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Recovery Method for Mites Discovered in Mummified Human Tissue

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Recovery Method for Mites Discovered in Mummified Human Tissue

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Much like other arthropods, mites have been discovered in a wide variety of forensic and archaeological contexts featuring mummified remains. Their accurate identification has assisted forensic scientists and archaeologists in determining environmental, depositional, and taphonomic conditions that surrounded the mummified remains after death. Consequently, their close association with cadavers has led some researchers to intermittently advocate for the inclusion of mites in archaeological site analyses and forensic case studies. However, despite their potential value, mites have been underutilized with a variety of reasons for the lack of inclusion of mites in archaeological and forensic analyses. Chief amongst these reasons is the lack of a systematic method for extracting mite specimens from recovered remains, the absence of methods available to archaeologists and forensic scientists that can aid in specimen identification, and the difficulty of specimen identification. The purpose of this thesis is to present a unified method for sampling, recovering, and mounting mite specimens that have been recovered from mummified human tissue. The goal is, when used together, these methods will significantly reduce barriers often encountered by archaeologists and forensic scientists seeking to incorporate mites into archaeological and forensic analyses. Although the scope of this research was limited to mummified human tissue, the hope is that the methods presented in this thesis will provide a way forward for forensic scientists and archaeologists interested in incorporating mites into their analyses.

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Table of Contents

CHAPTER 1: INTRODUCTION 1

CHAPTER 2: MUMMY TAPHONOMY AND ARTHROPOD ASSOCIATES OF DECAY 5

Mite Affiliations with Mummies in Forensic Contexts 8

Mite Affiliations with Mummies in Archaeological Contexts 15

Current Research 19

Preliminary Findings 24

Conclusion 29

CHAPTER 3: ON THE HUNT FOR ACARI – SAMPLING FOR MITES IN MUMMIFIED HUMAN TISSUE 31

Material and Methods 32

Processing Method for Mummified Tissue Samples 32

Sampling Method 33

Results 33

Discussion/Conclusion 34

CHAPTER 4: A NOVEL METHOD FOR WHOLE MITE RECOVERY IN MUMMIFIED HUMAN TISSUE 35

Material and Methods 36

Processing Method for Mummified Tissue Samples 36

Recovery Method 37

Results 38

Discussion 38

Conclusion 39

CHAPTER 5: A COMPARISION OF POTENTIAL MOUNTING METHODS FOR MITES RECOVERED FROM MUMMIFIED HUMAN TISSUE 41

Materials and Methods 42

Mounting in PVA 43

Mounting in Gum Dammar 44

Mounting in Hoyer's 46

Photographing the Mite Specimens 46

Results 47

Discussion 53

Efficiency and Convenience 54

Definition and Clarity 56

Conclusion 64
CHAPTER 6: DISCUSSION AND CONCLUSION 66
REFERENCES CITED 69

Table of Figures

Figure 1. Microscope image of a mite recovered from tissue associated with the sacrum of a mummified homicide victim in Chester, Nebraska, U.S.A. Image captured by Reinhard *et al*. (2017)……14

Figure 2. Microscope image of a Glycyphagidae mite recovered from Lithuanian mummy samples…...25

Figure 3. Microscope image of a Tarsonemidae mite recovered from the Lithuanian mummy samples...27

Figures 4 and 5. Two examples of Glycyphagidae mite specimens……...48

Figures 6 and 7. Male (right) and female Tarsonemidae specimens……..50

Figure 7. An example of an Acaridae mite specimen…….51

Figures 8 and 9. Two examples of Cheyletidae mite specimens……53

Figure 10. Example of unidentified mite specimen mounted in PVA……57

Figure 11. Example of unidentified mite specimen mounted in PVA…….58

Figure 12. Example of a Glycyphagidae mite specimen mounted in PVA…….59

Figure 13. Example of unidentified mite specimen mounted in Gum Dammar……..60

Figure 14. Example of a Cheyletidae mite specimen mounted in Gum Dammar……...60

Figure 15. Example of unidentified mite mounted in Gum Dammar……..61

Figure 16. Example of a Glycyphagidae mite specimen mounted in Hoyer's……62

Figure 17. Example of unidentified mite specimen mounted in Hoyer's……....63

Figure 18. Example of unidentified mite specimen mounted in Hoyer's……64

CHAPTER 1: INTRODUCTION

For centuries, scientists have been fascinated by the postmortem changes that occur in human remains and the types of information these changes can provide about the environmental and depositional conditions present after death. This fascination led to a proliferation of scientists dedicating their lives to studying the history of the body after death, and the birth of the field of taphonomy. The term "taphonomy" was coined in the 1940s by the Russian scientist Ivan Efremov in his pioneering article, *Taphonomy: New Branch of Paleontology*. As a discipline, taphonomy began within the field of Palaeontology, "…where its goal was to explain taphonomic histories, the environmental factors that affect organic remains between an organism's death and its final representation in the fossil record" (Haglund, 2014: p.99). Palaeontologists were largely interested in the taphonomic changes that occur to organisms after death to explain how and why fossils became preserved and how contemporary processes could explain past phenomena (Efremov, 1940; Pokines, 2014). Since its inception, the goals of taphonomy have changed little. However, its influence has expanded far beyond the field of Palaeontology. Taphonomy and its principles have been integrated into several disciplines, but it has become especially influential in the fields of forensic science and archaeology.

Within archaeology and forensic science, taphonomy is more broadly defined as the study and analysis of the various alterations and changes biological remains are subjected to from the time of death until recovery. These taphonomic changes and alterations encompass a wide variety of natural, as well as artificial, processes that often work in tandem to modify biological remains before, during, and after decomposition. A substantial portion of the natural processes that impact taphonomic alterations in archaeological and forensic remains are linked to environmental factors and depositional contexts, such as temperature, weather, post-depositional

environment, erosion, etc. However, potential decomposer communities also play a significant role in the taphonomic modification of remains found in archaeological and forensic contexts.

One of the most prominent and taphonomically important decomposer communities associated with forensic and archaeological remains are arthropods. A substantial body of research focuses on the recovery of arthropods from bodies found in forensic and archaeological contexts. Unlike other types of decomposers, arthropod associates of decay have bodies that are largely comprised of chitin, which allows them to resist the taphonomic processes of decomposition (Morrow *et al*., 2016). This inherent resistance to these processes is invaluable to archaeologists and forensic scientists, as it often ensures that arthropod specimens are preserved in adequate condition to provide valuable insight into the potential taphonomic conditions that may have affected the remains. More specifically, arthropod species can provide contextual taphonomic information, or information about factors that have acted on or altered remains and which point to the life history of corpses from the time of death to the time of recovery (Pokines, 2014). This includes information about environmental and depositional contexts surrounding the remains after death, post-mortem burial rituals, the post-mortem interval, movement of the body, and potentially even the cause of death (Joseph *et al*., 2011; Morrow *et al*., 2015). In large part, this research has primarily focused on various species belonging to the orders Diptera (true flies) and Coleoptera (beetles), as they represent the most prominent types of arthropods recovered from archaeological and forensic remains. However, by largely concentrating on these two orders, researchers have overlooked another important taxon of arthropod that are often associated with biological remains, the Arachnida class known as acari, or mites.

Much like other arthropods, mites have been recovered from a variety of biological remains in both forensic contexts (Megnin, 1894; Braig and Perotti, 2009; Perotti, Braig, and

Goff, 2010; Barton, Weaver, and Manning, 2013; Salona- Bordas and Perotti, 2014; O'Connor *et al*., 2015; Pimsler *et al*., 2016; Reinhard *et al*., 2017; Kamaruzaman *et al*., 2018) and archaeological contexts (Baker 1990; Baker, 2009; Hidalgo- Argüello *et al*., 2003; Morrow *et al*., 2016; Morrow *et al*., 2017). This close association between mites and cadavers has led some researchers in the fields of archaeology and forensic science to intermittently advocate for the inclusion of mites in archaeological site analyses and forensic case studies. The main appeal of mites recovered from such contexts largely stems from their potential to be utilized as archaeological and forensic indicators. Like other arthropod species, mites can be utilized to provide valuable information on the taphonomic, environmental, and depositional conditions that can affect cadavers after death. However, despite recommendations for their inclusion, mites and the prospective taphonomic context and insights they can provide largely remains unexplored. Various explanations have been offered for this lack of inclusion of mites. Chief amongst these explanations is the lack of a systematic method for extracting mite specimens from recovered human remains, the difficulty of specimen identification (Baker, 2009), and the absence of methods available to archaeologists and forensic scientists that can aid in specimen identification (Baker, 1990; Baker, 2009).

Over the past two years, the University of Nebraska's Pathoecology Laboratory has been conducting research on mummified tissue samples to remedy the issues associated with the incorporation of mites into archaeological site analyses and forensic case studies. The purpose of this thesis is to present a unified method for sampling, recovering, and mounting whole and fragmentary mite specimens that have been recovered from mummified human tissue. The goal is when used together these methods will significantly reduce barriers often encountered by archaeologists and forensic scientists seeking to incorporate mites into archaeological and

forensic analyses. The second chapter presented in this thesis includes a literature review of forensic and archaeological cases that have utilized mites. This chapter also provides a historical background for the samples and details preliminary research that led to the creation of the sampling, recovery, and mounting methods. The third chapter presents a method for sampling for the presence of potential mite populations on mummified human tissue. The fourth chapter presents a practical method for the recovery of whole mite specimens from mummified human tissue and the fifth chapter introduces and compares three mounting methods that are commonly used to mount mite specimens. Finally, this thesis concludes with a discussion of potential steps future researchers may employ to further make the analyses and identification of mite specimens accessible to both archaeologists and forensic scientists.

