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Paleoparasitology – Human Parasites in Ancient Material

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Contents

Abstract

Parasite finds in ancient material launched a new field of science: paleoparasitology. Ever since the pioneering studies, parasites were identified in archaeological and paleontological remains, some preserved for millions of years by fossilization. However, the paleoparasitological record consists mainly of parasites found specifically in human archaeological material, preserved in ancient occupation sites, from prehistory until closer to 2015. The results include some helminth intestinal parasites

still commonly found in 2015, such as *Ascaris* lumbricoides, *Trichuris trichiura* and hookworms, besides others such as Amoebidae and *Giardia intestinalis*, as well as viruses, bacteria, fungi and arthropods. These parasites as a whole provide important data on health, diet, climate and living conditions among ancient populations. This chapter describes the principal findings and their importance for knowledge on the origin and dispersal of infectious diseases.

1. Introduction – Parasitism

This chapter uses the broad definition of parasitism. The concept ranges all the way from mobile fragments of genetic material, both transmissible and non-transmissible, up to higher plants and vertebrate animals (Trager, 1988; Araújo et al., 2003; Araújo and Ferreira, 2014; Schmid-Hempel, 2011; Ewald and Swain Ewald, 2014). The definition considers the complex parasite–host–environment system and its interdependent relations, where changes or alterations in one component can influence the others. This is an ecological focus to parasitism, since both the macroenvironment and microenvironment induce changes and influence relations. The study of parasitism should be based on ecology. These relations consider the classifications between symbionts, commensals, mutualists and parasites, among others, as a single concept called parasitism. This unified approach allows a better understanding of parasitic diseases in their evolutionary process.

The broad definition of parasitism is not new. Brazilian parasitologist Samuel B. Pessoa, in the first edition of his book *Parasitologia Médica* in 1945, already reminded parasitologists that 'parasitism, commensalism, and symbiosis are categories created by our spirit, all displaying characteristics of the same general laws' (Pessoa, 1951, p. 5).

When German scientists Karl Georg Friedrich Rudolf Leuckart and Heinrich Anton de Bary independently created the concepts of symbiosis and parasitism, respectively (both in 1879), they made no mention of diseases, benefits or harm that could result from the relationship between species. The same connotation was already revisited by various other authors, including French parasitologist Emile Brumpt (Brumpt, 1913). Beginning with the first editions of his book, Brumpt highlighted the fact that parasitism and symbiosis converged in a single concept, since beings that were considered parasites and those viewed as symbionts or commensals could all cause harm or benefit to their hosts.

We thus propose the concept of parasitism as an organism that finds its ecological niche in another, namely, its host (Araújo et al., 2003; Araújo and Ferreira, 2014). The disease or harm does not result inexorably from the parasite's presence in the host, but can also result from conditions of the parasite, the host or the environment.

This article mainly features protozoa, helminths and arthropods, always highlighting the ecological characteristics of parasitism. Other parasites will be mentioned in passing.

The article assumes Aidan Cockburn's ideas on parasitic diseases: "Infectious disease is composed of three variables, the host, the pathogen and the environment. It is a constant state of flux, capable of changing in step with any variation in any one of its components. New diseases appear, old ones alter, and some may disappear completely" (Cockburn, 1963). The process is dynamic. Parasitic infection means a parasite's presence in a given host, but parasitic (or infectious) disease is the set of signs and symptoms that characterize an alteration resulting from changes in one or more of the components in the parasite–host–environment system. Infection and disease are thus distinct phenomena in the host, both dependent on the parasite's presence. Parasitic disease can occur in an individual or become a population-based phenomenon, characterized as an endemic or epidemic, with different overall impacts on the hosts. On the one hand, infection does not necessarily lead to disease, and many parasite species can be present in the host, often throughout life, without any symptoms. Infection is not synonymous with disease, and parasitic disease is the same as infectious disease (Araújo et al., 2003).

We use the concept of virulence as the parasite's capacity to multiply and spread in a given host population, while pathogenicity refers to the capacity to induce a lesion or disease (Araújo et al., 2003). Other authors use the terms synonymously (Poulin and Combes, 1999). However, the term pathogen becomes inapplicable, since the development of signs and symptoms in the host depends on the three components (parasite, host and environment), and the parasite's presence is thus not the exclusive determinant of the disease. We will always use the term 'parasite', both for infections and for their capacity to cause lesions in the host.

There are various examples of the above-mentioned issues, and they can be exemplified by the classical experiment on infection with *Entamoeba invadens* in snakes (Barrow and Stockton, 1960). When the amoebae remain in the host at an adequate temperature, they behave without causing any clinical manifestations, feeding on bacteria and

other elements from the intestinal content. However, when raising the temperature alters the environment, the parasites invade the tissues and the snake can die. Barrow and Stockton (1960) conducted experiments in eight species of snakes infected with this parasite under different temperatures, from 13 ºC to 25 ºC. No lesions were observed at lower temperatures, although the amoebae continued to be isolated in culture. However, at a temperature ≥25 ºC, the snakes presented lesions, and even those that did not show lesions at low temperatures became sick at higher temperatures.

When Carlos Chagas described the signs and symptoms of American trypanosomiasis, later named Chagas disease in his honor, the first case of an infected patient was a child with an acute manifestation of the disease, in whom trypanosomes were found in the bloodstream (Coura, 1997). The child was Berenice, and she died at 79 years of age from a cause other than Chagas disease. For years she attended congresses during which she was examined without presenting severe symptoms, but it was always possible to isolate *Trypanosoma cruzi* from her peripheral blood during her >70 years of life (Salgado, 1980).

2. Humans and Parasites

Throughout human evolution, from prehominids to present-day *Homo sapiens*, changes occurred in the relationship between humans and their parasites due to territorial occupations and changes made to environments by a host capable of creating technologies, migrating and altering newly occupied territories in a complex sociobiological relationship with nature. In this context, paleoparasitology consists of methods and techniques capable of recovering, in space and time, diverse moments in the trajectory of human hosts and their parasites, both those inherited from ancestors and those acquired from the environment while humans have roamed the planet.

Like all biological species, parasites have a single origin. When they become species-specific in a given host species, they accompany it wherever the environment allows the maintenance of their life cycle. Parasites are excellent markers of prehistoric migrations, since they are found in occupation sites in events and landscapes traversed by ancestral human groups (Araújo et al., 2008). These are called phylogenetically inherited parasite species, when they are shared by phylogenetically proximate hosts, as exemplified by *Enterobius vermicularis* infection in humans and phylogenetically closely related primates.

There are also species of parasites that originated from phylogenetically remote hosts and that adapted to humans over the course of their evolutionary history and the conquest of new territories. An example is infection with *Trypanosoma cruzi*, a protozoan whose original life cycle included wild mammals and the insect vector. However, *T. cruzi* adapted perfectly to the human host, including the domiciliation of triatomine species, ever since the first human migrations to the Americas (Ferreira et al., 2011). These are called ecologically acquired parasites, having adapted to (or infected) humans during part of their cycle by mechanisms in which humans approached their natural niche of infection (Pavlovsky, 1964) or pathocenosis (Grmek, 1983). Pathoecology has been defined as reconstruction of the ecology of a disease based on evidence recovered from archaeological sites (Reinhard and Pucu, 2014), which can also explain some parasites acquired during the social and biological evolution of the human species.

3. Paleoparasitology

Paleoparasitology is the study of parasites found in archaeological or paleontological material (Ferreira, 2014). This branch of paleopathology and parasitology opened new avenues for studying the evolution of the health-disease process in the human species, as well as in other hosts (Mitchell, 2013). Paleoparasitological findings provide a consistent source for paleoepidemiology by revealing the presence of infection in a given archaeological context and the possible consequences for the emergence or disappearance of infectious diseases in prehistoric populations.

Franco-British physician and microbiologist Sir Marc Armand Ruffer was the pioneer of paleoparasitology. Ruffer studied Egyptian mummies in the early twentieth century, developing rehydration techniques for mummified tissues, allowing their visual examination in histological sections with staining used in routine histopathology. He found calcified and well-preserved *Schistosoma haematobium* eggs in the kidney tissue of a mummy dated 3200 BC (Ruffer, 1910). This was the first parasite find in ancient human material, shedding light on haematuria in Egypt, which had been described in ancient texts but not previously proven. Although Ruffer's article does not show an image of the egg, his diagnosis was confirmed by German parasitologist Arthur Looss and Scottish pathologist Dr Alexander R. Ferguson, Ruffer's faculty colleagues at the Cairo School of Medicine.

For years, little was published in the field of paleoparasitology, although some studies were done with coprolites from extinct animals and with human coprolites, aimed at describing ancient diet. The first results that demonstrated the presence of intestinal helminths in preserved human remains came from cooperation between archaeologists and parasitologists. Lothar Szidat described *Ascaris lumbricoides* and *Trichuris trichiura* eggs in a naturally mummified human body discovered during excavations of peat fields in Prussia (Szidat, 1944), in a swampy region with low pH that facilitated the preservation of bodies sacrificed hundreds of years ago.

Other collaborations between archaeologists and parasitologists occurred during this initial period, as reviewed by Dittmar (2009) and Reinhard and Araújo (2012), but it was the partnership between botanist Eric Callen and parasitologist Thomas Cameron that laid the ground for the technique used to study parasites in coprolites. Callen and Cameron (1960) developed the rehydration of desiccated materials with trisodium phosphate aqueous solution. With this technique, desiccated materials like unlithified coprolites (and others potentially containing parasites) recover their original morphology, allowing parasitological testing using routine techniques. Rehydration does not alter the parasite structure measurements, and the standard measurements of current parasites can be used (Fugassa, 2014). Since then, parasitological tests in coprolites have multiplied, with growing collaboration between parasitologists and archaeologists. Coprolites are found in archaeological layers (Figures 1–3) or in the intestinal contents of preserved bodies.

In 1978, research in paleoparasitology in Brazil began at the Oswaldo Cruz Foundation, and the term paleoparasitology was coined

Figure 1. Archaeological layers at the Site of Toca do Boqueirão da Pedra Furada, 1986 (Piauí state, Brazilian northeast).

Figure 2. The Italian archaeologist Fabio Parenti excavating a coprolite in the Site of Toca do Boqueirão da Pedra Furada, 1986 (Piauí state, Brazilian northeast).

to refer to this field of knowledge (Ferreira et al., 1979). At the time, there was little research production in the area, with just a few articles on parasite finds published by North American authors in important journals or even mimeographed copies (Samuels, 1965; Heizer and Napton, 1969; Fry, 1970) and reviews on analyses in human coprolites in the United States, especially focusing on diet and food remains (Wilke and Hall, 1975), while England and a few other European countries featured studies on parasites in ancient material (Gooch, 1972; Jones, 1982). As with the first studies in these countries, Brazilian researchers developed collaborations with archaeology teams, since the entire paleoparasitological analysis hinged on painstaking

Figure 3. Archaeological layer showing the coprolite evidenced in the Site of Toca do Boqueirão da Pedra Furada, 1986 (Piauí state, Brazilian northeast).

archaeological excavations. It is indispensable to determine the sample's age, either through radiocarbon dating or another applicable technique, as well as a detailed description of the archaeological context.

Brazil's first samples from coprolites and mummified bodies came from the Institute of Brazilian Archaeology in Rio de Janeiro and soon afterwards from sites in Minas Gerais State and the archaeological region of São Raimundo Nonato, Piauí State, in Northeast Brazil, where we found hookworm and *Trichuris trichiura* eggs (Ferreira et al., 1980, 1983; Araújo et al., 1981). Our research group began collaboration with archaeologists from other countries early on: in 1983, we received samples of coprolites from Dr Lautaro Nuñez at Universidad del Norte in Chile. These were human coprolites containing *Diphyllobothrium pacificum* eggs, according to morphometric diagnosis. This was the first diagnosis of *D. pacificum* in prehistoric groups from the Andean coast, followed by a series of other finds, confirming the ingestion of raw sea fish by the local population (Araújo et al., 1983; Patrucco et al., 1983). These eggs differ only in size from those of another species, *Diphyllobothrium latum*, that has infected the human host since the Neolithic and whose cycle takes place in freshwater fish and is common in Europe.

Collaboration between parasitologists and archaeologists led to new progress in the field (Reinhard, 1992a; Ferreira et al., 1984; Dittmar, 2009), concentrating on the study of parasites in ancient material and their interpretations. Paleoparasitology is a broad field, not necessarily limited to the human host or to animals that may transmit their parasites to humans but also including studies of parasites in extinct or current animals, whose remains can be found and which contribute to the study of the paleoclimate and the origins of parasitism in current hosts (Poinar and Boucot, 2006; Dentzien-Dias et al., 2013; Poinar, 2014; Silva et al., 2014).

