and Demography in the Americas* edited by J.W. Verano and D.H. Ubelaker Smithsonian Institution Press, Washington, D.C., pp. 249-255.

Boyd, Robert. 1999. *The Coming of the Spirit of Pestilence: Introduced Infectious Diseases and Population Decline among Northwest Coast Indians, 1774-1874*. University of Washington Press, Seattle.

Bradley, George H. 1966. A Review of Malaria Control and Eradication in the United States. *Mosquito News* 46(4):462-470.

Bruce-Chwatt, LJ, and J. de Zulueta. 1980. *The Rise and Fall of Malaria in Europe: A Historico-Epidemiological Study.* Oxford University Press, Oxford.

Burnett, Barbara A. and Katherine A. Murray. 1993. Death, Drought, and de Soto: The Bioarcheology of Depopulation. In *The Expedition of Hernando de Soto West of the Mississippi*, *1541-1543*, edited by G.A. Young and M.P. Hoffman. University of Arkansas Press, Fayetteville, pp. 227-236.

Carter, Richard. 2003. Speculations on the Origins of Plasmodium Vivax Malaria. *Trends in Parasitology* 19(5):214-219.

Centers for Disease Control and Prevention. 2018. Parasites - Malaria. www.cdc.gov/parasites/malaria/index.html.

Cormier, Loretta A. 2011. *The Ten-Thousand Year Fever*. Left Coast Press, Walnut Creek.

Crosby, Alfred W. 1972. *The Columbian Exchange: Biological and Cultural Consequences of 1492*. Greenwood Press, Westport.

Crosby, Alfred W. 1986. *Ecological Imperialism: The Biological Expansion of Europe, 900-1900*. Cambridge University Press, Cambridge.

Davis, Christopher B. 2015. Paleopathology at the Shady Grove Site (22QU525): A Study of Health in the Upper Yazoo Basin During the Middle Mississippian Period. Master's Thesis, University of Southern Mississippi.

Dobyns, Henry F. 1983. *Their Numbers Became Thinned*. University of Tennessee Press, Knoxville.

D'Souza, Benedicta, Rajeevalochana Parthasarathy, Sreekantha, and Vivian D'Souza. 2011. Acid Phosphatase as a Marker in Malaria. *Indian Journal of Clinical Biochemistry* 26:396–399.

Dunn, Frederick L. 1965. On the Antiquity of Malaria in the Western Hemisphere. *Human Biology* 37(4):385-393.

Dye, David H. and Cheryl Anne Cox (editors). 1990. *Towns and Temples along the Mississippi*. University of Alabama Press, Tuscaloosa.

El-Najjar, Mahmoud Y., Betsy Lozoff, and Dennis J. Ryan. 1975. The Paleoepidemiology of Porotic Hyperostosis in the American Southwest: Radiological and Ecological Considerations. *American Journal of Roentgenology* 125(4):918-924.

El-Najjar Mahmoud Y., Dennis J. Ryan, Christy G. Turner, and Betsy Lozoff. 1976. The Etiology of Porotic Hyperostosis among the Prehistoric and Historic Anasazi Indians of Southwestern United States. *American Journal of Physical Anthropology* 44:477–487.

Faust, Ernest Carroll. 1951. The History of Malaria in the United States. *American Scientist* 39(1):121-130.

Gilles HM. 1997. Pathology of Malaria. In *Handbook of Malaria Infection in the Tropics,* edited by G. Carosi and F. Castelli. Associazione Italiana "Amici di R. Follereau," Bologna, Italy.

Giles, Bretton, Jennifer Bauder, and Marta P. Alfonso-Durruty. 2010. Revisiting the Dead at Helena Crossing, Arkansas. *Southeastern Archaeology* 29(2):323-340.

Gowland, Rebecca L. and A.G. Western. 2012. Morbidity in the Marshes: Using Spatial Epidemiology to Investigate Skeletal Evidence for Malaria in Anglo-Saxon England (AD 410-1050). *American Journal of Physical Anthropology* 147:301–311.

Hengen, Otto P. 1971. Cribra Orbitalia: Pathogenesis and Probable Etiology. *Homo* 22:57-75.

Holland, Thomas D. and Michael J. O'Brien. 1997. Parasites, Porotic Hyperostosis, and the Implications of Changing Perspectives. *American Antiquity* 62:183–193.

Hogue, S. Homes. 2007. Mississippian and Protohistoric/Early Contact Diet and Health: Biological and Cultural Continuity and Change in Oktibbeha County, Mississippi. *Southeastern Archaeology* 26(2):246-268.

Hudson, Charles. 1997. *Knights of Spain, Warriors of the Sun: Hernando de Soto and the South's Ancient Chiefdoms*. University of Georgia Press, Athens.