CHAPTER 2: MUMMY TAPHONOMY AND ARTHROPOD ASSOCIATES OF DECAY[1]

Arthropods have long been an important component of the study of mummified human remains. Unlike many other invertebrates, arthropod associates of decay have bodies that are largely comprised of chitin, which allows them to better resist the taphonomic and natural processes of decomposition (Morrow *et al*., 2016). The corpocenosis (community of organisms found in association with corpses) is comprised of a wide variety of active arthropod decomposers, including various taxonomic families in the order Dipteran and various taxonomic families in the order Coleoptera (Morrow *et al*., 2016). However, despite the large body of research that has been produced regarding this corpocenosis, there is one important taxon of arthropod that remains largely overlooked by the literature, and that is the Arachnida class known as acari, or mites.

Mites are morphologically tiny, but diverse joint-legged invertebrate arthropods of the class Arachnida and are related to spiders and scorpions. Roughly 55,000 species, 5,500 genera, and 1,200 subgenera representing approximately 540 families in 124 superfamilies of mites have been identified and described based on distinct morphological and behavioural characteristics (Krantz and Walter, 2009). Many acarologists, however, believe that the estimated number of mite species only accounts for a small fraction of the extant mite species that inhabit the Earth. Some estimates suggest the actual number of extant mite species could range anywhere between 500,000 to 1,000,000 (Krantz and Walter, 2009).

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[1] Based on Smith, Gipson, Piombino-Mascali, and Jankauskas (2021).
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Due to their noteworthy abundance, evolutionary plasticity, and relatively diminutive size, mites have been remarkably successful at colonizing a wide range of terrestrial, aquatic, and marine environments. Mites are a cosmopolitan taxon adept at flourishing in virtually every habitat capable of sustaining life. They can be found in freshwater, saltwater, oceanic trenches, tropical and temperate forests, grasslands, soil, arctic tundras, deserts, and human domiciles. Similarly, mites are quite adaptable and exploit an extensive variety of microhabitats, or small specialized habitats contained within larger surrounding habitats or ecosystems. The types of microhabitats mites can utilize include, but are not limited to, leaf litter, humus, decaying logs, puddles, lichen and mosses, hydrophytes, algae, beds and bed linens, clothing, skin, and fresh and mummified animal carcasses, and human corpses. Accordingly, mites occupy many different ecological niches in these microhabitats and are adept at adapting to the ecosystems they inhabit. Because mites can exploit a wide variety of microhabitats, they must practice a broad range of feeding strategies. Mites are known to be predators, parasites, scavengers, carnivores, herbivores, and fungivores and can subsist on anything from plants, bacteria, and fungi to other mites and invertebrates, to the flesh of both human and non-human animals.

The ubiquitous nature of mites and their propensity for colonizing and feeding on human cadavers has generated a substantial amount of interest from the disciplines of Forensic Science and Archaeology. So much so that, in recent years, forensic acarology has become one of the fastest-growing branches within the field of forensic science. Forensic acarology takes the scientific study of mites one step further by using information provided by the acari in the investigations of crime scenes (Rasmy, 2011). Mites from forensic contexts are recovered from corpses in active decay, as well as corpses that have been partially or fully mummified. Previous research conducted within the field of forensic acarology has suggested that mites have the

potential to make considerable contributions to modern criminal investigations and can serve as trace evidence and indicators of crime due to their close associations with corpses (Braig and Perotti, 2009; Medina *et al*., 2013, Perotti *et al*., 2009; Perotti and Braig, 2009; Salona-Bordas and Perotti, 2014; Rasmy, 2011; Russell *et al*., 2004). More specifically, this research proposes that mites may be useful in the estimation of post-mortem interval, as the life and death cycles of the mites can sometimes provide valuable information on how long a body has been in a specific location; to help determine whether a corpse has been moved or relocated, as mites are often microhabitat specific (Medina *et al*., 2013); provide or reinforce information about the types of arthropod decomposers that have visited the body since deposition (Salona-Bordas and Perotti, 2014); and in establishing cases of abuse and neglect (Rasmy, 2008). Unfortunately, despite growing interest in forensic acarology, the field remains in its infancy as far as research and practical applications are concerned.

Archaeoacarology is the study of mites that have been recovered from archaeological contexts. These mites are generally recovered from mummified human remains and associated grave goods. Much like forensic acarology, archaeoacarology is still very new and remains relatively unexplored save for a few isolated publications. Despite the paucity of literature surrounding archaeoacarology, some limited observations have been made about how it might be useful for the study of mummies in antiquity. Prior research has suggested that mites, when analysed in conjunction with other arthropod associates of decay, could be valuable in providing insights into the taphonomic conditions associated with mummies, the environmental and depositional contexts surrounding the mummies after death, post-mortem burial rituals and environmental conditions, and could also assist curators in assessing the curatorial needs of mummies in their care (Morrow *et al*., 2015).

The purpose of this chapter is to provide a brief overview of published cases in which mites have been found in association with mummified human remains in forensic and archaeological contexts. This chapter will begin with a discussion of contemporary forensic cases and historical archaeological cases that feature mites recovered from mummies. These sections will also include a review of other arthropod associates of decay that have been located alongside mites recovered from these contexts. The chapter will conclude with a section discussing current research that is being conducted in the field of archaeoacarology with mites recovered from mummies found in a crypt beneath the Dominican Church of the Holy Spirit in Vilnius, Lithuania.

Mite Affiliations with Mummies in Forensic Contexts

The roots of forensic acarology can be traced back to the winter of 1878 when the corpse of a new-born infant was found on the waste-ground of the rue Rochebrune in Paris. The corpse was described as having been "…covered in some kind of linen cloth soaked with moisture and rotten at the points that made contact with the soil" (Perotti *et al*., 2009: p.5). An autopsy was performed on the body of the infant on January 15, 1878, by the famed French pathologist Paul C. H. Brouardel. Brouardel reported that the body had been completely mummified and was inhabited by various invertebrate arthropods, including mites and butterfly larvae (Benecke, 2008). The substantial amount of colonization by arthropods prompted Brouardel to request the assistance of Edmond Perrier, a professor at the Museum National d' Histoire Naturelle, and Jean Pierre Megnin, a veterinary surgeon and entomologist. Monsieur Perrier was tasked with the examination of the butterfly larvae, while Jean Pierre Megnin was asked to examine the mites.

During his examination, Megnin found that the outside of the corpse was covered in "…a layer of fine brown powder that is exclusively composed of [casings/shed layers] of the mites

and their feces…" (Megnin, 1894: p.100). Additionally, he noted that the mites found on the outside of the corpse were not alive at the time of discovery but that a "…large, writhing, active colony…" (Megnin, 1894: p.134) of live mites was still present in the interior of the infant's cranium. Megnin found that all the mites recovered from the cranium and body belonged to a single species *Tyroglyphus longior* (Gervais), also known as *Tyrophagus longior* (Gervais). *T. longior* belongs to the family Acaridae (Astigmata) and was originally described by the French acarologists Paul Gervais in 1844 (Perotti, 2009). *T. longior* was reported as a mite that is associated with dry meats and cheeses (ibid) but was also known to consume the fatty acids and soapy materials comprised of ammonia that are produced during dry decomposition (Brouardel, 1879; Perotti et al., 2009). Megnin theorized that the colony of mites discovered on both the body and within the cranium must have initially originated from a small number of phoretic hypopial nymphs that were deposited on the corpse by other arthropods (Benecke, 2008), such as dipterans, coleopteran, or myriapods. Overall, Megnin calculated that a population of approximately 2.4 million living or dead mites were present on the body of the infant (Benecke, 2008). He also determined that after 15 days the first generation which was assumed to contain 10 females and 5 males matured and developed; after 30 days, 100 females and 50 males were present; after 45 days, 1,000 females and 500 males were present; and after 90-days, 1 million females and 500,000 males were present on the body (Benecke, 2008). Based on Megnin's discovery, as well as the discovery and classification of the butterfly larvae by Monsieur Perrier, the infant was estimated to have died approximately five to eight months before her discovery. In the following years, Megnin continued to publish articles about mites (Megnin, 1887; Megnin, 1889; Megnin, 1894; Megnin, 1896) and began developing "…his theory of predictable, ecological waves of insect life on corpses" (Benecke, 2008: p.23), which culminated in the

publication of his pioneering work “La Faune des cadavres: Application de l'entomologie à la médecine légale” in 1894. In this book, Megnin expanded and advanced his theory that corpses are exposed to eight successive waves of insects (discussed in more detail below), provided a description of the larval and adult stages for numerous families of insects, and provided drawings of the overall anatomy of the insects as well as drawings that focused on wing venation and posterior spiracles (Benecke, 2008). The book also featured 19 case reports describing insects found in association with cadavers.