Paleoparasitology now intersects with various other fields of science, based on the extensive current literature, featuring special sections in mainstream scientific journals such as *The Journal of Parasitology* (Faulkner and Reinhard, 2014) and the *International Journal of Paleopathology* (Buikstra, 2013; Dittmar et al., 2012; Dittmar, 2013). A major portion of the research focuses on the reconstitution of eating habits and health in the past, inferred by finding parasites that are specific to certain animals (Le Bailly and Bouchet, 2013; Arriaza et al., 2013a; Sianto et al., 2012; Jimenez et al., 2012; Reinhard et al., 2013); diet, parasites and health of populations (Reinhard et al., 2007); description of specific human parasites, the finding of which allowed new approaches to theories on migrations and prehistoric contacts between Asian and American populations by transpacific navigations (Araújo et

al., 1981, 1988, 2008) and other great waves of human groups moving around the medieval world (Anastasiou and Mitchell, 2013), and studies in paleoepidemiology and the origin of infectious diseases (Hugot et al., 1999; Araújo et al., 2009; Ferreira et al., 2011; Baum and Bar-Gal, 2003), among others.

In addition to these advances in knowledge obtained by microscopic analysis of ancient material, new techniques, particularly in molecular biology, have allowed diagnoses that were previously imprecise or false negative, like the detection of infections by intestinal and systemic protozoa. The diagnosis of protozoa can now be done with specific enzyme-linked immunosorbent assays kits, as with *Giardia intestinalis*, *Cryptosporidium* sp. and *Entamoeba histolytica* (Gonçalves et al., 2004; Le Bailly et al., 2008; Frías et al., 2013), and molecular biology techniques for systemic protozoa (Fernandes et al., 2008; Lima et al., 2008; Guhl et al., 2014).

There is debate on the origin and dispersal of some diseases such as syphilis, tuberculosis and the plague in Greece during the siege of Athens around 400 BC. The debate on the Athens plague involves speculation, that is, whether it was an epidemic of smallpox, epidemic typhus, typhoid fever or bubonic plague itself. Researchers from the University of Athens published the results of the analysis of dental pulp from skeletons dated to that time and showed the presence of DNA fragments from *Salmonella enterica* serovar Typhi, the etiological agent of typhoid fever, but their conclusions were challenged by Shapiro et al. (2006). As for syphilis and tuberculosis, the discussion focuses on their origin or absence in the Americas before the arrival of Columbus and his sailors. Syphilis purportedly originated in the Americas, but tuberculosis was believed to have been introduced during colonial times. Data from paleoparasitology now leave no doubt that the tuberculosis bacillus already circulated in the prehistoric population of the Americas (Gómez i Prati et al., 2003; Wilbur and Buikstra, 2006; Wilbur et al., 2008; Stone et al., 2009; Jaeger et al., 2013), but the jury is still out on syphilis, although the origin of *Treponema* species in the African continent is well established (Harper et al., 2008).

Archaeologists, with the exception of excavations performed by paleontologists, sponsored all paleoparasitological studies. Each archaeologist had his or her own research goal to uncover ancient cultural practices and to understand the vitality of those cultures. Recognizing this, Reinhard (1992a,b) called attention to parasitology as a tool for archaeologists (Reinhard et al., 1985). As reviewed by Bryant and Reinhard, several archaeologists adjusted their research designs specifically to recover coprolites (Bryant and Reinhard, 2012; Reinhard and

Bryant, 2008). Parasitologists recover data that have been unique in fulfilling archaeological research goals (Reinhard, 1992a,b).

The vitality of archaeological populations has been an inherent theme in archaeology that was codified in "bioarchaeology," which focuses on the analysis of skeletal human remains (Buikstra and Beck, 2008). By comparative analysis of bone pathology, bioarchaeologists assess the relative adaptive success of past populations. Because skeletons were the source of data, evidence of infectious disease was often limited to nonspecific indicators of stress, periosteal bone reaction and porotic hyperostosis. The evidence for actual infectious organisms comes from analysis of sediments associated with skeletons (Fugassa, 2014) or from coprolites (Jiménez et al., 2012).

A long-standing archaeological hypothesis was that ancient hunter-gatherers, relative to later agricultural peoples, were free of infections (Diamond, 1987). Testing this hypothesis was a research design of many coprolite recovery excavations in the 1960s and 1970s. To a large degree, archaeology and ethnography support this hypothesis (Reinhard, 1988; London and Hruschka, 2014). The low prevalence of parasitism led to a desire to explore the basis for hunter-gatherer avoidance of parasitism. One aspect is related to the presence of medicinal plants in the diet (Reinhard, 1985). Beyond the use of anthelmintics among hunter-gatherers, small band size, diffuse population and frequent movement of camps helped reduce parasite infection (Reinhard, 1988). The agricultural revolution was recognized by archaeologists as an abrupt change in the human condition that gave rise to a dramatic increase in infection (Diamond, 1987). The bone pathology, porotic hyperostosis, generally exhibits a higher prevalence among agricultural skeletal series. Porotic hyperostosis is exhibited as "spongy" expansions of the cranial diploe coinciding with an erosion of the outer cranial table of the parietals and occipital of effected skulls. The etiology of the pathology was long debated and finally resolved by Walker et al. (2009) who concluded that porotic hyperostosis lesions "are a result of the megaloblastic anemia acquired by nursing infants through the synergistic effects of depleted maternal vitamin B12 reserves and unsanitary living conditions that are conducive to additional nutrient losses from gastrointestinal infections around the time of weaning" (Walker et al., 2009, p. 119). The fluctuations of porotic hyperostosis have been a key issue in archaeology, especially in the Americas, that now can be asserted to have an origin in large part to parasitism (Reinhard, 1992a,b). Jiménez et al. (2012) underscored Walker et al. (2009) model in their characterization of extreme helminth prevalence at a site in Durango, Mexico, the skeletons from which exhibited porotic hyperostosis.

The period of agriculture is itself nuanced with the emergence of complex societies and empire civilizations. These nuances have been explored in the Andes for the Inca Empire and the earlier Chiribaya culture (Martinson et al., 2003; Santoro et al., 2003; Reinhard and Buikstra, 2003). The differences in louse and helminth infections were compared between three villages in the Ilo area of southern Peru based on the analysis of coprolites from burials, mummies and latrines. The sites included a coastal fishing village, an inland farming village and an administrative town. Social differentiation was best defined at the town where a wide variety of social status was evidenced by burial offerings. Fish tapeworm, *Diphyllobothrium pacificum* was the most common helminth found. This tapeworm infection was most common among the farmers (69%), less common among the fishers (25%) and lowest at the administrative center (20%). The presence of tapeworm at all sites indicates that fish was traded to all habitations, but control of the infection was best accomplished for the ruling elite and among the source fisher population itself (Martinson et al., 2003). The Chiribaya population was joined by refugees from the collapse of the Tiwanaku Empire of the Lake Titicaca region. Analysis of head lice from the refugee population mummies compared to the Chiribaya mummies showed that the refugees were more infested than even the most infested Chiribaya demographic group. This suggests that the lifestyle of the refugees was cramped and stressful such that grooming was reduced and reinfestation was unavailable (Reinhard and Buikstra, 2003). The Chiribaya culture farmers of the Atacama Desert of northern Chile were sometimes subsumed in the Inca Empire. This was the fate of Mitas Chiribaya farmers in the Lluta Valley of Chile. Because of the high corn production of the valley, it was absorbed into the Empire. Previously, farmers had lived in dispersed, small communities. Under the Incas, the farmers were aggregated into large concentrated towns. The corn production of the Inca farmers was largely taken away in taxes so that the farmers had to diversify their subsistence with fishing. Parasitologically, both Inca and Chiribaya farmers were parasitized by whipworm (*Trichuris trichiura*). However, differences emerged as the farmers became incorporated into the Empire. Hymenolepidid infection, present in the Chiribaya farmers, disappeared among the Inca farmers. Pinworm infection (*Enterobius vermicularis*) was absent among Chiribaya farmers but subsequently reached a prevalence of 21% among the Inca farmers. Thus, the parasite record signals crowding and subsistence diversification as the Empire expanded.

Catastrophic climate events can have paleoparasitological implications. Arriaza et al. (2010) found this to be the case for preagriculture

Chinchorro peoples of the coast of northern Chile. The data suggest that El Niño events caused fluctuation in coastal fish resources that in turn led to varying susceptibility of fishers to fish tapeworm infection. But drought in another part of the hemisphere is reflected in the archaeoparasite record. The excavation of Antelope House in Canyon de Chelly, Arizona, revealed the hundreds of years of prehistory of a Puebloan village that terminated with the Great Drought that lasted from 1276 and continued through 1299 (Morris, 1988). Parasite prevalence and diversity increased at the end of the occupation as people aggregated at the last villages with persistent water sources (Reinhard, 2008). This was mirrored at Elden Pueblo near Flagstaff, Arizona, that showed the highest concentration of helminth eggs in the final occupation strata of the site. Parasitism peaked around the time of abandonment.

The emergence and control of parasitism in historic times, and relation of ethnicity to infection, is a topic among historic archaeologists. Charles Fisher and his colleagues directed excavations of latrine sediments, as well as sediments from yards, streets, drains and other contexts to reconstruct the emergence and control of parasitism in Albany, New York. The analysis of hundreds of samples showed the introduction of geohelminths (soil-transmitted helminths) with the original Dutch colony. Infection peaked in the late 1700s and early 1800s but began to abate in the late 1800s as drainages were covered and as water projects brought clean water into the city. Interestingly, the water projects were designed first to provide water for fighting fires. By the turn of the twentieth century, fecal-borne parasitism was controlled in both the elite and poor neighborhoods (Fischer et al., 2007).

The longer-term emergence and control of fecal-borne geohelminths have been a source of research for many analysts working in Europe (Reinhard and Pucu, 2014). This research falls into the archaeological construct of paleoepidemiological transitions. There were two paleoepidemiological transitions. The first was associated with the Neolithic Revolution, which separated the ancient hunter-gatherer prehistory with the agricultural period. The Neolithic Revolution is thought to have resulted in a rise in infectious diseases. The Industrial Revolution is thought to represent reduction of infectious disease and a rise of occupational disease. This scheme was tested by Reinhard and Pucu (2014) for both Europe and the Americas. Interestingly, the paleoparasitology record of Europe bears out the hypothesized changes. The Neolithic Revolution is accompanied by the ubiquitous presence of whipworm and roundworm (*Ascaris lumbricoides*) infections. By

Roman times, these parasites are found wherever archaeologists sample latrines, burials or mummies. This transition, strangely, does not exist in the Americas where geohelminths, although introduced long in prehistory, never take hold as ubiquitous parasites among agriculturalists. The reasons for a relatively geohelminth free American prehistory have yet to be elucidated. Reinhard and Pucu (2014) suggest that Native American medicinal treatments, settlement patterns, fecal avoidance and lower population density may have helped maintain post-agricultural revolution parasitism at low levels. In addition, the European use of human feces as fertilizer and dependence on the humoral theory of disease may have contributed to spread of infections and failure to effectively treat infections (Reinhard and Pucu, 2014).

When ideal archaeological preservation can be combined with historical documents, very detailed reconstruction of infection patterns can be reconstructed. Kim et al. (2013) and Seo et al. (2014b) accomplished this for the city of Hansung, the capital area of the Joseon Dynasty, which is now located within Seoul, South Korea. By examining legal documents relating to the distribution of night soil (latrine contents) from the city to farmers, and the economic interdependence between major cities and nearby farmlands, researchers discovered that the night soils produced in a major city were recycled by farmers as fertilizers for fields. With that night soil, soil-transmitted parasites were spread on the fields to contaminate vegetables that were then marketed back to the city. High levels of geohelminth infection were an unavoidable result of this legally defined recycling process. Flood-deposited sediments in the city that were washed in from farm fields during periodic torrential rains in ancient times show that parasite eggs were indeed contaminants of the fields.

Remarkable preservation of Joseon Dynasty mummies provides fascinating evidence of food-borne fluke infection. Eighteen Joseon mummies have been studied for parasites as reviewed by Seo et al. (2014a,b). Four species of trematodes were found. Two species, *Clonorchis sinensis* (Chinese liver fluke) and *Metagonimus yokogawai*, are similar in that they are transferred by the consumption of fish. A total of six mummies were infected with one or both of these flukes, five with *C. sinensis* and three with *M. yokogawai*. *Paragonimus westermani*, a lung fluke, was found in four of the mummies. Its final intermediate host includes species of freshwater crabs. Finally, oyster consumption was a source of infection with *Gymnophalloides seoi*, an intestinal fluke. Two mummies were found to be positive for eggs of this parasite (Shin et al., 2012). In total, 11 of 18 Joseon mummies, representing the elite of this society,

were infected with one or more species of fluke and underscores the importance of uncooked meat in Ancient Korean diet.

The studies summarized above present a sampling of the "big picture" issues that have been addressed by cooperation of parasitologists and archaeologists. Other studies provide "vignettes" of ancient parasitism. Such studies focus on brief archaeological moments or give a detailed glimpse of human–parasite interactions. Kristjánsdóttir and Collins (2011) excavated cemeteries from Skriðuklaustur, a medieval monastic site and hospital used between AD 1493 and 1554 in Eastern Iceland. During this brief period, eight individuals died with hydatid cysts from eight of 160 burials recovered. The eight burials were clustered together in one part of the cemetery, which suggests that they were recognized as a specific symptom complex and were treated as such. A cyst in one of the skeletons was 17 cm in diameter. The cysts are a result of infection with *Echinococcus granulosus*. It was probably introduced in Iceland sometime after the late ninth century and became endemic by AD 1200. Although some of infected individuals lived with other diseases, including syphilis, the authors note that "they seem to have first been buried according to this shared condition and not relative to any of the other identified illnesses or age. The indications are that this ailment may have been recognized in medieval Iceland as having its own classification and perhaps requiring distinct treatment if not in life, at least in death" (Kristjánsdóttir and Collins, 2011, p. 485). The implication is that hydatid cyst disease was recognized early in Icelandic medical history. Thus, this excavation provides a "vignette" of a symptom complex recognized by monastic scholars.