Hudson, Charles, Marvin T. Smith, and Chester DePratter. 1990. The Hernando de Soto Expedition: From Mabila to the Mississippi River. In *Towns and Temples along the Mississippi*, edited by D.H. Dye and C.A. Cox. University of Alabama Press, Tuscaloosa, pp. 181-207.

Hume, Jennifer C.C., Emily J. Lyons, and Karen P. Day. 2003. Malaria in Antiquity: A Genetics Perspective. *World Archaeology* 35:180–192.

Humphreys, Margaret. 2001. *Malaria: Poverty, Race, and Public Health in the United States*. Johns Hopkins University Press, Baltimore.

Kelley, David B., Donald G. Hunter, Paul S. Gardner, Angela Tine, and Larry L. Tieszen. 1996. The McLelland and Joe Clark Sites: Protohistoric-Historic Caddo Farmsteads in the Red River Valley of Northwest Louisiana. *Southeastern Archaeology* 15(1):81-102.

Kelton, Paul. 2007. *Epidemics and Enslavement: Biological Catastrophe in the Native Southeast, 1492-1715*. University of Nebraska Press, Lincoln.

Lallo, J.W., George J. Armelagos, and R.P. Mensforth. 1977. The Role of Diet, Disease, and Physiology in the Origin of Porotic Hyperostosis. *Human Biology* 49:471–483.

Larsen, Clark Spencer. 1994. In the Wake of Columbus: Native Population Biology in the Postcontact Americas. *Yearbook of Physical Anthropology* 37:109-154.

Larsen, Clark Spencer and George R. Milner. 1994. *In the Wake of Contact: Biological Responses to Conquest*. Wiley-Liss, New York.

Larsen, Clark Spencer. 1995. Biological Changes in Human Populations with Agriculture. *Annual Review of Anthropology* 24:185-213.

Larsen, Clark Spencer, Mark C. Griffin, Dale L. Hutchinson, Vivian E. Noble, Lynette Norr, Robert F. Pastor, Christopher B. Ruff, Katherine F. Russell, Margaret J. Schoeninger, Michael Schultz, Scott W. Simpson, and Mark F. Teaford. 2001. Frontiers of Contact: Bioarchaeology of Spanish Florida. *Journal of World Prehistory* 15(1):69-123.

Listi, Ginesse A. 2013. Bioarchaeological Analysis of Subsistence and Health at the Lake George Site, Mississippi (22YZ557). *Southeastern Archaeology* 32:111-128.

Lallo, John W., George J. Armelagos, and Robert P. Mensforth. 1977. The Role of Diet, Disease, and Physiology in the Origin of Porotic Hyperostosis. *Human biology* 471-483.

Loveland, Carol J., John B. Gregg and William M. Bass. 1985. Ancient Osteopathology from the Caddoan Burials at the Kaufman-Williams Site, Texas. *Plains Anthropologist* 30(107):29-43.

Loy, Dorthy, Weimin Liu, Yingying Li, Gerald H. Learn, Lindsey J. Plenderleith, Sesh A. Sundararaman, Paul M. Sharp, and Beatrice H. Hahn. 2017. Out of Africa: Origins and Evolution of the Human Malaria Parasites Plasmodium falciparum and Plasmodium vivax. *International Journal for Parasitology* 47:87–97.

Masterson, K. M. 2014. *The Malaria Project: The U.S. Government's Secret Mission to Find a Miracle Cure*. New American Library, New York.

Mensforth, Robert P., C. Owen Lovejoy, John W. Lallo, and George J. Armelagos. 1978. Part Two: The Role of Constitutional Factors, Diet, and Infectious Disease in the Etiology of Porotic Hyperostosis and Periosteal Reactions in Prehistoric Infants and Children. *Medical Anthropology* 2:1-59.

Mathers, Clay, Jeffery M. Mitchem, and Charles M. Haecker, eds. 2013. *Native and Spanish New Worlds : Sixteenth-Century Entradas in the American Southwest and Southeast*. University of Arizona Press, Tuscon.

Maxcy, Kenneth F. 1923. The Distribution of Malaria in the United States as Indicated by Mortality Reports. *Public Health Reports (1896-1970)* 38(21):1125-1138.

Milanich, Jerald T. 1993. The Hernando de Soto Expedition and Spain's Efforts to Colonize North America. In *The Expedition of Hernando de Soto West of the Mississippi, 1541-1543,* edited by G.A. Young and M.P. Hoffman, pp. 11-28. University of Arkansas Press, Fayetteville.

Miquel-Feucht M.J., M. Polo-Cerdá, and J.D. Villalaín-Blanco. 1999. El síndrome criboso: Criba Femoral vs Criba Orbitaria. In *Sistematización metodológica en Paleopatología*, edited by J.A. Sánchez, pp. 221-237. Actas V Congreso Nacional AEP, Spain.