The publications produced by Jean Pierre Megnin were instrumental in advancing and popularizing forensic entomology, and to a lesser extent forensic acarology, as they contributed a substantial amount of information about the types of arthropod fauna associated with graves and mummified or decomposing corpses. In Megnin’s system, the first wave of arthropod succession coincides with the fresh stage of decomposition (as described in Damann and Carter, 2014, p.39) and is characterized by the arrival of species belonging to the families Muscidae, Phoridae, and Calliphoridae. The second wave, which most closely correlates to the bloat or active decay stage of decomposition (ibid:40-41), is characterized by the presence of Calliphoridae and Sarcophagidae. Wave three, which most closely corresponds to the active or advanced stages of decomposition (ibid: 41-42), is marked by the arrival of species from the Dermestidae and Pyralidae families. Wave four, which corresponds with the advanced stage of decomposition, (ibid: 41-42), is characterized by the arrival of species from the Piophilidae, Anthomyiidae, Cleridae and Fannidae families. Wave five also corresponds to the advanced stage of decay and is marked by the presence of Piophilidae, Lonchaeidae, Muscidae, Phoridae, Silphidae, and Histeridae. The final waves (six, seven, and eight) can be correlated to the skeletal remains stage of decomposition (ibid:41-42). Wave six is marked by the arrival of various species of mites,

including those from the families Dinychidae (Uropodidae), Trachytidae, Acaridae, Histiostomatidae, and Glycyphagidae. Wave seven is characterized by the presence of insect families Pyralidae, Tineidae, and Dermestidae. Finally, wave eight is marked by the arrival of species from the families Tenebriomedae and Anobiidae. Over time, the arthropod waves of succession created by Megnin have been expanded and altered to fully encapsulate the often-transitory nature of arthropod colonization of corpses. The mummified infant case was especially influential because it represented the second case in France's history in which entomology was used in a forensic context and the first-time mites were used to estimate post-mortem interval (PMI). Thus, opening the field of entomology and acarology up to forensic investigators.

After Megnin's pioneering work, forensic acarology experienced a considerable lull in its use in forensic casework. For nearly one hundred years, forensic acarology fell by the wayside and was overshadowed by forensic entomology. This is because mites are microscopic decomposers that do not share the obvious nature of proliferating insects. It wasn't until the 1980s that mites were reintroduced as a viable source of evidence for forensic casework and the late 2000s when case reports were published that featured mites recovered from mummified human remains.

Pimsler *et al*. (2016) described a fly-mite association discovered during routine indoor medicolegal death investigations in Texas, U.S.A. The first case described by the research team involved an individual that was found deceased in his bedroom. At the time of discovery, the decedent was reported to be in an advanced state of decomposition and most of the remaining soft tissue was observed to be desiccated and mummified (Pimsler *et al*., 2016). During the autopsy, the Medical Examiner recovered dipteran (true fly) larvae and pupae from the body. Dipteran pupae were also recovered from the hair and scalp of the decedent. The pupae

recovered from the hair and scalp were stored in tangled hair in a honeycomb arrangement to be reared to the adult stage so that they could be identified, while the larvae and pupae recovered from the body were divided into two samples, with half of the sample being preserved and the other half being kept alive (Pimsler *et al*., 2016). All adults reared from the hair sample were identified as *Synthesiomyia nudiseta* (Wulp) and displayed a substantial population of mites. Adults reared from the pupal samples collected from the body were identified as S. *nudiseta* (Wulp) and Sarcophagidae and did not exhibit any populations of mites.

Initially, the mites were identified by Pimsler and her colleagues as an unknown species of the genus *Myianoetus* (Acariformes: Histiostomatidae). *Myianoetus spp.* includes species that specialize on ephemeral resources, such as dung and vertebrate carrion, and whose appearance in the environment is oftentimes erratic (Russell *et al*., 2004; O'Connor, 2009; Pimsler *et al*., 2016). This genus of mite is known to be phoretic on flies that belong to several dipteran families, including Sphaeroceridae (Fain *et al*., 1980), Ceratopogonidae (Fain and Domrow, 1980), Muscidae (Negm and Alatawi, 2011), and Calliphoridae (Miranda and Bermudez, 2008). Phoretic mites are those mites that use other organisms for transportation purposes, without becoming parasitic to the transporting organism. Phoresy is a common and convenient strategy for mites, as they do not have wings and are often less mobile than their insect counterparts. As with other free-living Astigmatid mites, *Myianoetus* spp. show a morphologically and behaviourally specialized deutonymph instar which assists in dispersal by allowing the instar to persistently affix to suitable hosts (Krantz and Walter, 2009; Pimsler *et al*., 2016). Later comparisons of the specimens collected in this case with comparative voucher specimens of deutonymphs and adult mites permitted Pimsler and colleagues to identify the mite population associated with S. *nudiseta* (Wulp) on the samples of hair as *Myianoetus muscarum*.

Reinhard *et al*. (2017) identified 2,357mites and 1,425 mite eggs per ml of sample in tissue associated with the sacrum of a mummified homicide victim in Nebraska, U.S.A. The mummified decedent examined was that of an adult female who expired abruptly from blunt force trauma to the skull. The decedent was discovered six years after her death in her residence in the town of Chester, which is located on the border of Nebraska and Kansas. The body of the decedent was found unclothed and laying in the prone position on an unsecured section of carpet. Dirt that had accumulated on the carpet had been displaced onto the surface of the decedent's skin. When found, the decedent was in a near-perfect state of mummification. At the time of autopsy, 100% of the body was covered by preserved skin and the body hair, head hair, and fingernails remained preserved and in place on the body. However, minimal preservation of the internal organs was observed. Reinhard *et al*. (2017) noted that insect activity was limited on the body, with only 37 dermestid-type burrows being recorded. Unfortunately, because the original goal of the analysis was to determine whether the mummified decedent was killed in or near her Nebraska residence, or if she had been killed at another place and transported back to her residence based on an evaluation of pollen and botanical evidence, the mites were not evaluated for the purpose of identification (Figure 1).

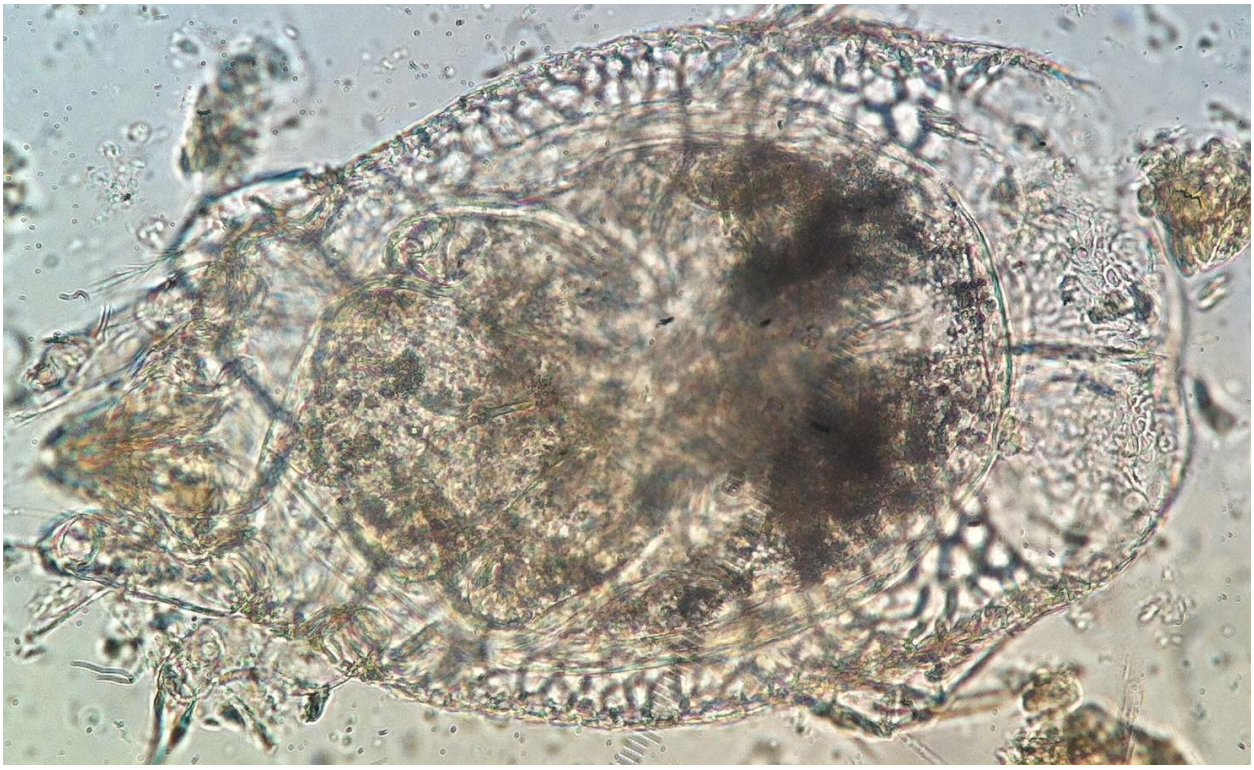

Figure 1. Microscope image of a mite recovered from tissue associated with the sacrum of a mummified homicide victim in Chester, Nebraska, U.S.A. Image captured by Reinhard et al. (2017).