Latrines provide other "vignettes" of human parasitism. The diet and parasitism of a single Roman Centurion were presented by Kuijper and Turner (1992). Along with a great diversity of pollen and seeds of spices and food, the eggs of intestinal parasites were found. The authors report that "many thousands" of eggs were present per cubic centimeter. Whipworm and *Ascaris lumbricoides* were most common, but taeniid eggs, consistent with the genera *Taenia* or *Echinococcus*, were also present. Thus, the dietary habits and intestinal infections of a single officer were evidenced in the latrine sediments. Another vignette of parasites carried by pilgrims comes from a thirteenth century latrine in the city of Acre, Israel. At that time, Acre was part of the Frankish Kingdom of Jerusalem. The fish tapeworm (eggs consistent with *Diphyllobothrium latum*) was identified. The authors note that fish tapeworm was also found in the latrine block of the Hospital of St John. The Order of St John was a religious order that cared for crusaders and pilgrims. The presence of fish tapeworm eggs in a crusader period latrine in the Levant

indicates that infected pilgrims from northern Europe travelled to Acre with active fish tapeworm infections (Mitchell et al., 2011). This sampling of studies reflects the popularity of parasitology as a tool for archaeologists (Reinhard, 1992a). The productive collaboration of archaeology and parasitology will continue to grow in regional distribution and in the development of new research questions beneficial to both fields.

4. Recommended Material and Techniques for Microscopic Examination in Paleoparasitology

4.1 Light microscopy techniques

It is always important to highlight the use of trisodium phosphate aqueous solution (Na_3PO_4), introduced by Callen and Cameron (1960) and based on the experiments by Van Cleave and Ross (1947) for the rehydration of desiccated samples. Based on the rehydration time recommended by the authors (72 h of immersion of the coprolite), bacterial or fungal contamination can occur and jeopardize the test results. Thus, in our laboratory, we began to use several drops of acetic formalin to prevent the growth of contaminant microorganisms (Araújo et al., 1998). Since the advent of molecular biology techniques, formalin is no longer recommended, and samples submitted to rehydration should preferably be refrigerated to avoid the development of current microorganisms (Fugassa, 2014).

Samuels (1965) conducted the first tests in various solutions and concluded by recommending trisodium phosphate solution. Experiments in our laboratories that used other rehydration solutions such as distilled water, potassium or sodium hydroxide, physiological saline, and others resulted in significant losses of parasite elements previously counted in fresh feces after experimental desiccation and rehydration. The results have not been published, but serve as recommendations for other authors. For example, *Giardia intestinalis* cysts practically disappeared after important warping of the outer membrane, leading to breakage. The same occurred with hookworm eggs, which are known to be fragile and prone to breaking. We thus reemphasize using the rehydration solution proposed by Callen and Cameron (1960).

After rehydration of the material, any parasitological technique for microscopic examination can be used. However, the Kato-Katz technique (Siqueira et al., 2011) should be avoided, since the reagents destroy some eggs after about 2 h, such as those of hookworms, or alter the outer shell in the case of *Ascaris lumbricoides*. Other concentration

techniques that use strong acid or alkaline reagents can also alter the results. Our laboratories have successfully used two techniques for parasitological examination. Concentration by spontaneous sedimentation is a simple, efficient and inexpensive technique developed by Adolpho Lutz in 1919 (Camacho et al., 2013) for the diagnosis of *schistosomiasis mansoni* by finding the parasite's eggs. It is still used in clinical test laboratories for both protozoa and intestinal helminths and was introduced in paleoparasitology because it is efficient, does not use any reagent except for the rehydration solution, and allows saving all the material used in the test. Nothing is lost.

The technique consists of taking 2–3 g of rehydrated material, crushing it with a glass pestle, filtering the suspension through gauze folded four times, and pouring it through a glass funnel into a 300- to 400-mL conical glass jar; the suspension is then left to settle for at least 2 h with the rehydrating liquid touching the lower part of the gauze (Figure 4). Next, the sediment is collected from the glass jar using a disposable Pasteur pipette to prepare the microscope slides. An additional recommendation is to add a drop of glycerol to improve the transparency under microscopy. The slides can be sealed with modified Noyer seal, a 1:1 mixture of resin and beeswax, boiled until the
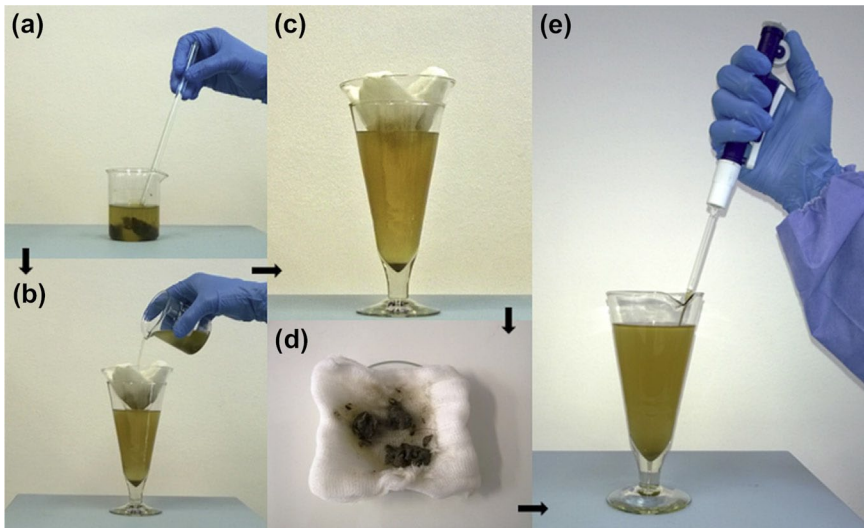
Figure 4. Lutz' sedimentation technique steps e (a) mixing the sediment, after rehydrating the coprolite or the sediment; (b) pouring the rehydrated sediment through the gauze to the conical glass jar; (c) after 2–4 h, the sedimentation process is completed; (d) preserved macroresidues; (e) collecting the sediment for microscopic analysis.

mixture become homogeneous, without emitting any smoke. Extreme care is needed in preparing the mixture, which is highly flammable. The slide covers are sealed using a metal loop heated over a Bunsen burner, spreading the melted mixture over the edges of the slide cover with the hot metal loop. Before applying the mixture, it is crucial to dry the edges of the slide cover in order to avoid spilling liquid over it and preventing the proper sealing. Under microscopic examination, all the parasite structures are measured and photographed and the other microfossils are observed. We recommend examining 20 slides in all the fields for each sample preserved by desiccation and at least 500 slides for permineralized (fossil) material, but in the latter case using acids or ultrasound to dissociate the material (Silva et al., 2014).

All the unused material settled in the glass jar should be collected with a pipette and stored separately, refrigerated or even frozen, for future use. The same is true for the material trapped in the gauze, which can be examined for food remains and occasionally fragments of helminths. In this case to preserve the material, we recommend drying the material in an oven at 45 °C or even at room temperature under low relative humidity, on absorbent paper, until there is no longer any change in weight.

For samples collected from the pelvic cavity of skeletons or sediment from archaeological sites, we recommend the sugar solution flotation techniques (Fugassa, 2014), which offers good recovery of parasite structures. The following is a description of details for counting remains found under the microscope.

4.2 Counting remains under the microscope

4.2.1 Analysis of sediments
Shaft features are remains of wells, latrines and other vertical pits. Much of the earliest work in England and Germany focused on the recovery of eggs from shaft latrines. The challenge in analyzing sediments from such features is separating the parasite evidence from inert sand and other organic remains. A similar challenge is encountered when recovering eggs from skeletonized burials. In many sites, the sand and organics are held together in a calcium carbonate matrix. Two methods from that period are still used today. Jones (1985) modified the Stoll's dilution method from clinical parasitology to archaeological samples. There has been a renewed interest in this simple, rapid and efficient method (Fugassa et al., 2006, 2008). Another older method is derived from palynology. Hevly et al. (1979) discovered five species of parasites represented in sediment samples from

an archaeological site in northern Arizona. This was a significant discovery showing that sediment digestion is successful in recovering eggs from calcareous soils. In turn this was a breakthrough because parasite egg distribution could be traced stratigraphically using long-established pollen analysis techniques. The inert mineral components are removed by hydrochloric acid, sedimentation and finally hydrofluoric acid baths. Finally, the cellulose component is removed by acetylation. The different steps of this method were tried on eggs from five parasite species. It was shown that nematode cestode, and trematode eggs survived the first stages of the processing until acetolysis. There was some destruction of cestode and trematode eggs in acetolysis solution (Fischer et al., 2007). From calcareous sediments, the eggs were more readily identifiable after palynological processing than established flotation methods. This became a quantitative method when combined with another palynological method called pollen concentration. This method is based on adding known numbers of exotic spores to known amounts of sample and was presented by Warnock and Reinhard (1992). The counted ratio of parasite eggs to spores can then be used to quantify the numbers of eggs per gram and milliliter of sediment. Most recently, Florenzano et al. (2012) showed that careful application of acetolysis, called "light acetolysis," results in remarkably cleansed eggs with clear surface morphology. This palynological–parasitological hybrid method is advantageous for several reasons. It is a rapid method designed for processing dozens of samples simultaneously. It results in the recovery of pollen from diet, medicines and environmental sources. The data can be used to generate graphs of parasite egg distribution vertically and horizontally. The disadvantage of this method is that specialized equipment, such as an acid-rated fume hood, is needed for the hydrofluoric acid treatments.

The basic method for the analysis of coprolites has been rehydration in trisodium phosphate. After rehydration, the methods varied. By 1986, two main methods had emerged. The Lutz spontaneous sedimentation method, described above, uses gravitation-based, passive filtration of microscopic remains through gauze mesh (Camacho et al., 2013; Ferreira et al., 1980; Reinhard et al., 1986; Sianto et al., 2005). The second method is based on disaggregating the samples on standard stir plates and screening the disaggregated residues through metal screens (Fugassa et al., 2011; Reinhard et al., 1986; Reinhard, 1988). The disaggregation/screening method was derived from previous dietary methods (Bryant, 1974; Reinhard and Bryant, 1992). Both methods had the goal of separating remains larger than about one-third of a millimeter from microscopic remains. When the *Lycopodium* parasite

egg concentration method was introduced (Sianto et al., 2009), re-searchers rapidly incorporated egg concentration with either Lutz or disaggregation/ screening. Recently, Jiménez et al. (2012) compared these two methods and found them comparable with regard to para-site egg recovery. Currently, the combination of parasite quantification using exotic spores is combined with either the Lutz spontaneous sed-imentation or the disaggregation screen processing methods by most researchers (Camacho et al., 2013; Fugassa et al., 2011; Jiménez et al., 2012; Kumm et al., 2010; Martinson et al., 2003; Reinhard and Bry-ant, 2008; Reinhard and Urban, 2003; Santoro et al., 2003; Sianto et al., 2005; Dufour and Le Bailly, 2013).

Bouchet et al. (1999) use ultrasound for disaggregation of rehy-drated material, which must be done carefully to avoid breaking the parasite structures. This technique is not often recommended, except in particular cases when there is a lot of mineral aggregate with the coprolite or latrine sediments. It can also be used for permineralized (fossil) coprolites.

These techniques have been described by Reinhard et al. (1986), Araújo et al. (1998), Bouchet et al. (2003), Silva et al. (2014), Hugot et al. (2014) and Fugassa (2014), with comments and indications for each of them.

4.2.2 Coprolites in mummies

Some mummies contain coprolites. These can be analyzed as de-scribed above for coprolites. However, many mummy coprolites are saturated with breakdown products that inhibit rehydration. If rehy-dration does not occur with trisodium phosphate, we have found that a 4.0% solution of KOH is effective in dispersing the breakdown prod-ucts allowing for the disaggregation of the sample.

It has been our experience that less than half of mummies contain coprolites. Aufderheide (2003) reports that even fewer sub-adult mum-mies contain coprolites. The intestinal tract is sometimes completely decomposed in spontaneously mummified remains. In other mummies, only fragments of the large intestine remain. When the large intes-tine is preserved, but is empty of coprolites, microfossils and macro-fossils can still be recovered for the mummy. The challenge relates to finding a control sample. In our laboratory, we sometimes need to re-solve this problem by deriving two samples from the same intestine: intestine interior and intestine exterior washes (Searcey et al., 2013). After rehydration in 0.5% trisodium phosphate solution, we wash the exterior of the intestine and maintain the recovered microfossils as a "control." We then open the section and wash the interior to recover

parasite remains as well as dietary and medicinal residue. We call this method "intestinal wash." Quantification of microfossils is done by adding a known number of exotic *Lycopodium* spores to a known volume or weight of sediment (Reinhard et al., 2006).