McIlvaine, Britney Kyle. 2015. Implications of Reappraising the Iron-deficiency Anemia Hypothesis. *International Journal of Osteoarchaeology* 25(6):997-1000.

Mitchem, Jeffery M., Timothy S. Mulvihill, Jami J. Lockhart, and David W. Stahle. 2016. We Think We Found Hernando De Soto's Cross at Casqui. A Paper presented at the 73rd Annual Meeting of the Southeastern Archaeological Conference, Athens, Georgia, October 28, 2016.

Moreau R, Malu D. Tshikudi, M. Dumais, E. Dalko, V. Gaudreault, H. Roméro, C. Martineau, O. Kevorkova, J.S. Dardon, E.L. Dodd, et al. 2012. Alterations in Bone and Erythropoiesis in Hemolytic Anemia: Comparative Study in Bled, Phenylhydrazine-treated and Plasmodium-infected mice. *PLOS ONE* 7:e46101.

Morse, Phyllis A. 1981. *Parkin: The 1978-1979 Archeological Investigations of a Cross County, Arkansas Site*. No. 13. Arkansas Archeological Survey, University of Arkansas.

Morse, Phyllis A. 1993. The Parkin Archaeological Site and its Role in Determining the Rout of the do Soto Expedition. In *The Expedition of Hernando de Sot West of the Mississippi, 1541-1543*, edited by G.A. Young and M.P Hoffman. University of Arkansas Press, Fayetteville. pp. 58-67.

Murray, K. A. *Bioanthropological Analysis of Parkin (3CS29).* Unpublished Contract Report submitted to Arkansas Archaeological Survey, Fayetteville.

National Institute of Health. 2016. Malaria. www.niaid.nih.gov/diseases-conditions/malaria-parasite.

Nerlich, Andreas G., Bettina Schraut, Sabine Dittrich, Thomas Jelinek, and Albert R. Zink. 2008. Plasmodium falciparum in ancient Egypt. *Emerging Infectious Diseases* 14:1317–1319.

Nunn, John F., and Eddie Tapp. 2000. Tropical Diseases in Ancient Egypt. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 94(2):147-153.

Offenbecker, A.M., and D.T. Case. 2016. Health Consequences of European Contact in the Great Plains: A Comparison of Systemic Stress Levels in Pre- and Post-Contact Arikara Populations. *International Journal of Osteoarchaeology* 26:502-513.

Phillips, Phillip, James A. Ford, and James B. Griffin. 1951. *Archaeological Survey in the Lower Mississippi Alluvial Valley*. Peabody Museum of American Ethnology and Archaeology 25, Cambridge.

Porter, Larry. 2016. *Salvage Excavations at the Wild Violet Site, 3LO226, a Woodland Period Site in Logan County, Arkansas*. Submitted to U.S. Army Corps of Engineers and the Caddo Nation. Arkansas Archaeological Survey, Fayetteville.

Powell, Mary L. 1992. Health and Disease in the Late Prehistoric Southeast. In *Disease and Demography in the Americas*, edited by J.W. Verano and D.H. Ubelaker. Smithsonian Institution Press, Washington DC, pp. 41-54.

Powell, Mary L. P.S. Bridges, and A.M.W. Mires, editors. 1991. *What Mean These Bones? Studies in Southeastern Bioarchaeology.* University of Alabama Press, Tuscaloosa.

Rabino Massa, Emma, Nicoletta Cerutti, and Marin D. Savoia. 2000. Malaria in Ancient Egypt: Paleoimmunological Investigation on Predynastic Mummified Remains. *Chungará (Arica)* 32(1):7-9.

Ramenofsky, Ann F. 1987. *Vectors of Death: The Archaeology of European Contact*. University of New Mexico Press, Albuquerque.

Ramenofsky, Ann F. and Patricia Galloway. 1997. Disease and the Soto Entrada. In *The Hernando De Soto Expedition : History, Historiography, and "Discovery" in the Southeast,* edited by P.K. Galloway. University of Nebraska Press, Lincoln, pp. 259-282.

Ramenofsky, Ann F., Alicia K. Wilbur and Anne C. Stone. 2003. Native American Disease History: Past, Present and Future Directions. *World Archaeology* 35(2):241-257.

Rivera, Frances, and Marta Mirazón Lahr. 2017. New Evidence Suggesting a Dissociated Etiology for Cribra Orbitalia and Porotic Hyperostosis. *American Journal of Physical Anthropology* 164(1):76-96.

Roberts, Charlotte A. 2000. Infectious Disease in Biocultural Perspective: Past, Present and Future Work in Britain. *Human osteology in archaeology and forensic science, London, Greenwich Medical Media* pp.145-162.