Kamaruzaman *et al*. (2018) described the biology of a species of Macrochelidae mite found on the corpse of a homeless man. In this case, the decedent was found outdoors on a patch of land close to a popular beach in 'Solar Vistahermosa', Alicante, southeast Spain. The body was discovered in a face-up position lying on the ground under an umbrella. At the time of discovery, the decedent was fully clothed and was covered by a blanket up to his neck, with only his head exposed to the elements. The cadaver was noted to be in an advanced state of decay, with some partial mummification of the body. During the autopsy, the pathologist collected empty puparia and blowfly adults from the family Calliphoridae, including *Calliphora vicina, Chysomya albiceps, Lucilia sericata*; the larvae, pupae, and puparia of *Hydrotaea capensis*; the empty puparia, pupae, larvae, and adults of *Synthesiomyia nudiseta*; the larvae *from Fannia*

scalaris; and the pupae of *Conicera tibialis* and *Puliciphora rufipes* (Kamaruzaman *et al*., 2018). Mites were also recovered from the body and were sent to the Acarology Lab at the University of Reading for preparation and identification. Two female mites belonging to *Macrocheles muscaedomesticae* were recovered from the body. *M. muscaedomesticae* is a cosmopolitan species commonly found in domestic, urban, and semirural habitats (Kamaruzaman *et al*., 2018, Durkin *et al*., 2019). This species of mite specializes in rotting organic matter, such as dung and compost (*Krantz* and Walter, 2009). However, *M. muscaedomesticae* is also known to be a highly synanthropic species of mite and will often inhabit poultry farms (Perotti, 1998; Kamaruzaman *et al*., 2018). Additionally, *M. muscaedomesticae* is phoretic on other synanthropic animals, with a preference for flies of the Muscidae and Fanniidae families that associate with humans. *M. muscaedomesticae* adults frequently feed on the eggs of Muscidae and Fanniidae, as well as acarid mites (Kamaruzaman *et al*., 2018). *M. muscaedomesticae* has only been reported on human corpses in two other cases in Europe. In the first case, *M. muscaedomesticae* were excised from the brain of a boy following a failed operation in France (Kamaruzaman *et al*., 2018). In the second case, one female mite was recovered from a human corpse found in a small, wooded area on the North Downs in southeast England (Kamaruzaman *et al*., 2018).

Mite Affiliations with Mummies in Archaeological Contexts

Mites have long been utilized by archaeologists and paleopathologists to help study past populations and archaeological assemblages. Research employing mite remains from archaeological contexts began in the 1960s with coprolites recovered from archaic mummified human remains. Mites retrieved from these coprolites allowed researchers to reconstruct conditions that existed at the time of defecation, identify available food and dietary conditions,

and understand the dynamics and ecology of past diseases (Baker, 2009; Radovsky, 1970; Guerra *et al*., 2003; Chaves da Rocha and Maués da Serra-Freire, 2014). By the 1970s, archaeologists had begun to retrieve and identify mites from human remains recovered during archaeological excavations. However, these early studies did not systematically incorporate the mites into their site analyses but merely mentioned that mites had been recovered (Radovsky, 1970). It wasn't until the 1990s and 2000s that this oversight was corrected, and mites began receiving a more thorough evaluation when uncovered in association with ancient, mummified human remains.

Baker (1990) was one of the first researchers to rigorously investigate mites found in association with a human mummy excavated from Tarapaca, Northern Chile. The recovered mites were extracted from the gut contents of a mummified individual by archaeologists at the Institute of Archaeology, University of London and were referred to Anne Baker for identification purposes. Baker was able to identify the specimens as a new species, *Lardoglyphus robustisetosus*. The mite family Lardoglyphidae includes two genera and nine species (O'Connor, 2009). These mites are known to infest stored food products, especially those food products that contain processed animal materials. Due to the high number of adults, nymphs, and hypopi contained within the intestines, it was hypothesized that *L. robustisetosus* was ingested with food infested by the mites and that this food possibly consisted of dried meat or pemmican (Holden and Nunez, 1993; Baker, 2009).

Hidalgo- Argüello *et al*. (2003) reported on mites recovered from thirteen tombs housing the mummified remains of Leonese royalty discovered at the Collegiate-Basilica of St. Isidoro in Leon, Spain. Samples taken from the bottom of the tomb, which included bones, hairs, clothing, dust, and teeth, were found to contain both mite eggs and the remnants of adult mites. The

researchers were able to identify nonparasitic mites belonging to the suborder Oribatida (described as *O. oribatei*) and the family Aphelacaridae (*Aphelacarus acarinus*). Mites in the suborder Oribatida frequently occur in the top layer of soil and litter debris, and can also be found on plants, lichens, and mosses. These mites consume a variety of organic materials, including fungi, algae, decomposing plant material, dead collembolans, and occasionally nematodes. *A. acarinus* mites are a semi-cosmopolitan species that have been recorded in a wide variety of habitats, including mattress dust, stored grains, soil, bat guano, and the nests of subterranean termites (Baker and Craven, 2003; Baker, 2009).

Other mites recovered from the Leonese samples include those from the superfamily Tarsonemoidea and the subfamily Podapolipidae. Mites of the subfamily Podapolipidae are known to be parasites of arthropods. Their hosts include cockroaches (Dictyoptera, Blattodea), hide beetles (*Dermestes ater*), and carnivorous ground beetles (Coleoptera, Carabidae) (Baker, 2009). Mites from the Cheyletidae family were also found. Cheyletid mites can frequently be found in synanthropic habitats, such as grain stores, nests, farm detritus, and house dust. These mites often predate other mites, as well as the early instars of storage moths (Lepidoptera) and beetles (Baker, 2009).

While Hidalgo- Argüello *et al*. (2003) did not elaborate on the possible origins of the mites found in the sample, Anne Baker (2009) hypothesized that the prevalence of mite fragments and the absence of whole, live mites indicates that the infestation of mites was most likely not recent. Rather, Anne Baker (2009) believes that the mites gained access to the mummies and tombs when Napoleon's troops occupied the basilica in the 19th century. During this time, the tombs were broken into and looted, and parts of the basilica were converted into barns and stables for horses.

Morrow *et al*. (2016) reported the occurrence of a variety of mites, and other arthropods, in embalming jars containing the visceral remains of at least two prominent members of the Medici family and the materials used to prepare their bodies after death, such as sponges and cloths. Fragmentary mite remains were found in eight (Samples 2-7, 9, and 10) of the ten samples processed for the study. However, due to poor preservation of the recovered mite fragments and an absence of whole specimens, the researchers were unable to identify the mites taxonomically. In lieu of identification, Morrow et al. (2016) opted for the quantification of mite densities via counts of mite capitula and eggs observed in the samples. The mites were quantified using the *Lycopodium* Quantification Technique (Camacho *et al*., 2018) frequently employed in palynological and archaeoparasitological analyses. The use of this quantification technique allowed the researchers to demonstrate large mite densities in seven (Samples 3-7, 9, and 10) of the ten samples. Morrow *et al*. (2016) were also able to establish the presence of a host of other invertebrate arthropods that were present alongside the mites in the embalming jars. A total of six out of the ten samples processed contained the remains of other non-mite arthropods. Samples taken from jars 4, 6, and 9, which contained viscera and textiles, included puparia from *Hydrotaea capensis* (Diptera: Muscidae). Samples taken from jars 5, 6, 7, and 10, which contained body tissue, vegetal remains, and fibers, included Phorid puparia. The researchers were also able to recover spider beetle (Coleoptera: Ptinidae) remains from jars 4 and 5.

Morrow *et al*. (2017) recovered the fragmentary remains of mites and pseudoscorpions from grave deposits extracted from burials located in the Ychsma Polity of Pachacamac on the Central Coast of Peru. Sediment samples were taken from twenty-one burials and rehydrated in 0.5% trisodium phosphate for 48 hours. After rehydration, the samples were screened through a 250 μm mesh. Any material larger than 250-microns was subsequently examined for the

presence of arthropods. In total, Morrow *et al*. (2017) were able to recover pseudoscorpion fragments from two of the samples, as well as a host of fragmentary mite remains. Based on their findings, the researchers were able to conclude that the decomposition process resulted in a proliferation of both insects and mites. The mites, however, were too badly preserved to allow taxonomic identification. Morrow and her colleagues hypothesize that the poor preservation of the mites can be attributed, in part, to predation by the pseudoscorpions that infested the Peruvian mummies at Pachacamac.

Current Research

The Dominican Church of the Holy Spirit sits atop several subterranean crypts containing the remains of clergymen and laypeople, dating back to the 18th/19th century AD (Piombion-Mascali *et al*., 2014). When the crypts were first constructed, they consisted of a principle subterranean area established under the central nave (Piombino-Mascali *et al*., 2015) with a series of side chambers erected under the sanctuary aisles. The bodies of the clergymen and laypeople were originally housed in multiple chambers that included separate entrances. However, the crypts were modified by the late 19th century to allow access via a single entrance.

During the last three centuries, the crypts have endured several disturbances (ibid). The first of these events occurred when Napoleon's army established a military hospital in the church (ibid). The crypts were used as makeshift sepulchres and the corpses of soldiers were deposited within them. By 1844, the Russian Empire had regained control of the church and began chronicling the crypt and its contents. Shortly thereafter, in 1854, a description of the crypts and their history was published by the Dominican Friar Wojciech Wincenty Bagiński (ibid). This publication provided a list of the aristocrats and religious figures entombed within the crypts. The document also noted that during the mid-1800s the human remains contained within the

crypts were moved to two side chambers which were subsequently walled up. Additionally, the main accessway to the crypts was sealed.