The quantification of the structures found in the process adds important information to the results, but caution is recommended in the interpretations. Intestinal helminths and protozoa vary in the deposition or elimination of parasite structures in the feces. There are intervals in the deposition, with enormous variation in the number of forms eliminated per day. Importantly, the variation in preservation in archaeological sites or mummified bodies can result in either paucity or abundance of parasite structures (Piombino-Mascali et al., 2013; Kumm et al., 2010; Shin et al., 2009). Even in tests in modern patients, helminth egg count is no longer used to estimate parasite burden, except in specific cases such as *schistosomiasis mansoni* (Caldeira et al., 2012).

4.3 Molecular techniques applied to paleoparasitology

4.3.1 Molecular diagnosis

Firstly, an experimental test is done to determine the extraction standard and amplification methodology. The most appropriate techniques are thus verified before applying them to the material from the collection. After testing, the technique is applied to the selected samples, depending on the parasite to be investigated.

Preparation of experimental material under different conditions. These require simulating the state of preservation of the samples to be studied (desiccation, formalin, alcohol and other procedures).

1. DNA extraction in experimental samples
2. Definition of the appropriate technique and protocols
3. DNA extraction from different samples in the collections
4. Data analysis and comparisons
5. Paleoparasitological identification and interpretation
6. Selection of protocols and recommended techniques

Depending on the type of preservation of the material, the extraction can proceed according to the recommendations by Campos and Gilbert (2012). Multicopy targets, <300 bp, can be prioritized (Poinar et al., 2006). There are some in-house targets for parasites and hosts, and others have been described in the literature and used in previous studies by different authors. For purification, we use the GFX DNA and Gel Band GE Healthcare kit according to the manufacturer's protocol.

Nucleotide sequencing: amplification products are sequenced directly in both directions using the automatic sequencer Applied Biosystems® 3130 Genetic Analyser (FIOCRUZ Platform). The products can also be cloned prior to direct sequencing. To analyze and edit the sequences, we use the programs Chromas Lite version 2.1 (Technelysium Pty Ltd 2007), BioEdit v7.1.11.

As exemplified by Dittmar (2009, 2014), novel techniques are able to recover and analyze ancient DNA (aDNA). As is explained, multiplex DNA techniques allow one to obtain aDNA sequences of different parasite species after a single polymerase chain reaction. Other techniques, such as metagenomics, amplify all DNA of the sample, including contaminants. Metagenomics has been applied with success by authors (Khairat et al., 2013; Kay et al., 2014) for recovering DNAs of different parasites from mummies, and Wood et al. (2013) in coprolites of extinct birds of New Zealand.

5. Parasite Finds in Human Archaeological Remains

5.1 Ascaris lumbricoides and Trichuris trichiura

Two intestinal helminths stand out as the most common in human archaeological material, whether in coprolites, intestinal content of mummified bodies or sediment from skeletons (a comprehensive table was published by Leles et al., 2010). They are *Ascaris lumbricoides* and *Trichuris trichiura*, both perhaps inherited from ancestral humans in Africa (Mitchell, 2013; Araújo and Ferreira, 2014). However, their distribution differs, with the first found more frequently in Europe, especially during the medieval period, but very rarely in the New World. Meanwhile, *T. trichiura* is common in both the Old and New Worlds (Leles et al., 2010). This created a paradox for the paleoepidemiology of *Ascaris* infection. Why are *A. lumbricoides* eggs so rare in New World material when the mechanism of transmission, by the ingestion of infective eggs, is similar to that of *T. trichiura*? Although this question has not been totally elucidated, it was discussed by Leles et al. (2010), showing that it is possible to detect the presence of the infection by molecular biology techniques and confirming its presence in prehistoric New World groups. Even so, it is still rare to find this parasite's eggs or genetic material in archaeological sites of the Americas, perhaps due to the conditions of preservation, since most of the sites in which its presence was diagnosed by recovering genetic material are located in semiarid regions (Leles et al., 2010).

The discussion on the origin of parasitism by *Ascaris lumbricoides* in humans has sparked debate as to whether it originated after the domestication of swine, thus resulting from the transfer of *A. suum* and its subsequent speciation in the human host, or the opposite, that is, humans transmitted the infection to swine. Recent studies in current and archaeological materials showed that it is really the same species in both hosts, while *A. lumbricoides* has assumed priority in the scientific nomenclature (Leles et al., 2012).

Some evidence points to an ancient origin of the parasite in the human host, with findings suggestive of the parasite's eggs in caves inhabited by Pleistocene groups (Bouchet et al., 1996) and finds in Africa in coprolites dated 12,000–9,000 years BP (Evans et al., 1996). There is growing evidence that the two purported *Ascaris* species circulate between humans and swine, with minor variations in the haplotypes and indiscriminate transmission between the two hosts (Betson et al., 2014; Cavallero et al., 2013; Dutto and Petrosillo, 2013; Vlaminck et al., 2014).

Infection by *Ascaris lumbricoides* and in certain regions by *Trichuris trichiura* appears to have been very abundant in Europe during the medieval period. In some cases, the eggs from these parasites reach incalculable levels, as in the case of a Sicilian mummy, in which *T. trichiura* eggs exceeded the number of food remains (Kumm et al., 2010; Piombino-Mascali et al., 2013). The same occurred with *A. lumbricoides*, although with lower numbers (but still significant), in the analysis of latrines in medieval Belgium (Fernandes et al., 2005). Infection by these two intestinal helminths was part of life in medieval Europe (Reinhard et al., 2013; Reinhard and Pucu, 2014).

A. lumbricoides and *Trichuris trichiura* dispersed not only in Europe, but wherever the human hosts migrated, possibly from Africa (Evans et al., 1996), having been found in various regions of medieval Asia (Matsui et al., 2003; Han et al., 2003; Shin et al., 2009; Oh et al., 2010; Kim et al., 2013; Seo et al., 2014a). Both infections reached the Americas during the prehistoric period (Araújo et al., 1981; Ferreira et al., 1980, 1983; Gonçalves et al., 2003; Leles et al., 2010; Reinhard et al., 1986). The evidence is more consistent for *Trichuris*, but we can state that both became cosmopolitan infections since ancient times.

Ascaris infection can cause severe harm in malnourished children, including intestinal obstruction and even death from acute septicemia due to rupture of the intestine by the formation of a bolus of *Ascaris lumbricoides* in the digestive tract lumen. The Wirsung canal in the pancreas can also become obstructed by erratic penetration of an adult worm, the length of which can exceed 30–40 cm in the case of

females and with a low worm burden. Adult worms consume a considerable amount of sugar ingested by the host, which can lead to low blood glucose when the parasite burden is high, especially in children.

As for *Trichuris* infection, clinical manifestations are very rare, but can be complicated in the case of a high parasite burden. In such circumstances, the helminths extend beyond the ascending colon (where they are normally located), reaching the final segments of the large intestine, where they cause a loss of muscle elasticity and prolapsed rectum. This occurs mainly in malnourished children and the elderly. The individuals themselves often reduce the prolapse until this becomes too difficult or a bacterial infection occurs, often with concomitant myiasis. The prolapse is quite peculiar, probably related to a condition called *maculo*, described among indigenous people and even among the European colonizers and African slaves (Rezende, 2003; Bianucci et al., 2015).

5.2 Hookworms

The hookworms that most frequently parasitize humans, *Ancylostoma duodenale* and *Necator americanus*, contributed to the debate on transpacific contacts or migrations of Asian populations to the Americas (Araújo et al., 1981, 1988, 2008; Ferreira and Araújo, 1996; Reinhard et al., 2001). The two species have probably accompanied the human host since the first human migrations from Africa (Ferreira et al., 2014). Like *Ascaris lumbricoides* and *Trichuris trichiura*, the hookworms that parasitize humans dispersed throughout the globe in regions that allowed maintenance of their life cycle. However, hookworm egg finds are rare in archaeological material from Europe and Africa, but are more frequent in both South America and North America (Gonçalves et al., 2003). These parasites require passage through the soil for approximately two weeks until their soil cycle is complete, from the elimination of the eggs in the feces until the release of infective larvae. These changes require temperatures >20 °C and moist soil. Only then are the larvae capable of penetrating the host and continuing their life cycle. Under microscopy, the eggs are nearly indistinguishable, but *A. duodenale* eggs are slightly smaller (46.4–77.5 × 23.5–61 µm) than those of *N. americanus* (54.2–85.3 × 30–54 µm), based on statistical analyses (Rep, 1963).

Due to these biological characteristics, the humans that crossed the Bering land-and-ice bridge could not have introduced hookworm infection by this route. Prehistoric transpacific migrations or contacts by seafaring Asian populations to the Americas were thus hypothesized (Araújo et al., 1981, 2008; Montenegro et al., 2006).

Infections with high parasite burden, associated with deficiencies in the human host's health and nutrition, cause severe cases of ancylostomiasis, with intense anemia due to intestinal blood loss through feces, edema of the lower limbs, paleness, weakness, and other signs and symptoms. Texts by naturalists and chroniclers from the colonial period in the Americas include descriptions consistent with ancylostomiasis, with such details as generalized edema and the habit of eating dirt or clay among indigenous people (Sousa, n.d.; Araújo et al., 1981). Geophagy is one of the symptoms of hypochromic microcytic anemia, associated with hookworm infection and deficient iron intake, especially in malnourished children and young people and pregnant women with iron deficiency.

5.3 *Enterobius vermicularis*

Another nematode, specific to humans and their closest primate relatives, *Enterobius vermicularis* (pinworm) has a peculiar life cycle with no need for passage through the soil for the eggs to be able to infect new hosts. The mechanism of transmission can occur by ingestion of eggs eliminated in the feces and contaminating food or water, by the host's own hands, through the air or by autoinfection. Thus, the pinworm cycle is not altered by high or very low temperatures, but the infection rates are facilitated by the agglomeration of hosts.

Infection by *Enterobius vermicularis* originated from African hosts and dates to prehominids, since it is shared by primates close to humans, such as the chimpanzee (*Pan troglodytes*). From Africa, pinworm infection spread to the rest of the globe, reaching the Americas with the first migrants. *E. vermicularis* eggs were found in an Egyptian mummy, proving ancient infection in Africa (Horne, 2002), with some rare finds in Europe (Herrmann, 1985) and Asia (Shin et al., 2011), but with most finds concentrated in the Americas (Ferreira et al., 1997; Hugot et al., 1999).

Based on the biological characteristics of its life cycle, *Enterobius vermicularis* infection was introduced in the Americas both by prehistoric migrations across the Bering route (Ferreira et al., 1997) and transpacific contacts of Asian groups with the Americas (Iñiguez et al., 2003).

Enterobiasis, or oxyuriasis, rarely produces major clinical manifestations, but there are characteristic symptoms such as anal itching, resulting from the presence of gravid females that migrate close to the area of oviposition. The females cause local irritation, leading to pruritus, the intensity of which varies between individuals. More serious

clinical cases only occur rarely, caused by bacterial invasion of the lesions provoked by the itching. Transmission of this helminthic infection is facilitated by the agglomeration of hosts and is easily diagnosed in day care centers, nursing homes, and other locations that can facilitate transmission directly from host to host or by dispersal of eggs in circulating air or contaminated food, in addition to autoinfection by eggs carried directly from hand to mouth or the simple penetration of larvae hatched from the eggs deposited in the perianal region. Prehistoric infection in North America was very well documented by Hugot et al. (1999), studying this parasite among the Ancestral Puebloans that inhabited the dwellings built of clay and wood in the rock shelters and caves in the canyons, which were clustered and with intercommunications. There were also collective rooms with directed air circulation, which facilitated aerosol transmission of the parasite's eggs.

5.4 Diphyllobothrium sp.

Diphyllobothriasis was also an emblematic parasitic infection in certain regions of the prehistoric Americas, as well as in Europe during the Neolithic (Le Bailly et al., 2005). There are various finds of eggs from *Diphyllobothrium latum* and related species of this cestode in Europe, showing infection associated with the ingestion of raw or poorly cooked lacustrine fish. This zoonosis became rare after the Middle Ages, certainly due to changes in dietary customs and sanitary control developed in the countries (Bouchet and Le Bailly, 2014).

Along the Pacific Coast of South America, especially among peoples in the Atacama Desert, diphyllobothriasis has persisted since prehistory. There are various records of *Diphyllobothrium pacificum* eggs in the prehistoric coastal population, especially in Chile and Peru. This parasite's cycle occurs in crustaceans and saltwater fish, showing that marine resources were used frequently, even among groups located far from the sea (Araújo et al., 1983). The habit of eating raw fish has persisted from prehistory till 2015. Parasitologist Jean Baer recorded this species in Peruvian population in 2015 when he was called to investigate an outbreak of diarrhea, vomiting and abdominal pain in the 1960s (Baer et al., 1967). Importantly, he referred to the antiquity of this parasite infection after visiting the Museum of Archaeology in Lima, where the collection includes pottery similar to the current ceramic ware for serving *ceviche*, a typical dish made of raw fish and spices. Proof of the observation by Baer et al. (1967) came when the parasite eggs were found in human coprolites from the Atacama

Desert (Araújo et al., 1983). It was later shown that the infection was quite common in the different human groups that inhabited the region (Reinhard and Urban, 2003).