Roberts, Charlotte A., and Jane E. Buikstra. 2003. *The Bioarchaeology of Tuberculosis*. University Press of Florida, Gainesville.

Rodrigues, Priscila T., Hugo O. Valdivia, Thais C. de Oliveira, João Marcelo P. Alves, Ana Maria R. C. Duarte, Crispim Cerutti-Junior, Julyana C. Buery, Cristiana F. A. Brito, et al. 2018. Human Migration and the Spread of Malaria Parasites to the New World. *Nature Scientific Reports,* 8:1993.

Rose, Jerome C. 1984. Bioarchaeology of the Cedar Grove Site. In *Cedar Grove: An Interdisciplinary Investigation of the Late Caddo Farmstead in the Red River,* edited by N.L. Turbowitz, pp. 227-256. Research Series No.23. Arkansas Archaeological Survey, Fayetteville.

Rose, Jerome C., editor. 1999. Bioarchaeology of the South Central United States. Arkansas Archaeological Survey Research Report No. 55. University of Arkansas.

Rothschild, Bruce. 2012. Extirpolation of the Mythology that Porotic Hyperostosis is Caused by Iron Deficiency Secondary to Dietary Shift to Maize. *Advances in Anthropology* 2:157–160.

Setzer, Teddi J. 2010. Malaria in Prehistoric Sardinia (Italy): An Examination of Skeletal Remains from the Middle Bronze Age. *ProQuest Dissertations and Theses* 338.

Setzer, Teddi J. 2014. Malaria detection in the field of paleopathology: A Meta-analysis of the State of the Art. *Acta Tropica* 140:97–104.

Sherman, I.W., editor. 1998. *Malaria: Parasite Biology, Pathogenesis, and Protection*. ASM Press, Washington, D.C.

Smith-Guzmán, Nicole E. 2015a. Cribra Orbitalia in the Ancient Nile Valley and its Connection to Malaria. *International Journal of Paleopathology* 10:1–12.

Smith-Guzmán, Nicole E. 2015b. The Skeletal Manifestation of Malaria: An Epidemiological Approach Using Documented Skeletal Collections. *American Journal of Physical Anthropology* 158(4):624–635.

Stuart-Macadam, P. 1987. Porotic Hyperostosis: New Evidence to Support the Anemia Theory. *American Journal of Physical Anthropology* 74:521–526.

Stuart-Macadam, P. 1989. Porotic Hyperostosis: Relationship between Orbital and Vault Lesions. *American Journal of Physical Anthropology* 80:187–193.

Steckel, Richard H., and Jerome C. Rose (editors). 2002. *The Backbone of History: Health and Nutrition in the Western Hemisphere,* Cambridge University Press: Cambridge.

Sundararaman, Sesh A., Lindsey J. Plenderleith, Weimin Liu, Dorothy E. Loy, Gerald H. Learn, Yingying Li, Katharina S. Shaw, Ahidjo Ayouba, Martine Peeters, Sheri Speede, George M. Shaw, Frederic D. Bushman, Dustin Brisson, Julian C. Rayner, Paul M. Sharp & Beatrice H. Hahn. 2016. Genomes of Cryptic chimpanzee Plasmodium Species Reveal Key Evolutionary Events Leading to Human Malaria. *Nature Communications* 7:11078.

Verano, John W. and Douglass H. Ubelaker (editors). 1992. *Disease and Demography in the Americas*. Smithsonian Institution Press, Washington.

Walker, Phillip L., Rhonda R. Bathurst, Rebecca Richman, Thor Gjerdrum, and Valerie A. Andrushko. 2009. The Causes of Porotic Hyperostosis and Cribra Orbitalia: A Reappraisal of the Iron-Deficiency-Anemia Hypothesis. *American Journal of Physical Anthropology* 139:109–125.

Webb, J.L.A. 2009. *Humanity's Burden: A Global History of Malaria*. Cambridge University Press, Cambridge.

Whayne, Jeannie M., Thomas A. DeBlack, George Sabo, Joseph P. Swain, and Joseph Swain. 2013. *Arkansas: A Narrative History*. University of Arkansas Press, Fayetteville.

World Health Organization. 2018. Malaria. www.who.int/malaria/en.

Young, Gloria A. and Michael P. Hoffman (editors). 1993. *The Expedition of Hernando de Soto West of the Mississippi, 1541-1543*. University of Arkansas Press, Fayetteville.

Zaino, Edward C. 1964. Paleontologic Thalassemia. *Annals of the New York Academy of Sciences* 119:402–412.

Zinke, M. 1975. *An Analysis of Mississippian Burial Components from the Hazel Site, Poinsett County, Arkansas.* Unpublished Master's Thesis. Department of Anthropology, University of Arkansas, Fayetteville.