In 1858, the archaeologist Eustachy Tyszkiewicz was granted permission to examine the crypts to determine whether the body of Grand Duke Alexander had been entombed there (ibid). In his report, the archaeologist noted that columns of coffins had been created by stacking the coffins on top of one another and that a number of bodies, including those of unburied French soldiers from 1812, lay piled up within the crypt. Preserved bodies, numbering in the hundreds, were documented in the church cellars, with multiple rows of bodies being leaned against walls. In 1901, students of the Nazarene Gymnasium were successfully able to reorganize two of the crypts and managed to reinter some of the scattered bones in coffins. In 1906, noted historian Władysław Zahorski provided in-depth descriptions of the bodies, textiles, and coffins located in the crypts. However, at this time Zahorski also reported that the corpses of the Napoleonic soldiers were no longer present.

In the 1930s, during Lithuania's occupation by Poland, more extensive efforts were made to organize the subterranean vaults by students at Stefan Batory University (Piombino-Mascali *et al*., 2015). During this time debris was removed from the chambers. Coffins were moved from the side rooms to the main crypt and historical documents were recovered, which included the dates for coffins from the late 17th and 18th centuries. However, work on the project was terminated before its completion. By 1941, National Socialist Germany had assumed control over Lithuania in the midst of World War II (Piombino-Mascali *et al*., 2015) and in 1944 the crypts were designated as bomb shelters.

In 1962, during a period of Soviet occupation in Lithuania, there was renewed interest in cataloging and preserving the contents of the crypts by government officials (Piombino-Mascali

et al., 2015). In late 1962 it was reported that the bodies contained within the crypts were in bad condition due to contact with air. The infiltration of air was linked to the presence of a storeroom for vegetables. It was also at this time that concerns over contamination of the crypts due to the presence of mice and flies were expressed (Piombino-Mascali *et al*., 2015). The committee of government officials overseeing the project decided that closing the chambers and sealing the mummies behind a glass window would be advisable. Thus, the mummies were sealed.

Beginning in 1963, artifact information was collected, and coffin hardware and associated textiles were documented by a team of historians, ethnographers, and forensic scientists. Some articles of clothing and religious artifacts found in the crypts were also collected for exhibition. At this time, forensic scientist Juozas Markuli registered the remains of 500 individuals as being present within the crypts, with 200 of these individuals being found to have undergone mummification. Analysis of these individuals showed that mummification occurred spontaneously due to low constant temperatures and environmental ventilation (Piombino-Mascali *et al*., 2015). Some bodies mummified in the early stages of decomposition and exhibited bloating or adiopocere formation.

By 1967, conditions in the crypt had significantly deteriorated. Markuli noted at the time that it felt as if humidity levels within the crypt had drastically increased. It was also reported that fungal- induced decomposition of the soft tissues on some of the bodies had initiated (Piombino-Mascali *et al*. 2015). Appeals were made to the Ministry of Culture to re-establish the initial environmental conditions by restoring airflow to the crypts. However, no actions were taken to save the mummies and the site was eventually closed.

Finally, in 2004 an anthropological survey of the chamber containing the greatest number of corpses was conducted (Piombino-Mascali *et al*., 2015). This study revealed that all

mummies contained within the crypts were decomposing. Sealing the mummies behind glass had been ill-advised. In 2008, the glass was removed, and efforts to conserve the remaining mummies were made.

In 2011, officials from the Dominican Church of the Holy Spirit requested research be performed that enabled the analysis, documentation, and preservation of 23 mummies and their associated body parts (Piombino-Mascali *et al*., 2015). Thus, leading to the creation of the Lithuanian Mummy Project[2]. This project was designed to allow researchers to analyse and document various aspects of the lives of those individuals interred in the crypt, as well as provide analyses and documentation of the environment surrounding them in death. The investigations performed by the Lithuanian Mummy project have become instrumental in uncovering information about the decomposition process of the mummies and provide a framework for surveying the organisms that can potentially operate as active decomposers to the mummies housed within the crypts.

The University of Nebraska's Pathoecology Laboratory is currently researching mummified tissue samples that were recovered and submitted by the Lithuanian Mummy Project for analysis. Previous research indicated that the mummified tissue samples were infested with a wide diversity of mites. This finding was confirmed by student volunteers during the Fall of 2018 when the superficial layers of the mummified tissue samples were dusted for adherent detritus. The detritus collected from the tissue samples was then microscopically examined for the presence of mites. Both whole and fragmentary mites were discovered during this preliminary examination. This research project constitutes a pioneering study on mites that are

[2] For more information on the Lithuanian mummies and the Lithuanian Mummy Project see: Piombino-Mascali *et al*., 2014; Piombino-Mascali *et al*., 2015; Piombino-Mascali *et al*., 2015; Weintraub, 2015; and St. Fleur, 2017.

associated with corpses. As such, four primary goals were derived for this project. The first goal seeks to ascertain whether different tissue samples will exhibit a differential diversity and abundance of mite assemblages related to coffin microhabitats. The second goal is to evaluate whether the mummies were thoroughly infested with mites to determine whether the mites colonized the mummies early in the history of the crypt or later when the mummies were disturbed and damaged. The third goal seeks to establish whether a brown powdery substance found on some of the mummies is mold, as it was previously identified, or layers of mites. Finally, the fourth goal of the study will be to attempt to identify whether predatory mite species and grazers both appear in the samples, which will indicate that a community of mites infested the corpses for a substantial amount of time. Moreover, a survey of other arthropod remains found in association with mites will be conducted.

The novelty of this research project has also required the researchers to experiment with multiple recovery techniques to find the ideal method for extracting mites from mummified tissue. Early attempts at extracting the mites involved micro-pipetting individual mites from processed samples and placing them onto microscope slides. However, this method proved too time-consuming and was abandoned. Additionally, kerosene-floatation was attempted (Proctor, 2001) but was found ineffective due to the abundance of lipids present in the samples. The current method being applied is soaking the mummified tissue in 0.5% trisodium phosphate and then screening the samples through a 50, 160, and 250 μm meshes. The resulting mites are concentrated by microcentrifugation into size classes based on mesh size. Microscope mounts are made with either glycerine or Hoyer's solution for slide preparation. Slides are then viewed using a Nikon Eclipse microscope with an Infinity Image Capture system. A further attempt to

mount mite specimens for Scanning Electron Microscopy was made to facilitate more accurate identification of the recovered mite species.

Preliminary Findings

The recovery technique described above has allowed researchers to successfully recover a wide array of both whole and fragmentary mite remains, as well as mite eggs, from the mummified tissue samples. So far, only two of the recovered specimen types have been positively identified to the family level. However, with the use of SEM, the researchers anticipate more identifications soon.

The first specimen recovered belonged to the family Glycyphagidae (Figure 2). Glycyphagidae is the largest in the superfamily Glycyphagoidea with 41 genera and 192 species (Krantz and Walter, 2009). Mites in this family are known to occupy the nests of vertebrates, with specific species preferring the nests of rodents, insectivores, and Didelphimorph marsupials that inhabit the New World. Glycophagidae often exhibit a variety of body morphologies and setal form. However, virtually all mites within the family Glycyphagidae will display a pattern of external ridges on the venter surface of the subcapitulum (Krantz and Walter, 2009). Other characteristics that are generally shared among Glycyphagidae include a rounded, soft body; extended and barbed idiosomatic setae; a cuticle that is covered with pointed microtrichia or lobed protrusions; elongated tarsi; and a shield-like, reduced, or absent prodorsal sclerotization (Krantz and Walter, 2009). Some species in this family have also been found to colonize human domiciles, where they make up a significant part of the acarofauna of both house dust and stored food products (Krantz and Walter, 2009). In recent decades, members of the Glycyphagidae family have been implicated as a source of clinically significant allergens, as well as

occupational allergens among agricultural workers, bakers and pastry chefs, shopkeepers, cheesemakers, horse riders, millers, etc.

Figure 2. Microscope image of a Glycyphagidae mite recovered from the Lithuanian mummy samples.

The second specimen recovered belonged to the family Tarsonemidae (Figure 3). The Tarsonemidae is a family of mite which was established in 1877 (Baker and Wharton, 1952).

Tarsonemidae has since been re-examined and reassigned into a complex system of super- and subfamilies. These families are based upon morphological and biological factors; for example, the subfamily of *Podapolipinae* is classified by its parasitic relationship with insects. Other subfamilies are classified on parasitism with plant species or necrophagous species. These classifications were established in 1939 by H.E. Ewing. In 1953, Robert E. Beer rearranged genera to different families. He then defined Tarsonemidae with the addition of the subfamily *Tarsoneminae* which were comprised of two of Ewing's subfamilies; Beer also included three other subfamilies: *Steneotarsonemus*, *Xenotarsonemus*, and *Rhynchotarsonemus* (Jeppson *et al*., 1975).

Tarsonemidae mites have been affiliated with agriculture since 1877 when the *Steneotarsonemus bancrofti* was associated with sugar cane plants. As well as the *S. bancrofti*, many other Tarsonemidae mites have been implicated in the agricultural world as pests. Produce often associated with mite infestation include strawberries, pineapples, mushrooms, and other fungi (Baker and Wharton, 1952; Ewing, 1922; Jeppson *et al*., 1975). This family of mites can also be parasitic to insects, small and large animals, or even humans (Jeppson *et al*., 1975).