6. Other Parasites: Parasites of Animals Found in Human Coprolites; Parasites in Prehistoric Asia

Prehistoric parasitic infections in Asia displayed a different profile from that of other continents. Although parasitism by *Ascaris lumbricoides* and *Trichuris trichiura* was also prevalent, infections with parasites of fish in humans were quite common (Seo et al., 2008). These findings show a dependency on (or preference for) eating raw saltwater fish, sometimes leading to important zoonotic infections in the population. There are reports of significant changes between countries, for example, when *Ascaris* and *Trichuris* infections were introduced from China into Japan along with rice farming (Matsui et al., 2003).

There are interesting finds of parasites of animals in human coprolites. Parasites of animals found in human feces identify the consumption of foods of animal origin, ingested raw or poorly cooked, where the parasite eggs can cross the digestive tract without infecting the human host. Meanwhile, some parasites of animals infect humans and can cause diseases. The most common infection of this type in Europe and in certain regions of the Americas is diphyllobothriasis, but other parasites of fish consumed traditionally in Asia are also frequently found in archaeological remains. They reveal the persistence of a diet based on raw or poorly cooked fish from prehistory to the present.

False parasitism, or the presence of eggs or other parasite structures in human feces and pertaining to parasites that are not capable of infecting the human host, can indicate the consumption of raw or poorly cooked animals, or even plants, as in the case of eggs from *Meloidogyne* sp., a parasite of plants found in human coprolites (unpublished). In some cases, it is possible to identify the species or group of animals that served as food. This was the case described by Sianto et al. (2012), showing that the ingestion of small lizards or geckos was common in prehistoric Brazil. Eggs of Pharyngodonidae, a parasite of lizards, were found in human coprolites, which allowed a discussion on the ingestion, in prehistory, of live or recently killed geckos. This is still a habit among isolated human groups exposed to food shortages in semiarid regions of Brazil's hinterlands (Sianto et al., 2012).

7. Origin and Evolution of Trypanosomatids in Humans and the Paradigm Shift from Results in Paleoparasitology

Trypanosoma cruzi infection occurs among insect vectors, wild and domestic animals and humans. It is considered a zooanthroponosis (Coura and Dias, 2009; Coura and Borges-Pereira, 2010, 2012), since the parasite circulated primarily only among wild animals. Humans encroached on the parasite's cycle when they reached the Americas and occupied natural foci of the *T. cruzi* life cycle (Araújo et al., 2009).

According to the classical theory on the origin of Chagas disease, it began after the domestication of small rodents from genus *Cavia* (or "*cuyes*") in the Bolivian altiplano, spreading from there southward and reaching what is now Brazil much later (Pinto-Dias, 2013). Based on recent data from paleoparasitology, we now know that the parasite already infected humans 7000 years ago in central South America, in the Brazilian *cerrado*, including the form of the disease characterized by digestive tract lesions (Lima et al., 2008), and in the Cohuailla Desert along the border between the southern United States and northern Mexico (Reinhard et al., 2003).

The human groups that used the same caves where infected vectors and animals were found were exposed to the vectors and to maintenance of the wild cycle. American trypanosomiasis appears to have a very ancient origin, and according to Poinar (2014) its life cycle has occurred in triatomines and mammals since the Cenozoic. The occurrence of *Trypanosoma antiquae* in triatomines found in Dominican amber (Poinar, 2005), now dated to the Miocene (Iturralde-Vinent and MacPhee, 1996; Iturralde-Vinent, 2001), and associated with bat hair indicates the antiquity of the infection and probably its origin in these mammals (Poinar, 2014). Other Trypanosomatidae may potentially be traced back to the Cretaceous Burmese Amber (Poinar and Poinar, 2004a,b; Poinar, 2007).

Infection in humans probably dates to soon after their arrival in the Americas (Ferreira et al., 2011), wherever they occupied existing transmission foci among wild mammals (Araújo et al., 2009). With the increasing transformation of spaces by humans and their domestic animals, new adaptations of the parasite to new hosts occurred, as well as to new habitats, in a process called domiciliation (Lardeux, 2013). *T. cruzi* is known as a single species, but with different lineages that were characterized over the course of this long evolutionary process (Guhl et al., 2014).

Findings show that the infection circulated among prehistoric groups that inhabited the rock shelters and caves, or wherever they created favorable conditions for colonization by triatomines (Araújo et al., 2009), in both North and South America, in various biomes where the vector and other animal hosts were found. The occurrence of lesions in the digestive tract (Lima et al., 2008; Reinhard et al., 2003) and heart (Fornaciari et al., 1992; Rothhammer et al., 1985) has raised speculation on the repercussions or impact of the disease in prehistoric groups, which included small bands of hunter-gatherers. Sudden death from cardiac arrhythmia, especially among young and apparently healthy individuals, would have had a significant impact on nomadic hunter-gatherer groups. Meanwhile, the chronic manifestations of Chagas disease, such as heart failure and the development of megas (extensive dilations) in the digestive tract, would have caused limitations in the individuals, with the need for special care, as in the case of the mummy from Coahuaila (Reinhard et al., 2003).

Further in relation to kinetoplastids, little is known about the origins of infections by species from genus *Leishmania* in humans. There are various gaps in the knowledge, especially in relation to asymptomatic cases, often more numerous than symptomatic ones (Marzochi et al., 1985). The origins of *Leishmania* species purportedly resulted from a process of speciation in the Old World (Tuon et al., 2008). As with genus *Trypanosoma*, a putative *Leishmania* species has been found in vectors preserved in amber (Poinar and Poinar, 2004a,b). An international research project is under way, coordinated in Italy, on the origins and evolution of *Leishmania* species (Nerlich et al., 2012).

8. Ectoparasites

This group of parasites includes different classes of arthropods and some other invertebrates. The most common classes in humans are Insecta and Arachnida (mites and ticks). Some of these ectoparasites, such as head lice *Pediculus humanus*, are also shared with nonhuman primates, and thus likely infested humans since the prehominids (Araújo et al., 2003; Mitchell, 2013). According to Reed et al. (2004) and Raoult et al. (2008), lice have parasitized primates for some 25 million years, and when chimpanzees diverged from humans 6 million years ago, genus *Pediculus* remained in both species. This parasite has been found in Egyptian mummies and in archaeological sites in North America and South America. Infestation became as widespread

in the Andean region (Arriaza et al., 2012; Dutra et al., 2014) as in medieval Europe, where the hairdos of the nobility were veritable breeding grounds for lice (Fornaciari et al., 2009). The same was true of the refined hairdos of the Tiwanaku: when the Incas arrived as conquerors of other Andean empires, they levied 'lice taxes', now interpreted as a way to control infestation or simply as a form of human head count (Souffez, 2001).

There is extensive research on the origin and evolution of pediculosis. Reed et al. (2004) and Raoult et al. (2008) provided important evidence on the antiquity and evolution of this parasite infection in humans, with the oldest finding thus far in the Americas (Araújo et al., 2000). Studies on the meaning and impact of mass lice infestations were conducted in Andean populations (Dutra et al., 2014; Arriaza et al., 2012, 2013b), where exceedingly well-preserved mummified bodies are found, including well-preserved hair.

The preservation of eggs or lice is so extraordinary that they were found on human shrunken heads from the Jívaro, an indigenous group on the border of Brazil with Ecuador, dated to the colonial period and the Second Empire in Brazil, in the nineteenth century (Araújo et al., 2005). Eggs of ectoparasites have in rare cases even been found on dinosaur feathers or mammal hair preserved in amber (e.g. Martill and Davis, 1998; Poinar, 2014). Both lice (Order Phthiraptera) and fleas (Order Siphonaptera), which currently parasitize bird and mammals, can be traced back to the Cretaceous at least (see the review in Nagler and Haug, 2015).

9. Conclusions

Paleoparasitology contributes new data to knowledge on the origin and evolution of parasitic diseases. It situates – in time and space – the presence of infection in human groups that have already disappeared, situating their distribution and tracing prehistoric migrations across the continents through parasites preserved in the occupation sites.

Paleoparasitology has also contributed greatly to studies on the evolution of parasites and their hosts. Despite previous claims that parasites do not leave fossils (e.g. Dorris et al., 1999), parasite finds have become frequent in remains from extinct animals dating back millions of years (Littlewood and Donovan, 2003; Poinar, 2014), particularly those preserved in amber and in coprolites found in paleontological sites (Poinar and Boucot, 2006; Dentzien-Dias et al., 2013). It is thus

possible to study the presence of parasites millions of years old and their relations with current species, as in the case of Oxyurid and Ascarid eggs found in cynodonts, dated 240 million years BP (Silva et al., 2014; Hugot et al., 2014). This group of parasites has persisted to 2015, sharing a long evolutionary history with its hosts.

The development and growing interest of research groups in paleoparasitology have stimulated progress in knowledge on parasite–host–environment relations, reinforcing the ecological approach that should be encouraged in studies on parasites and hosts.

References

Anastasiou, E., Mitchell, P.D., 2013. Human intestinal parasites from a latrine in the 12th century Frankish castle of Saranda Kolones in Cyprus. Int. J. Paleopathol. 3, 218–223.

Araújo, A., Ferreira, L.F., Confalonieri, U., 1981. A contribution to the study of helminth findings in archaeological material in Brazil. Rev. Bras. Biol. 41, 873–881.

Araújo, A., Ferreira, L.F., Confalonieri, U., Nunez, L., 1983. Eggs of *Diphyllobothrium pacificum* in pre-Columbian human coprolites. Paleopathol. News 41, 11–13.

Araújo, A., Ferreira, L.F., Confalonieri, U., Chame, M., 1988. Hookworm and the peopling of America. Cad. Saúde Pública 2, 226–233.

Araújo, A., Reinhard, K., Bastos, O.M., Costa, L.C., Pirmez, C., Iniguez, A.M., Vicente, A.C., Morel, C.M., Ferreira, L.F., 1998. Paleoparasitology: perspectives with new techniques. Rev. Inst. Med. Trop. São Paulo 40, 371–376.

Araujo, A., Ferreira, L.F., Guidon, N., Freire, N.M.S., Reinhard, K., Dittmar, K., 2000. Ten thousand years of head lice infection. Parasitol. Today 16, 269–270.

Araújo, A., Jansen, A.M., Bouchet, F., Reinhard, K., Ferreira, L.F., 2003. Parasites, the diversity of life, and paleoparasitology. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 5–11.

Araújo, A., Mendonça de Souza, S.M., Nascimento, F., Reinhard, K., 2005. The Jivaro shrunken heads from the National Museum, Rio de Janeiro, Brazil: authentic or counterfeits. J. Biol. Res. 80, 152–157.

Araújo, A., Reinhard, K., Ferreira, L.F., Gardner, S., 2008. Parasites as probes for prehistoric migrations? Trends Parasitol. 24, 112–115.

Araújo, A., Jansen, A.M., Reinhard, K., Ferreira, L.F., 2009. Paleoparasitology of Chagas disease: a review. Mem. Inst. Oswaldo Cruz 104, 9–16.

Araújo, A., Ferreira, L.F., 2014. Parasitism. In: Ferreira, L.F., Reinhard, K., Araújo, A. (Eds.), Foundations of Paleoparasitology. Editora Fiocruz/International Federation of Tropical Medicine, pp. 111–119.

Arriaza, B.T., Reinhard, K.J., Araújo, A., Orellana, N.C., Standen, V.G., 2010. Possible influence of the ENSO phenomenon on the pathoecology of diphyllobothriasis and anisakiasis in ancient Chinchorro populations. Mem. Inst. Oswaldo Cruz 105, 66–72.

Arriaza, B., Orellana, N., Barbosa, H.S., Menna Barreto, R., Araújo, A., Standen, V., 2012. Severe head lice infestation in an Andean mummy of Arica, Chile. J. Parasitol. 98, 433–436.

Arriaza, B., Standen, V., Reinhard, Karl, Araújo, A., Heukelbach, J., Dittmar, K., 2013a. On head lice and social interaction in archaic Andean coastal populations. Int. J. Paleopathol. 10, 1–15.

Arriaza, B., Standen, V., Núñez, H., Reinhard, K., 2013b. Study of archaeological nits/eggs of *Pediculus humanus capitis* by scanning electron microscopy. Micron 45, 145–149. Aufderheide, A., 2003. The Scientific Study of Mummies. Cambridge University Press, p. 320.

Baer, J.G., Miranda, H., Fernandez, W., Medina, J., 1967. Human diphyllobothriasis in Peru. Z. Parasitenkd 28, 277–289.

Barrow Jr., J.H., Stockton, J.J., 1960. The influences of temperature on the host–parasite relationships of several species of snakes infected with *Entamoeba invadens*. J. Protozool. 7, 377–383.

Baum, J., Bat-Gal, G.K., 2003. The emergence and co-evolution of human pathogens. In: Greenblatt, C., Spigelman, M. (Eds.), Emerging Pathogens. Oxford University Press, Oxford, pp. 67–78.