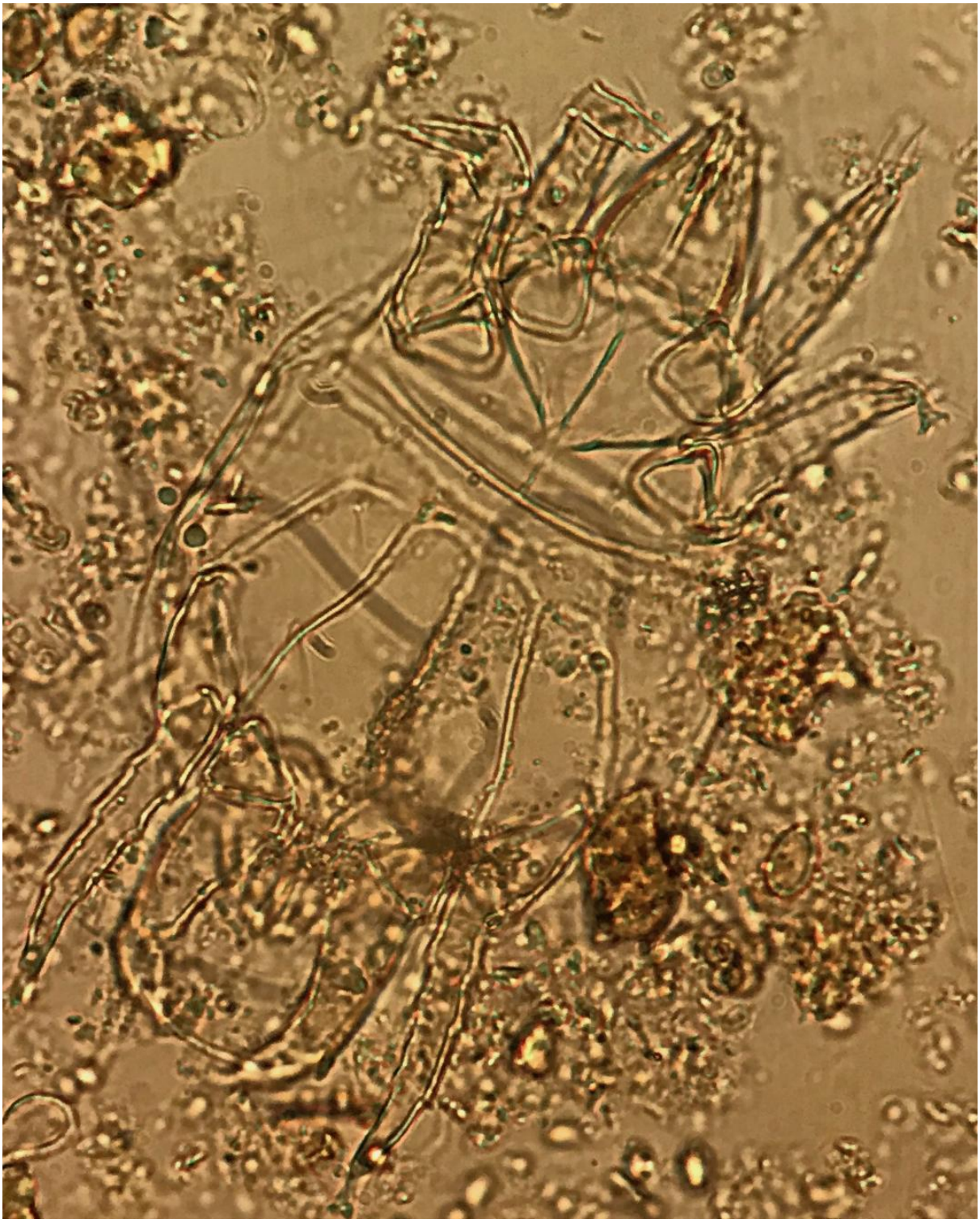

Figure 3. Microscope image of a Tarsonemidae mite recovered from the Lithuanian mummy samples.

Tarsonemid mites are generally very small creatures ranging anywhere from 100 to 300 micrometers, with males being much smaller than the females. Females of the Tarsonemidae family tend to be more oval in body-shape, while the males tend to be rounder. The bodies of these mites are called the idosoma which is transected by a suture. This suture divides the anterior two pairs of legs and the posterior two pairs of legs. The anterior section of the body is called the propodosoma, while the posterior is known as the hysterosoma; the latter section is further divided into the metapososoma and the opisthosoma. Males of the Tarsonemid mites have genital papilla or plates which contain aedeagus. Females of the species have special organs located between coxae 1 and 2. These organs are referred to as clavate sense organs, or psuedostigmatic organs paired with sensible trichodea, and may be linked to the tracheal system (Jeppson *et al*., 1975). The mites also have setae which, on the females, connect to the fourth set of legs with a whip-like shape. The mouthparts of a Tarsonemid mite are located in the capsular head known as the capitulum (Jeppson *et al*., 1975). The capitulum contains a pair of chelicerae which, in some species, are inserted into the plant cells for feeding purposes, as well as paired palpi (Denmark, 2000; Jeppson *et al*., 1975).

The classification of mites in the Tarsonemidae family for the males is based primarily on the characteristics of the posterior legs due to the variability of these features. The fourth pair of legs in this species are considered accessory copulatory appendages due to their use in the mite's mating and premating behaviours. These legs are also generally separated into 4 segments, although a fusion of the tibia and tarsus can occur giving the appearance of 3 segmentations. Claws at the end of these legs can also vary from large, pronounced claws to almost non-existent. The femur may also vary with the species as spurs and other modifications may be present to help with mating as the fourth pair of legs has little to do with movement. Setae may also be used

to classify species within the family as the morphology and location of these appendages may range from normal characteristics to modified characteristics. Normal is classified as narrow at the base to tapered at the ends, while modified may include various other shaping (Jeppson *et al*., 1975).

The life cycle of the Tarsonemid mites has four separate stages (Baker and Wharton, 1952; Jeppson *et al*., 1975). These stages include the egg, larva, quiescent nymphal stage, and the adult (Baker and Wharton, 1952). However, it was originally thought that these stages were separated into egg, larva, immature female, adult male, and adult female (Ewing, 1922). The latter stages were combined into a single category and the immature female was renamed as the nymph stage (Ewing, 1922). Female mites can only lay one egg per reproductive event, and the egg tends to be relatively large in comparison to the female with a white opaque appearance (Baker and Wharton, 1952; Jeppson *et al*., 1975). Once the mite is ready to emerge from the egg, it hatches as a white opaque 6-legged larva with the males being much smaller than the females (Jeppson *et al*., 1975). After this stage is complete, the mites enter a pupal or nymph stage where the mites transform into adults with the development of the genitalia and the fourth pair of legs (ibid). Once these features have developed, the mite is classified as an adult and may range in color depending upon its food sources (ibid).

Conclusion

Like insects, mites have been discovered in a wide variety of forensic and archaeological contexts featuring mummified human remains. Their accurate identification has aided forensic scientists in estimating post-mortem interval, determining whether a body has been moved or relocated, and reinforced information about the types of arthropod decomposers that have visited the body since deposition. Likewise, mites have also assisted archaeologists by providing

valuable insights into the taphonomic conditions associated with mummies, the environmental and depositional contexts surrounding the mummies after death, post-mortem burial rituals and environmental conditions, and the curatorial needs of mummies housed in museum collections (Morrow *et al*., 2015). Despite their ubiquity and the potential value they hold for analysing crime scenes and archaeological assemblages, mites have rarely been utilized for these purposes. This is due in large part to two factors. The first factor is the difficulty in extracting mite specimens from recovered human remains. The second factor is the lack of experience among archaeologists and forensic scientists with mite taxon. Current research being conducted in the fields of forensic acarology and archaeoacarology is helping to remedy the first factor. The second factor, however, might prove more difficult to resolve. One suggestion for future researchers in the fields of forensic acarology and archaeoacarology is to focus their endeavours on constructing user-friendly dichotomous keys that make it easier for those who are unfamiliar with mites, and their associated anatomy and taxonomy to identify them when they are discovered during crime scene or site analyses.

CHAPTER 3: ON THE HUNT FOR ACARI – SAMPLING FOR MITES IN MUMMIFIED HUMAN TISSUE[3]

Arthropods have played a pivotal role in the study of mummified human remains. Unlike many other types of invertebrates, the bodies of arthropods largely consist of chitin, which allows them to better withstand the destructive nature of both natural and taphonomic processes of decomposition (*Morrow et al*., 2016). This inherent resistance to these processes is invaluable to researchers, as it can provide a snapshot into the corpocenosis surrounding mummified individuals. The corpocenosis (or the community of organisms found in association with corpses) consists of a wide range of arthropod decomposers (Morrow *et al*., 2016). However, two orders, Diptera (true flies) and Coleoptera (beetles), have been the primary focus of research regarding corpocenosis. The preoccupation with species belonging to the orders Diptera and Coleoptera have led many researchers to overlook other categories of arthropod that potentially compromise the corpocenosis of mummified individuals.

The purpose of this study is to contribute further information about other types of arthropods that make up the corpocenosis of mummified human remains. Based on previous research which demonstrated acari, or mites, are often found in close association with mummified remains (Megnin, 1894; Baker 1990; Hidalgo- Argüello *et al*., 2003; Baker, 2009; Morrow *et al*., 2016; Pimsler *et al*., 2016; Morrow *et al*., 2017; Reinhard *et al*., 2017; Kamaruzaman *et al*., 2018), it was hypothesized by the authors that the presence of human tissue provides nutrient-rich resource islands that create the potential for a range of mite species to

[3] Based on manuscript by Smith, Reinhard, and Gipson (n.d.).
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colonize and proliferate the bodies of mummies. To test this hypothesis, mummified tissue samples that were recovered and submitted by the Lithuanian Mummy Project were processed and analysed to definitively determine whether mites were present on the mummified individuals buried within the subterranean crypts located under the Dominican Church of the Holy Spirit and to establish whether a diverse number of mite species had colonized the mummies. Additionally, this study sought to develop a practical method for sampling for potential mite populations that may occupy mummified human tissue.

Material and Methods

Processing Method for Mummified Tissue Samples

For this study, three samples of particulate sediment were taken from the abdomens of three mummified individuals buried at the Dominican Church of the Holy Spirit. Two of the three samples (VD-2 and VD-11) consisted of the abdominal cavity contents of two mummified adult individuals, while the third sample (VD-5) consisted of tissue recovered from the abdominal cavity of a third mummified adult individual.

Three subsamples taken from the original VD-2, 11, and 5 samples were screened through a 500 μm sieve to remove large organic matter. The resulting fine particulate sediment was weighed, and each subsample received .50g of the fine particulate sediment. The fine particulate sediment from each subsample was then placed into three separate plastic 50mL centrifuge tubes for a rehydration series. One Lycopodium tablet was added to each subsample for the purpose of later quantification of mites. All subsamples were rehydrated in 15mL of 0.5% trisodium phosphate for 24 hours.

After rehydration, 36% hydrochloric acid (HCL) was incrementally added to each subsample to reduce any existing calcium carbonate. Ethanol was also incrementally added to the subsamples to mitigate the frothing reaction that occurs when HCL is exposed to calcium carbonate. HCL was incorporated until no further frothing reaction was observed.

After 24 hours, 4.0% potassium hydroxide (KOH) was added to each subsample to clear away lipids that could obscure any potential mites present. The subsamples were left to sit in the KOH solution for 40 minutes. Once cleared, the subsamples were screened through a 250μm mesh using distilled water and collected in a 250mL beaker. All material smaller than 250μm (microremains) that were collected in the 250mL beaker were then poured back into 50mL tubes. These 50mL tubes were then repeatedly centrifuged to concentrate the microremains.

Sampling Method

Following the rehydration series, 5 to 10 microscope slides were prepared for each subsample to determine whether mites were present on the mummified individuals. To survey for mites, one to two drops of Hoyer's Slide Mounting Medium was applied to a 75-x-25-x-1mm microscope slide. A wooden applicator stick was then submerged in the wet microremains and the microremain residue was mixed into the Hoyer's Slide Mounting Medium. The microscope slides were subsequently analysed for mites using a Nikon Eclipse microscope.

Results

Slide preparations made via the sampling method confirmed the presence of mites in mummies interred within the crypts of the Dominican Church of the Holy Spirit. Mite remains were observed in each of the processed samples; fragmentary mite remains were the most common type of remains encountered, followed closely by mite eggs. The fragmentary remains

observed consisted of various portions of disarticulated gnathosoma, idiosoma, and legs, while the mite eggs were largely whole. Although rare, a wide array of whole mite specimens was also observed in each of the subsamples indicating that multiple species of mites had in fact colonized the mummies. Three of the whole specimen types found in the subsamples were able to be positively identified to the family level. These included mites from the Glycyphagidae, Tarsonemidae, and Acaridae families.

Discussion/Conclusion

The results of this study demonstrate that mites are another type of arthropod that can potentially make up the corpocenosis of mummified individuals. Analysis of the slide preparations indicate that a multitude of mite species were able to comfortably colonize and proliferate within the abdominal cavities of at least three of the mummified individuals enclosed within the crypts. This suggests that the individuals housed within the subterranean crypts located beneath the Dominican Church of the Holy Spirit acted as attractive resource islands to a wide range of mite species. However, further research is needed to isolate whole mite specimens to gain a more accurate understanding of the various mite species that populate the mummies. Moreover, the study was able to successfully develop a practical method for sampling for potential mite populations that inhabit mummified human tissue.

CHAPTER 4: A NOVEL METHOD FOR WHOLE MITE RECOVERY IN MUMMIFIED HUMAN TISSUE[4]

A substantial body of research has been produced that focuses on the recovery of arthropods present in mummified human remains. This preoccupation with arthropods mainly stems from the capacity of their chitinous exoskeletons to withstand both natural and taphonomic processes of decomposition (Morrow *et al.*, 2016). This resistance often ensures that arthropod specimens are preserved in good enough condition to provide valuable insight into the environmental, depositional, and taphonomic conditions that surrounded the mummified remains after death. In large part, these studies have primarily focused on various species belonging to the orders Diptera (true flies) and Coleoptera (beetles), as they represent the most prominent types of decomposers recovered from mummified remains. However, by largely concentrating on these two orders researchers have overlooked another important taxon of arthropod that are often found on mummified human remains, the Arachnida class known as acari, or mites.

Much like Diptera and Coleoptera, mites have been recovered from a variety of mummified human remains in both forensic contexts (Megnin, 1894; Pimsler *et al*., 2016; Reinhard *et al*., 2017; Kamaruzaman *et al*., 2018) and archaeological contexts (Baker 1990; Baker, 2009; Hidalgo- Argüello *et al*., 2003; Morrow *et al*., 2016; Morrow *et al*., 2017). This close association between mites and mummified tissue has led some researchers in the fields of archaeology and forensic science to intermittently advocate for the inclusion of mites in archaeological site analyses and forensic entomological case studies in which mummified human

[4] Based on manuscript by Smith, Gipson, and Reinhard (n.d.)
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remains have been recovered. However, despite recommendations for their inclusion, mites and the prospective context and insights they can provide for mummified individuals largely remains unexplored.

Various explanations have been offered for the lack of inclusion of mites in forensic and archaeological analyses. Chief amongst these explanations is the lack of a systematic method for recovering whole mite specimens from mummified tissue, the difficulty of specimen identification (Baker, 2009), and the absence of methods available to archaeologists and forensic scientists that can aid in specimen identification (Baker, 1990; Baker, 2009). The purpose of this chapter is to provide archaeologists and forensic scientists with a practical, cost-effective, and systematic method for recovering whole mite specimens from mummified human tissue. The intent is that this chapter will serve as the first step in making the analyses and identification of mite specimens more accessible to researchers working in the fields of archaeology and forensic science.

Material and Methods

Processing Method for Mummified Tissue Samples

For this study, four subsamples of particulate sediment were taken from the recovered abdominal cavity contents of a mummified adult individual (VD-11) buried in subterranean crypts located under the Dominican Church of the Holy Spirit (Vilnius, Lithuania). All four subsamples taken from the original VD-11 sample were screened through a 500μm sieve to remove large organic matter and the resulting fine particulate sediment was weighed out so that each subsample received .50g of the fine particulate sediment. The fine particulate sediment from each subsample was then placed into four separate plastic 50mL centrifuge tubes for a

rehydration series. All subsamples were rehydrated in 15mL of 0.5% trisodium phosphate for 24 hours.

After rehydration, 3mL of 36% hydrochloric acid (HCL) was incrementally added to each subsample to produce a frothing reaction, which occurs when HCL is exposed to calcium carbonate. The froth produced by each subsample was immediately pipetted into a second plastic 50mL tube, and 2-4mL of 95% ethanol was added to sink the particulate matter contained in the froth. The froth samples were then allowed to sit for 24 hours.

Once 24 hours had elapsed, a 160μm and 315μm sieve were stacked on top of one another and the subsamples were screened through the sieves one at a time, with 95% alcohol being used to filter the particulate matter through the sieves. A large petri dish was placed under the sieves to collect the resultant sample. The sample collected in the large petri dish was then decanted through a 100μm mesh into a 250mL beaker. The liquid sample, which was collected in the beaker, is typically murky and turbid, making it difficult to find and recover whole mite specimens. However, it is not impossible, and this sample should be retained for later analysis. The mesh and the particulate matter caught in it were moved to a second 250mL beaker and 95% alcohol was used to filter the particulate matter into the beaker. This sample, which is much clearer than the initial one, was poured into a medium sized petri dish and immediately analysed using a dissecting microscope. The turbid and clear samples that are produced via this method may be screened through the 100μm mesh multiple times.

Recovery Method

The petri dish containing the sample was placed under a dissecting microscope and a micropipette was used to extract individual mites observed floating in the sample. The mite was

then transferred to a microcentrifuge tube and a small amount of 95% alcohol was added to the microcentrifuge tube, so that the mite specimen did not dry out before it could be mounted.

Results

Over 225 individual mite specimens were able to be recovered from the four VD-11 subsamples using the method described above. Among the 225 individual specimens that were extracted from the four samples, at least six to seven different species of mite were observed. Four of the species, belonging to the Glycophagidae, Tarsonemidae, Acaridae, and Cheyletidae families, were immediately recognizable. The identity of the other two to three species, however, could not be determined straight away.

Discussion

The recovery method described in this chapter serves as a convenient and efficient technique for extracting whole mite specimens from mummified human tissue. Overall, this procedure is relatively fast, easy, and cost-effective, as it only requires a minimal amount of basic equipment and reagents. The streamlined nature of the method also ensures that it can successfully be employed by those who do not have a background in entomology or acarology. Thus, making it a practical solution for archaeologists and forensic scientists that wish to recover mite specimens present in mummified human remains.