Betson, M., Nejsum, P., Bendall, R.P., Deb, R.M., Stothard, J.R., 2014. Molecular epidemiology of ascariasis: A global perspective on the transmission dynamics of *Ascaris* in people and pigs. J. Infect. Dis. 15, 932–941.

Bianucci, R., Torres, E.J.L., Dutra, J.M.F.D., Ferreira, L.F., Nerlich, A.G., Souza, S.M.M., Giuffra, V., Chieffi, P.P., Bastos, O.M., Travassos, R., Souza, W., Araújo, A., 2015. *Trichuris trichiura* in a post-Colonial Brazilian mummy. Mem. Inst. Oswaldo Cruz 110, 145–147.

Bouchet, F., Bafier, D., Girard, M., Morel, P., Paicheler, J.C., David, F., 1996. Paléoparasitologie en contexte Pleistocène: première observations à la Grande Grotte d'Arcy-sur-Cure (Yonne), France. C.R. Acad. Sci. III 319, 147–151.

Bouchet, F., Lefréve, C., West, D., Corbert, D., 1999. First paleoparasitological analysis of a midden in the Aleutian Islands (Alaska): results and limits. J. Parasitol. 85, 369–372.

Bouchet, F., Guidon, N., Dittmar, K., Harter, S., Ferreira, L.F., Chaves, S.M., Reinhard, K., Araújo, A., 2003. Parasite remains in archaeological sites. Mem. Inst. Oswaldo Cruz 98 (Suppl.1), 47–52.

Bouchet, F., Le Bailly, M., 2014. The findings in Europe. In: Ferreira, L.F., Reinhard, K., Araújo, A. (Eds.), Foundations of Paleoparasitology. Ed. Fiocruz/Int. Fed. Tropical Medicine, pp. 363–388.

Brumpt, E., 1913. Précis de Parasitologie. Masson, Paris, p. 9.

Bryant Jr., V.M., 1974. Prehistoric diet in southwest Texas: the coprolite evidence. Am. Antiq. 39, 407–420.

Bryant Jr., V.M., Reinhard, K.J., 2012. Coprolites and archaeology: the missing links in understanding human health. In: Hunt, A. (ed.), Vertebrate Coprolites. New Mexico Museum of Natural History and Science Bulletin 51.

Buikstra, J.E., Beck, J., 2008. In: Buikstra, Jane E., Beck, Lane A. (eds.), The Contextual Analysis of Human Remains. Academic Press/Elsevier, 347–258.

Buikstra, J., 2013. EIC editorial: Introducing the paleoparasitology issue. Int. J. Paleopathol. 3, 139.

Caldeira, K., Teixeira, C.F., Silveira, M.B., Fries, L.C.C., Romanzini, J., Bittencourt, H.R., Graeff-Teixeira, C., 2012. Comparison of the Kato-Katz and Helmintex methods for the diagnosis of schistosomiasis in a low-intensity transmission focus in Bandeirantes, Paraná, southern Brazil. Mem. Inst. Oswaldo Cruz 107, 690–692.

Callen, E.O., Cameron, T.W.M., 1960. A prehistoric diet revealed in coprolites. New Sci. 8, 35–40.

Camacho, M., Pessanha, T., Leles, D., Dutra, J., Silva, R., Mendonça de Souza, S.M., Araújo, A., 2013. Lutz' spontaneous sedimentation technique and the paleoparasitological analysis of sambaqui (shellmounds) sediments. Mem. Inst. Oswaldo Cruz 108, 155–159.

Campos, P.F., Gilbert, T.M., 2012. DNA extraction from formalin-fixed material. Methods Mol. Biol. 840, 81–85.

Cavallero, S., Snabel, V., Pacella, F., Perrone, V., D'Amelio, S., 2013. Phylogeographical studies of *Ascaris* spp. based on ribosomal and mitochondrial DNA sequences. PLoS Negl. Trop. Dis. 7, e2170. doi: 10.1371/journal.pntd.0002170.

Cockburn, A., 1963. The Evolution and Eradication of Infectious Diseases. The Johns Hopkins Press, Baltimore, pp. 255.

Coura, J.R., 1997. Síntese histórica e evolução dos conhecimentos sobre a doença de Chagas. In: Coura, J.R., Dias, J.C.P. (Eds.), Clínica e Terapênutica da Doença de Chagas. Editora Fiocruz, pp. 469–486.

Coura, J.R., Dias, J.C., 2009. Epidemiology, control and surveillance of Chagas disease: 100 years after its discovery. Mem. Inst. Oswaldo Cruz 104 (Suppl. 1), 31–40.

Coura, J.R., Borges-Pereira, J., 2010. Chagas disease: 100 years after its discovery. A systemic review. Acta. Trop. 115, 5–13.

Coura, J.R., Borges-Pereira, J., 2012. Chagas disease. What is known and what should be improved: A systemic review. Rev. Soc. Bras. Med. Trop. 45, 286–296.

Dentzien-Dias, P.C., Poinar Jr., G., de Figueiredo, A.E., Pacheco, A.C., Horn, B.L., Schultz, C.L., 2013. Tapeworm eggs in a 270 million-year-old shark coprolite. PLoS One 8, e55007. doi: 10.1371/journal.pone.0055007.

Diamond, J., 1987. The worst mistake in the history of the human race. Discov. Mag. 64–66.

Dittmar, K., 2009. Old parasites for a new world: the future of paleoparasitological research. A review. J. Parasitol. 95, 365–371.

Dittmar, K., Araújo, A., Reinhard, K., 2012. The study of parasites through time: Archaeoparasitology and paleoparasitology. In: Grauer, A.L. (ed.), A Companion to Paleopathology. Wiley-Blackwell, New Jersey, pp. 170–190.

Dittmar, K., 2013. Guest editorial. Int. J. Paleopathol. 3, 140–141.

Dittmar, K., 2014. Paleoparasitology and ancient DNA. In: Ferreira, L.F., Reinhard, K., Araújo, A. (Eds.), Foundations of Paleoparasitology. Editora Fiocruz/International Federation of Tropical Medicine, pp. 277–288.

Dorris, M., De Ley, P., Blaxter, M.L., 1999. Molecular analysis of nematode diversity and the evolution of parasitism. Parasitol. Today 15, 188–193.

Dufour, B., Le Bailly, M., 2013. Testing new parasite egg extraction methods in paleoparasitology and an attempt at quantification. Int. J. Paleopathol. 3, 199–203.

Dutra, J.M., Alves, A.D., Pessanha, T., Rachid, R., Souza, W., Linardi, P.M., Ferreira, L.F., Mendonça de Souza, S.M., Araújo, A., 2014. Prehistorical *Pediculus humanus capitis* infestation: quantitative data and low vacuum scanning microscopy. Rev. Inst. Med. Trop. São Paulo 56, 115–119.

Dutto, M., Petrosillo, N., 2013. Hybrid *Ascaris suum/lumbricoides* (Ascaridiae) infestation in a pig farmer: A rare case of zoonotic ascariasis. Centr. Eur. J. Public Health 21, 224–226.

Evans, A.C., Markus, M.B., Mason, R.J., Steel, R., 1996. Late stone-age coprolite reveals evidence of prehistoric parasitism. S. Afr. Med. J. 86, 274–275.

Ewald, P.W., Swain Ewald, H.A., 2014. Joint infectious causations of human cancer. Adv. Parasitol. 84, 1–26.

Faulkner, C.T., Reinhard, K.J., 2014. A retrospective examination of paleoparasitology and its establishment in the Journal of Parasitology. J. Parasitol. 100, 253–259.

Fernandes, A., Ferreira, L.F., Gonçalves, M.L.C., Bouchet, F., Klein, C.H., Iguchi, T., Sianto,

L., Araújo, A., 2005. Intestinal parasite analysis in organic sediments collected from a 16th Century Belgium archaeological site. Cad. Saúde Pública 21, 329–332.

Fernandes, A., Iñiguez, A.M., Lima, V.S., Souza, S.M., Ferreira, L.F., Vicente, A.C., Jansen, A.M., 2008. Pre-Columbian Chagas disease in Brazil: *Trypanosoma cruzi* I in the archaeological remains of a human in Peruaçu Valley, Minas Gerais, Brazil. Mem. Inst. Oswaldo Cruz 103, 514–516.

Ferreira, L.F., Araújo, A., Confalonieri, U., 1979. Subsidios para a Paleoparasitologia do Brasil I - Parasitos encontrados em coprólitos no município de Unai, Minas Gerais. In: Congr. Bras. Parasitol., Abstr.,Campinas, SP, p. 56.

Ferreira, L.F., Araújo, A.J.G., Confalonieri, U.E.C., 1980. The finding of eggs and larvae of parasitic helminths in archaeological material from Unai, Minas Gerais, Brazil. Trans. R. Soc. Trop. Med. Hyg. 74, 798–800.

Ferreira, L.F., Araújo, A., Confalonieri, U.E.C., 1983. The finding of helminth eggs in a Brazilian mummy. Trans. R. Soc. Trop. Med. Hyg. 77, 65–67.

Ferreira, L.F., Araújo, A., Confalonieri, U., Nuñez, L., 1984. The finding of eggs of *Diphyllobothrium pacificum* in human coprolites (4100-1950 B.C.) from northern Chile. Mem. Inst. Oswaldo Cruz 79, 175–180.

Ferreira, L., Araújo, A., 1996. On hookworms in the Americas and trans-pacific contact. Parasitol. Today 12, 454–455.

Ferreira, L.F., Reinhard, K., Araújo, A., Coura, L.C., 1997. Paleoparasitology of oxyuriasis. Acad. Nac. Med. 157, 20–24.

Ferreira, L.F., Jansen, A.M., Araújo, A., 2011. Chagas disease in prehistory. Acad. Bras. Ci. 83, 1041–1044.

Ferreira, L.F., 2014. An introduction to paleoparasitology. In: Ferreira, L.F., Reinhard, K., Araújo, A. (Eds.), Foundations of Paleoparasitology. Editora Fiocruz/International Federation of Tropical Medicine, pp. 27–41.

Ferreira, L.F., Reinhard, K.J., Araújo, A., 2014. The origin of parasites of humans. In: Ferreira, L.F., Reinhard, K., Araújo, A. (Eds.), Foundations of Paleoparasitology. Editora Fiocruz/International Federation of Tropical Medicine, pp. 121–139.

Fischer, C.L., Reinhard, K., Kirk, M., DiVirgilio, J., 2007. Privies and parasites: the archaeology of health conditions in Albany, New York. Hist. Archaeol. 41, 172–197.

Florenzano, A., Mercuri, A.M., Pederzoli, A., Torri, P., Bosi, G., Olmi, L., Rinaldi, R., Mazzanti, M.B., 2012. The significance of intestinal parasite remains in pollen samples from medieval pits in the Piazza Garibaldi of Parma, Emilia Romagna, Northern Italy. Geoarchaeology 27, 34–47.

Fornaciari, G., Castagna, M., Viacava, P., Tognetti, A., Bevilacqua, G., Segura, E.L., 1992. Chagas' disease in Peruvian Inca mummy. Lancet 339, 128–129.

Fornaciari, G., Giuffra, V., Marinozzi, S., Picchi, M.S., Masetti, M., 2009. 'Royal' pediculosis in Renaissance Italy: lice in the mummy of the King of Naples Ferdinand II of Aragon (1467–1496). Mem. Inst. Oswaldo Cruz 104, 671–672.

Frías, L., Leles, D., Araújo, A., 2013. Studies on protozoa in ancient remains – A review. Mem. Inst. Oswaldo Cruz 108, 1–12.

Fry, G.F., 1970. Preliminary analysis of the Hogup Cave coprolites. In: Aïkens, M. (Ed.), Hogup Cave, Appendix III. University of Utah, Salt Lake City, pp. 247–250 (Anthropological Papers) 93.

Fugassa, M.H., Araújo, A., Guichón, R.A., 2006. Quantitative paleoparasitology applied to archaeological sediments. Mem. Inst. Oswaldo Cruz 101 (Suppl. 2), 29–33.

Fugassa, M.H., Taglioretti, V., Gonçalves, M.L., Araújo, A., Sardella, N.H., Denegri, G.M., 2008. *Capillaria* spp. eggs in Patagonian archaeological sites: statistical analysis of morphometric data. Mem. Inst. Oswaldo Cruz 103, 104–105.

Fugassa, M.H., Reinhard, K.J., Johnson, K.L., Gardner, S.L., Vieira, M., Araújo, A., 2011. Parasitism of prehistoric humans and companion animals from Antelope Cave, Mojave County, Northwest Arizona. J. Parasitol. 97, 862–867.

Fugassa, M., 2014. Paleoparasitological diagnosis. In: Ferreira, L.F., Reinhard, K., Araújo, A. (Eds.), Foundations of Paleoparasitology. Ed. Fiocruz/World Federation of Tropical Medicine, Rio de Janeiro, pp. 223–254.

Gómez i Prat, J., Mendonça de Souza, S.M.F., 2003. Prehistoric tuberculosis in America: adding comments to a literature review. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 151–159.

Gonçalves, M.L.C., Araújo, A., Ferreira, L.F., 2003. Human intestinal parasites in the past: new findings and a review. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 103–118.

Gonçalves, M.L.C., Silva, V.L., Andrade, C.M., Reinhard, K., Rocha, G.C., Le Bailly, M., Bouchet, F., Ferreira, L.F., Araújo, A., 2004. Amoebiasis distribution in the past: first steps using a immunoassay technique. Trans. R. Soc. Trop. Med. Hyg. 98, 88–91.

Gooch, P.S., 1972. Helminths in Archaeological and Prehistoric Deposits. Commonwealth Institute of Helminthology, St Albans, UK. Annotated Bibliography, Nº 9.

Grmek, M.D., 1983. Les Maladies à l'Aube de la Civilisation Occidentale. Payot, Paris.

Guhl, F., Auderheide, A., Ramírez, J.D., 2014. From ancient to contemporary molecular ecoepidemiology of Chagas disease in the Americas. Int. J. Parasitol. 44, 605–612.

Han, E.T., Guk, S.M., Kim, J.L., Jeong, H.J., Kim, S.N., Chai, J.Y., 2003. Detection of parasite eggs from archaeological excavations in the Republic of Korea. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 123–126.

Harper, K.N., Ocampo, P.S., Steiner, B.M., George, R.W., Silverman, M.S., Bolotin, S., Pillay, A., Saunders, N.J., Armelagos, G.J., 2008. On the origin of the Treponematoses: a phylogenetic approach. PLoS Negl. Trop. Dis. e148. doi: 10.1371/journal.pntd.0000148.

Heizer, R.F., Napton, L.K., 1969. Biological and cultural evidence from prehistoric human coprolites. Science 165, 563–568.

Herrmann, B., 1985. Parasitologisch-epidemiologische auswertungen mit- telalterlicher kloaken. Z. Archaol. Mittelalt. 13, 131–161.

Hevly, R.H., Kelly, R.E., Anderson, G.A., Olsen, S.J., 1979. Comparative effects of climate change, cultural impact, and volcanism in the paleoecology of Flagstaff, Arizona, A.D. 900–1300. In: Sheets, E., Grayson, D. (Eds.), Volcanic Activity and Human History. Academic Press, New York, pp. 487–523.

Horne, P.D., 2002. First evidence of enterobiasis in ancient Egypt. J. Parasitol. 88, 1019–1021.

Hugot, J.P., Reinhard, K., Gardner, S.L., Morand, S., 1999. Human enterobiasis in evolution: origin, specificity and transmission. Parasite 6, 201–208.

Hugot, J.P., Gardner, S.L., Borba, V.H., Araújo, P., Leles, D., Da-Rosa, A., Dutra, J., Ferreira, L.F., Araújo, A., 2014. Discovery of a 240 million year old nematode parasite egg in a cynodont coprolite sheds light on the early origin of pinworms in vertebrates. Parasit. Vectors 7, 486. doi: 10.1186/s13071-014-0486-6.

Iñiguez, A.M., Reinhard, K., Araújo, A., Ferreira, L.F., Vicente, A.C.P., 2003. *Enterobius vermicularis*: ancient DNA from North and South American human coprolites. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 67–69.

Iturralde-Vinent, M.A., MacPhee, R.D.E., 1996. Age and paleogeographical origin of dominican amber. Science 273, 1850–1852.

Iturralde-Vinent, M.A., 2001. Geology of the amber-bearing deposits of the greater Antilles. Carribean J. Sci. 37, 141–166.

Jaeger, L.H., Mendonça de Souza, S.M., Dias, O.F., Iñiguez, A.M., 2013. *Mycobacterium tuberculosis* complex in remains of 18th-19th century slaves. Braz. Emerg. Infect. Dis. 19, 837–839.

Jiménez, A.F., Gardner, S.L., Araújo, A., Fugassa, M., Brooks, R.H., Racz, E., Reinhard, K., 2012. Zoonotic and human parasites of inhabitants of Cueva de los Muertos Chiquitos, Rio Zape Valley, Durango, Mexico. J. Parasitol. 98, 304–309.

Jones, A.K.J., 1982. Recent finds of intestinal parasite ova at York, England. Presented at the Annual Meeting of the European Chapter of the Paleopathology Association.

Jones, A.K.G., 1985. Trichurid ova in archaeological deposits: Their value as indicators of ancient feces. In: Fieller, N.J.R., Gilbertson, D.D., Ralph, N.G.A. (eds.), Paleobiological investigations: Research design, methods and data analysis, BAR International Series 266. British Archaeological Reports, Oxford, pp. 105–114.

Kay, G.L., Sergeant, V.G., Bandiera, P., Milanese, M., Bramanti, B., Bianucci, R., Pallen, M.J., 2014. Recovery of a medieval *Brucella melitensis* genome using shotgun metagenomics. MBio 5, e01337-14. doi: 10.1128/mBio.01337-14.

Khairat, R., Ball, M., Chang, C.C., Bianucci, R., Nerlich, A.G., Trautman, M., Ismail, S., Shanab, G.M., Karim, A.M., Gad, Y.Z., Pusch, C.M., 2013. First insights into the metagenome of Egyptian mummies using next-generation sequencing. J. Appl. Genet. 54, 309–325.

Kim, M.J., Shin, D.H., Song, M.J., Song, H.Y., Seo, M., 2013. Paleoparasitological surveys for detection of helminth eggs in archaeological sites of Jeolla-do and Jeju-do. Korean J. Parasitol. 51, 489–492.

Kristjánsdóttir, S., Collins, C., 2011. Cases of hydatid disease in Medieval Iceland. Int. J. Osteoarchaeol. 21, 479–486.

Kuijper, W.J., Turner, H., 1992. Diet of a Roman centurion at Alphen aan den Rijn, The Netherlands, in the first century AD. Rev. Paleobot. Palynol. 73, 187–204.

Kumm, K., Reinhard, K., Piombino, D., Araújo, A., 2010. Archaeoparasitological investigation of a mummy from Sicily (18th–19th century AD). Anthropol. Int. J. Sci. Ma. 48, 177–184.

Lardeux, F., 2013. Niche invasion, competition and coexistence amongst wild and domestic Bolivian populations of Chagas vector *Triatoma infestans* (Hemiptera, Reduviidae, Triatominae). C. R. Biol. 336, 183–193.

Le Bailly, M., Leuzinger, U., Schlichtherle, H., Bouchet, F., 2005. *Diphyllobothrium*: neolithic parasite? J. Parasitol. 91, 957–959.

Le Bailly, M., Gonçalves, M.L.C., Harter-Lailheugue, S., Prodéo, F., Araújo, A., Bouchet, F., 2008. New finding of *Giardia intestinalis* (Eukaryote, Metamonad) in Old World archaeological site using immunofluorescence and enzyme-linked immunosorbent assays. Mem. Inst. Oswaldo Cruz 103, 298–300.

Le Bailly, M., Bouchet, F., 2013. *Diphyllobothrium* in the past: Review and new records. Int. J. Paleopathol. 3, 182–187.

Leles, D., Reinhard, K., Fugassa, M., Ferreira, L.F., Iñiguez, A.M., Araújo, A., 2010. A parasitological paradox: Why is ascarid infection so rare in the prehistoric Americas? J. Archaeol. Sci. 37, 1510–1520.

Leles, D., Gardner, S.L., Reinhard, K., Iñiguez, A.M., Araújo, A., 2012. Are *Ascaris lumbricoides* and *Ascaris suum* a single species? Parasit. Vectors 5, 42. doi: 10.1186/1756-3305-5-42.

Lima, V.S., Iñiguez, A.M., Otsuki, K., Ferreira, L.F., Araújo, A., Vicente, A.C.P., Jansen, A.M., 2008. Chagas disease by *Trypanosoma cruzi* lineage I in a hunter-gatherer ancient population in Brazil. Em. Inf. Dis. 14, 1001–1002.

Littlewood, T.J., Donovan, S.K., 2003. Fossil parasites: a case of identity. Geol. Today 19, 136–142.

London, D., Hruschka, D., 2014. Helminths and human ancestral immune ecology: what is the evidence for high helminth loads among foragers? Am. J. Hum. Biol. 26, 124–129.

Martill, D.M., Davis, P.G., 1998. Did dinosaurs come up to scratch? Nature 396, 528–529.

Martinson, E., Reinhard, K.J., Buikstra, J.E., Dittmar, K., 2003. Pathoecology of Chiribaya parasitism. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 195–205.

Marzochi, M.C., Coutinho, S.G., De Souza, W.J., Toledo, L.M., Grimaldi, G., Momen, H., Pacheco, R., Sabroza, P.C., Souza, M.A., Rangel, F.B., 1985. Canine visceral leishmaniasis in Rio de Janeiro, Brazil. Clinical, parasitological, therapeutical and epidemiological findings (1977-1983). Mem. Inst. Oswaldo Cruz 80, 349–357.

Matsui, A., Kanchara, M., Kanehara, M., 2003. Paleoparasitology in Japan – discovery of toilet features. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 127–136.

Mitchell, P., 2013. The origins of human parasites: exploring the evidence for endoparasitism throughout human evolution. Int. J. Paleopathol. 3, 191–198.

Mitchell, P.D., Anastasiou, E., Syon, D., 2011. Human intestinal parasites in crusader Acre: evidence for migration with disease in the medieval period. Int. J.Paleopathol. 1, 132–137.

Montenegro, A., Araújo, A., Hetherington, R., Ferreira, L.F., Weaver, A., Eby, M., 2006. Parasites, paleoclimate and the peopling of the Americas: using the hookworm to time the Clovis migration. Cur. Anthropol. 47, 193–198.

Morris, J.N., 1988. Excavations at Casa Bisecada (Site 5MT8829), a Pueblo I field House/ Pueblo II habitation. In: Kuckelman, K.A., Morris, J.N. (eds.), Archaeological Investigations on South Canal, vol. I. Complete Archaeological Service Associates, Cortez, Colorado, pp. 108–151. Four Corners Archaeological Project Report, no. 11.

Nagler, C., Haug, J.T., 2015. From fossil parasitoids to vectors: Insects as parasites and hosts. Adv. Parasitol. 90, 137–200.

Nerlich, A.G., Bianucci, R., Trisciuoglio, A., Sch€onian, G., Ball, M., Giuffra, V., Bachmeier, B., Pusch, C.M., Ferroglio, E., Fornaciari, G., 2012. Visceral leishmaniasis during Italian Renaissance, 1522–1562. Emerg. Infect. Dis. 18, 184–186.

Oh, C.S., Seo, M., Lim, N.J., Lee, S.J., Lee, E.J., Lee, S.D., Shin, D.H., 2010. Paleoparasitological report on *Ascaris* aDNA from an ancient East Asian sample. Mem. Inst. Oswaldo Cruz 105, 225–228.

Patrucco, R., Tello, R., Bonavia, D., 1983. Parasitological studies of coprolites of pre-Hispanic Peruvian populations. Curr. Anthropol. 24, 393–394.

Pavlovsky, E.N., 1964. Natural Nidality of Transmissible Infections. Nauka, Moscow.

Pessoa, S.B., 1951. Parasitologia Médica. Ed. Guanabara, Waissman Koogan, Rio de Janeiro, BR, p. 11.

Piombino-Mascali, D., Zink, A.R., Reinhard, K., Lein, M., Panzer, S., Aufderheide, A.C., Rachid, R., De Souza, W., Araújo, A., Chaves, S.A.M., Leroy-Toren, S., Teixeira-Santos, I., Dutra, J.M.F., 2013. Dietary analysis of Piraino 1, Sicily, Italy: The role of archaeopalynology in forensic science. J. Archaeol. Sci. 40, 1935–1945.

Pinto Dias, J.C., 2013. Human Chagas disease and migration in the context of globalization: Some particular aspects. J. Trop. Med. 2013, 789758. doi: 10.1155/2013/789758.

Poinar, Jr., G., Poinar, R., 2004a. *Paleoleishmania proterus* n. gen., n. sp., (Trypanosomatidae: Kinetoplastida) from Cretaceous Burmese Amber. Protist 155, 305–310.

Poinar, Jr., G., Poinar, R., 2004b. Evidence of vector-borne disease of Early Cretaceous reptiles. Vector-Borne Zoonotic Dis. 4, 281–284.

Poinar, Jr., G., 2005. *Triatoma dominicana* sp. n. (Hemiptera: Reduviidae: Triatominae), and *Trypanosoma antiquus* sp. n. (Stercoraria: Trypanosomatidae), the first fossil evidence of a triatomine-trypanosomatid vector association. Vector-Borne Zoonotic Dis. 5, 72–81.

Poinar, G., Boucot, A.J., 2006. Evidence of intestinal parasites of dinosaurs. Parasitology 133, 245–249.

Poinar, Jr., G., 2007. Early Cretaceous trypanosomatids associated with fossil sand fly larvae in Burmese amber. Mem. Inst. Oswaldo Cruz 102, 635–637.

Poinar, H.N., Schwarz, C., Qi, J., Shapiro, B., MacPhee, R.D.E., Buigues, B., Tikhonov, A., Huson, D.H., Tomsho, L.P., Auch, A., Rampp, M., Miller, W., Schuster, S.C., 2006. Metagenomics to Paleogenomics: Large-scale sequencing of Mammoth DNA. Science 311, 392.

Poinar, G.O., 2014. Evolutionary history of terrestrial pathogens and endoparasites as revealed in fossils and subfossils. Adv. Biol. Article ID 181353, 29 pages doi: 10.1155/2014/181353.

Poulin, R., Combes, C., 1999. The concept of virulence: Interpretations and implications. Parasitol. Today 15, 474–475.

Raoult, D., Reed, D.L., Dittmar, K., Kirchman, J.J., Rolain, J.M., Guillen, S., Light, J.E., 2008. Molecular identification of lice from pre-Columbian mummies. J. Infect. Dis. 197, 535–543.

Reed, D.L., Smith, V.S., Hammond, S.L., Rogers, A.R., Clayton, D.H., 2004. Genetic analysis of lice supports direct contact between modern and archaic humans. PLoS Biol. 2, e340.

Reinhard, K., 1985. Parasitism at Antelope House: A Puebloan village in Canyon de Chelly, Arizona. In: Merbes, C.F., Miller, R.J. (Eds.), Health and Disease in the Prehistoric Southwest. Tempe, Arizona State University, Anthropol. Papers, 34.

Reinhard, K.J., Ambler, J.R., McGuffie, M., 1985. Diet and parasitism at Dust Devil cave. Am. Antiq. 50, 819–824.

Reinhard, R., Confalonieri, U., Ferreira, L.F., Herrmann, B., Araújo, A., 1986. Recovery of parasite remains from coprolites and latrines: Aspects of paleoparasitological technique. Homo (Stuttgart) 37, 217–239.

Reinhard, K., 1988. Cultural ecology of prehistoric parasitism on the Colorado Plateau as evidenced by coprology. Am. J. Phys. Anthropol. 77, 355–366.

Reinhard, K., 1992a. Parasitology as an interpretative tool in archaeology. Am. Antiq. 57, 231–245.

Reinhard, K., 1992b. Patterns of diet, parasitism, and anemia in prehistoric west North America. In: Stuart-Macadam, P., Kent, S. (eds.), Diet, Demography, and Disease: Changing Perspectives in Anemia. Aldine de Gruyter, New York, pp. 219–258.

Reinhard, K., Bryant Jr., V.M., 1992. Coprolites analysis: A biological perspective on archaeology. In: Schiffer, M.B. (ed.), Advances in Archaeological Method and Theory, vol. 4. Academic Press, New York, pp. 245–288.

Reinhard, K., Araújo, A., Ferreira, L.F., Coimbra, C.E., 2001. American hookworm antiquity. Med. Anthropol. 20, 96–101 discussion 101–104.

Reinhard, K., Urban, O., 2003. Diagnosing ancient diphyllobothriasis from Chinchorro mummies. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 191–193.

Reinhard, K., Fink, T.M., Skiles, J., 2003. A case of megacolon in Rio Grande Valley: preliminary data. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 165–172.

Reinhard, K., Buikstra, J., 2003. Louse infestation of the Chiribaya Culture, Southern Peru: Variation in prevalence by age and sex. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 173–179.

Reinhard, K., Damon, T., Edwards, S.K., Meier, D.K., 2006. Pollen concentration analysis of Ancestral Pueblo dietary variation. Paleogeogr. Paleoclimatol. Paleoecol. 237, 92–109.

Reinhard, K.J., Ambler, J.R., Szuter, C.R., 2007. Hunter-gatherer use of small animal food resources: Coprolite evidence. Int. J. Osteoarchaeol. 17, 416–428.

Reinhard, K.J., 2008. Pathoecology of two Ancestral Pueblo villages. In: Reitz, E.J., Scarry, C.M., Scudder, S.J. (eds.), Case Studies in Environmental Archaeology, second ed. Plenum Press, New York, pp. 191–210.

Reinhard, K.J., Bryant Jr., V., 2008. Pathoecology and the future of coprolite studies in bioarchaeology. In: Papers in Nat. Res., pp. 2–21; http://digitalcommons.unl.edu/natrespapers/43/

Reinhard, K., Araújo, A., 2012. Synthesising parasitology in archaeology with paleopathology. In: Buikstra, J., Roberts, C. (Eds.), The Global History of Paleopathology: Pioneers and Prospects, vol. 1. Oxford University Press, Oxford, pp. 751–764.

Reinhard, K., Ferreira, L.F., Bouchet, F., Sianto, L., Dutra, J., Iñiguez, A.M., Leles, D., Le Bailly, M., Fugassa, M., Pucu, E., Araújo, A., 2013. Food, parasites, and epidemiological transitions: a broad perspective. Int. J. Paleopathol. 86, 1–8.

Reinhard, K., Pucu, E., 2014. Comparative parasitological perspectives on epidemiologic transitions: the Americas and Europe. In: Zuckerman, M.K. (ed.), Modern Environments and Human Health. Wiley Blackwell Eds., John Wiley and Sons, Inc, Hoboken, New Jersey, pp. 321–336.

Rep, B.H., 1963. On the polyxenia of Ancylostomidae and the validity of the characters for their differentiation. (II). Trop. Geogr. Med. 12, 271–316.

Rezende, J., 2003. Maculo e sua variada sinonímia. Rev. Patol. Trop. 32, 131–135.

Rothhammer, F., Allison, M.J., Núñez, L., Standen, V., Arriaza, B., 1985. Chagas' disease in pre-Columbian South America. Am. J. Phys. Anthropol. 68, 495–498.

Ruffer, M.A., 1910. Note on the presence of *Bilharzia haematobia* in Egyptian mummies of the twentieth dynasty (1250–1000). Br. Med. J. 16. Part 1.

Salgado, J.A., 1980. O centenário de Carlos Chagas e a menina Berenice. Mem. Inst. Oswaldo Cruz 75, 193–195.

Samuels, R., 1965. Parasitological study of long dried fecal samples. In: Osborne, D., Katz, B.S. (eds.), Contributions of the Wetherill Mesa Archaeological Project. Society for American Archaeology, Memoirs, p. 19.

Santoro, C., Vinton, S.D., Reinhard, K., 2003. Inca expansion and parasitism in the Lutta Valley: Preliminary data. Mem. Inst. Oswaldo Cruz 98 (Suppl. 1), 161–163.

Schmid-Hempel, P., 2011. Evolutionary Parasitology. The Integrated Study of Infectious, Immunology, Ecology, and Genetics. Oxford University Press, UK, p. 5.

Searcey, N., Reinhard, K., Egarter-Vigl, E., Maixner, F., Piombino-Mascali, D., Zink, A.R., van der Sanden, W., Gardner, S.L., Bianucci, R., 2013. Parasitism of the Zweeloo Woman: Dicrocoeliasis evidenced in a Roman period bog mummy. Int. J. Paleopathol. 3, 224–228.

Seo, M., Shin, D.H., Guk, S.M., Oh, C.S., Lee, E.J., Shin, M.H., Kim, M.J., Lee, S.D., Kim, Y.S., Yi, Y.S., Spigelman, M., Chai, J.Y., 2008. *Gymnophalloides seoi* eggs from the stool of a 17th-century female mummy found in Hadong, Republic of Korea. J. Parasitol. 94, 467–472.

Seo, M., Oh, C.S., Chai, J.Y., Jeong, M.S., Hong, S.W., Seo, Y.M., Shin, D.H., 2014a. The changing pattern of parasitic infection among Korean populations by paleoparasitological study of Joseon Dynasty mummies. J. Parasitol. 100, 147–150.

Seo, M., Araújo, A., Reinhard, K.J., Chai, J.Y., Shin, D.H., 2014b. Paleoparasitological studies on mummies of the Joseon Dynasty, Korea. Korean J. Parasitol. 52, 235–242.

Shapiro, B., Rambaut, A., Gilbert, M.T., 2006. No proof that typhoid caused the Plague of Athens (a reply to Papagrigorakis et al.). Int. J. Infect. Dis. 10, 334–335.

Shin, D.H., Lim, D.S., Choi, K.J., Oh, C.S., Kim, M.J., Lee, I.S., Kim, S.B., Shin, J.E., Bok, G.D., Chai, J.Y., Seo, M., 2009. Scanning electron microscope study of ancient parasite eggs recovered from Korean mummies of the Joseon Dynasty. J. Parasitol. 95, 137–145.

Shin, D.H., Oh, C.S., Chai, J.Y., Lee, H.J., Seo, M., 2011. *Enterobius vermicularis* eggs discovered in coprolites from a medieval Korean mummy. Korean J. Parasitol. 49, 323–326.

Shin, D.H., Oh, C.S., Chai, J.Y., Ji, M.J., Lee, H.J., Seo, M., 2012. Sixteenth century *Gymnophalloides seoi* infection on the coast of the Korean Peninsula. J. Parasitol. 98, 1283–1286.

Sianto, L., Reinhard, K., Chame, M., Chaves, S.M., Mendonça de Souza, S.M., Gonçalves, M.L.C., Fernandes, A., Ferreira, L.F., Araújo, A., 2005. The finding of *Echinostoma* sp. (Trematoda: Digenea) and hookworm eggs in coprolites collected from a Brazilian mummified body dated of 600–1,200 years before present. J. Parasitol. 91, 972–975.

Sianto, L., Chame, M., Silva, C.S.P., Gonçalves, M.L.C., Reinhard, K., Fugassa, M.H., Araújo, A., 2009. Animal helminths in human archaeological remains: A review. Rev. Inst. Med. Trop. São Paulo 51, 119–130.

Sianto, L., Santos, I.T., Chame, M., Chaves, S.M., Souza, S.M.F.M., Ferreira, L.F., Reinhard, K., Araújo, A., 2012. Eating lizards: A millenary habit evidenced by Paleoparasitology. BMC Res. Notes 5, 586. doi: 10.1186/1756-0500-5-586.

Silva, P.A., Borba, V., Dutra, J., Leles, D., Da-Rosa, A.A.S., Ferreira, L.F., Araújo, A., 2014. A new ascarid species in cynodont coprolite dated of 240 million years. Acad. Bras. Ci 86, 265–270.

Siqueira, L.M., Coelho, P.M., Oliveira, A.A., Massara, C.L., Carneiro, N.F., Lima, A.C., Enk, M.J., 2011. Evaluation of two coproscopic techniques for the diagnosis of schistosomiasis in a low-transmission area in the state of Minas Gerais, Brazil. Mem. Inst. Oswaldo Cruz 106, 844–850.

Souffez, M., 2001. Des insects et des Homes: Répresentations du poux dans la culture andine. Thèse. Université de Paris X Nanterre, Paris.

Sousa, G.S., n.d. Notícia do Brasil. São Paulo, Livraria Martins Editora (originally published in 1587).

Stone, A.C., Wilbur, A.K., Buikstra, J.E., Roberts, C.A., 2009. Tuberculosis and leprosy in perspective. Am. J. Phys. Anthropol. 40 (Suppl. 49), 66–94.

Szidat, L., 1944. Über die Erhaltungsfähigkeit von Helmintheneierm in vor-und Fühgeschichtlichen Moorleichen. Z. Parasitenkd 13, 265–274.

Trager, W., 1988. Living Together. The Biology of Animal Parasites. Plenum Press, New York, N.Y., 467 pp., p. 1–3.

Tuon, F.F., Neto, V.A., Amato, V.S., 2008. *Leishmania*: Origin, evolution and future since the Precambrian. FEMS Immunol. Med. Microbiol. 54, 158–166.

Van Cleave, H.J., Ross, J.A., 1947. A method for reclaiming dried zoological specimens. Science 105, 318.

Vlaminck, J., Levecke, B., Vercruysse, J., Geldhof, P., 2014. Advances in the diagnosis of *Ascaris suum* infections in pigs and their possible applications in humans. Parasitology 28, 1–8.

Walker, P.L., Bathurst, R.R., Richman, R., Gjerdrum, T., Andrushko, V.A., 2009. The causes of porotic hyperostosis and cribra orbitalia: A reappraisal of the iron-deficiency anemia hypothesis. Am. J. Phys. Anthropol. 139, 109–125.

Warnock, P.J., Reinhard, K.J., 1992. Methods for extracting pollen and parasite eggs from latrine soils. J. Archaeol. Sci. 19, 261–264.

Wilbur, A.K., Buikstra, J.E., 2006. Patterns of tuberculosis in the Americas: How can modern biomedicine inform the ancient past? Mem. Inst. Oswaldo Cruz 101 (Suppl. 2), 59–66.

Wilbur, A.K., Farnbach, A.W., Knudson, K.J., Buikstra, J.E., 2008. Diet, tuberculosis, and the paleopathological record. Curr. Anthropol. 49, 963–977.

Wilke, J.P., Hall, H.G., 1975. Analysis of Ancient Feces: A Discussion and Annotated Bibliography. Archaeological Research Facility, Department of Anthropology, University California, Berkeley.

Wood, J.R., Wilmshurst, J.M., Rawlence, N.J., Bonner, K.I., Worthy, T.H., Kinsella, J.M., Cooper, A., 2013. A megafauna's microfauna: Gastrointestinal parasites of New Zealand's extinct moa (Aves: Dinornithiformes). PLoS One 8, e57315. doi: 10.1371/journal.pone.0057315.