As demonstrated, the method described in this chapter can be successfully utilized to recover both whole and fragmentary mite specimens. Many of the mite specimens were in such good condition that it was immediately evident various families of acari were represented in the sample. Furthermore, some mites retained important external identifiers, such as specialized setae, modified legs, etc., which allowed them to be classified into specific families. This

indicates that the processing method, and the reagents used during processing, are not detrimental or damaging to the mites.

Additionally, this method further limits the potential for mite specimens to be damaged or destroyed during the recovery process, as it requires the acarine material present in the mummified tissue to be handled as little as possible. This is important as both fresh and historical acarine material tends to be fragile, and taxonomically important morphological structures can be damaged or destroyed when the specimen is subjected to excessive handling.

One potential issue that was observed during the recovery process is that because no clearing agents are used in this method the samples are often murky or turbid, with large quantities of non-acarine material being present even after the samples have been screened. This can sometimes make it difficult to recognize mites present in the samples. KOH (4%) was added to some of the samples to try and mitigate this issue. However, the addition of KOH did not appear to produce a noticeable change in the clarity of the samples. In the future, it could be beneficial to experiment with other clearing agents to see if they can reduce excess debris.

Conclusion

The intent of this chapter was to provide a systematic, practical, and cost-effective method for recovering whole mite specimens from mummified human tissue. The whole mite recovery method described in this chapter proved to be remarkably effective at separating mite specimens from the mummified tissue. Over 225 individual whole mite specimens, and multiple fragmentary mite specimens, were able to be recovered. The recovered specimens were also found to be in relatively good condition after being processed, indicating that this whole mite recovery method is not detrimental or damaging to the mites. These results demonstrate that this

method can serve as an important first step in breaking down barriers that often hinder the use of mites in archaeological and forensic analyses. However, more work is needed to fully make the analyses and identification of mite specimens accessible to archaeologists and forensic scientists. It is imperative that future research involving the incorporation of mites into archaeological and forensic analyses of mummified human remains focus on creating practical methods that can make the identification of these mite specimens less challenging.

CHAPTER 5: A COMPARISION OF POTENTIAL MOUNTING METHODS FOR MITES RECOVERED FROM MUMMIFIED HUMAN TISSUE[5]

Since the 1960s, there has been a growing interest in archaeoacarology, the study of mites that have been recovered from archaeological contexts. Archaeologists and paleopathologists have often utilized mites found in coprolites, mummified human tissue, and grave goods to study past populations and archaeological assemblages. The main appeal of mites recovered from such contexts largely stems from their potential to be utilized as archaeological indicators. Mites make excellent archaeological indicators for several reasons. First and foremost, mites exhibit noteworthy abundance in all types of archaeological deposits, even those with small amounts of material or that are ill-suited to other kinds of indicators (Schelvis, 1992c; Baker, 2009). Their noteworthy abundance in archaeological deposits coupled with the capacity of their chitinous exoskeleton to resist taphonomic and natural decomposition ensures that mite specimens are preserved and obtainable in archaeological assemblages, making them reliable archaeological indicators (Schelvis, 1987 [1989]; Baker, 2009). Mites' geographic ubiquity and ability to successfully colonize and flourish in a wide range of terrestrial, aquatic, and marine environments also mark them as important archaeological indicators, as mites hold the potential to be recovered from a variety of archaeological sites, regardless of location. Additionally, mites are often microhabitat specific, which leads to many species having a limited habitat-range or a specific host (Schelvis, 1987; Baker, 2009). This microhabitat specificity extends to both human and non-human animal remains, which mites are adept at colonizing and exploiting. Finally, their

[5] Based on manuscript by Smith, Racz, and Reinhard (n.d.).
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short reproduction and propagation times make them invaluable indicators, as these cycles can emphasize both rapid and short-term changes to the environment (Baker, 2009). Mites have also previously been used by archaeologists and paleopathologists to help reconstruct past environments and conditions that were present when the mites were alive and at the time of deposition, establish available food resources and dietary conditions, and recognize the ecology and dynamics of past diseases (Radovsky, 1970; Schelvis, 1990b; Schlevis, 1990c; Baker, 2009; Guerra et al., 2003; Chaves da Rocha and Maués da Serra-Freire, 2014). However, despite decades of interest in this subfield of archaeology and intermittent championing of the prospective archaeological value of mites (Denford, 1978; Schelvis, 1987 [1989]; Schelvis, 1990a; Baker, 2009), archaeoacarology remains relatively unexplored save for a few isolated publications and mites are still rarely included in the analyses of archaeological sites.

Two major hinderances that are often cited as contributing to the lack of inclusion of mites in archaeological analyses are the difficulty of specimen identification (Baker, 2009) and the lack of methods available to archaeologists and paleopathologists that can aid in making specimen identification easier (Baker, 1990; Baker, 2009). The goal of this chapter is twofold: The first goal is to provide archaeologists and paleopathologists with three different mounting methods that can significantly reduce various barriers that are often encountered when trying to identify archaeological mite specimens. The second goal is to present a short comparison of the three methods of mite identification. This will aid archaeologists and paleopathologists interested in pursuing the identification of mites recovered from archaeological assemblages by providing a better understanding of the advantages and disadvantages associated with each method.

Materials and Methods

The mummified tissue sample used in this analysis was originally recovered from subterranean crypts located under the Dominican Church of the Holy Spirit (Vilnius, Lithuania). The sample (VD-11) consisted of the abdominal cavity contents of a mummified adult individual. The sample was submitted to the University of Nebraska's Pathoecology Laboratory in the Fall of 2014 by the Lithuanian Mummy Project for parasitological analysis. Preliminary research conducted in subsequent years, however, indicated that the mummified tissue sample had also been infested with a broad variety of mites. These findings were confirmed when detritus from the VD-11 sample was processed and microscopically examined for mites in 2020. During this analysis, both whole and fragmentary mites were recovered from the processed material. It was decided that the whole mites should be mounted to both preserve the specimens and to accentuate important external morphological and histological features that could be used in the identification of these mites. However, due to the paucity of literature that exists on the mounting of mites recovered from archaeological assemblages, there was debate as to which mounting medium would be most suitable for mounting the delicate mite specimens. This led the authors to conclude that it would be beneficial to mount a set number of these mite specimens using three common mounting mediums (PVA, Gum Dammar, and Hoyer's) that are often utilized by acarologists to mount fresh mite specimens.

Mounting in PVA

To mount archaeological mite specimens in PVA mounting medium, the mites were first stained using lactophenol cotton blue. This allows for easier visualization of the mite during the mounting process and further highlights important gross morphological and histological features during the microscopic examination of the mite. To stain the mite specimen, the mite was transferred to a microcentrifuge tube and covered with a small amount of 95% ethanol. One to

two drops of the lactophenol cotton blue were then added to the microcentrifuge tube, and the specimen was subsequently allowed to sit for 24 hours to allow the lactophenol cotton blue to permeate the body of the mite.

Once the mite specimen had been stained, the contents of the microcentrifuge tube were transferred to a spot plate. The mite specimen was located using a dissecting microscope and moved from the original lactophenol cotton blue/ethanol solution into clear 95% ethanol situated on the same spot plate. One to two drops (roughly an apple seed-sized amount) of the PVA mounting medium were added to a 75-x-25-x-1mm microscope slide using the tip of a metal dissecting probe. An insect pin, which had been affixed to the end of a wooden applicator stick using Gorilla-brand super glue, was then dipped into the PVA droplet, and used to extract the mite from the clear ethanol. The mite was subsequently transferred to the PVA droplet placed on the microscope slide. After the mite was securely deposited onto the microscope slide, the insect pin was further employed to push the mite down into the PVA and position the mite with its ventral side exposed. A round coverslip was then carefully placed over the mite. Finally, the slides were dried on a slide warmer, set between 32.5°F and 35.5°F, for five to eight days. Once the PVA was fully set, any excess PVA that pooled outside of the coverslip was removed using an X-Acto no. 10 blade. The microscope slide was then sealed using GC Electric Red Insulating Varnish. In total, 100 mite specimens were mounted using the PVA mounting medium.

Mounting in Gum Dammar

To mount archaeological mite specimens in Gum Dammar mounting medium, the mites were first stained using lactophenol cotton blue. Much like with PVA, this allows for easier visualization of the mite during the mounting process and further highlights important external morphological and histological features during the microscopic examination of the mite. To stain

the mite specimen, the mite was transferred to a microcentrifuge tube and covered with a small amount of 95% ethanol. One to two drops of the lactophenol cotton blue were then added to the microcentrifuge tube, and the specimen was subsequently allowed to sit for 24 hours to allow the lactophenol cotton blue to permeate the body of the mite.

After the mite specimen had been stained, a modified dehydration series was performed. The mite specimen was centrifuged once in 95% ethanol. The 95% ethanol was then poured off, and 100% ethanol was added to the mite specimen. The specimen was centrifuged, and the 100% ethanol was again poured off. The process of adding 100% ethanol to the specimen and centrifuging was repeated two more times, for a total of three centrifuge cycles. When the last centrifuge cycle was completed and the 100% ethanol was poured off, three to five drops (enough to adequately cover the specimen) of xylene were added to the microcentrifuge tube. The specimen was then allowed to sit in the xylene for 30 minutes. When 30 minutes had elapsed, the contents of the microcentrifuge tube were transferred to a spot plate. The mite specimen was located using a dissecting microscope. One to two drops (roughly an apple seed-sized amount) of the Gum Dammar mounting medium were added to a 75-x-25-x-1mm microscope slide using the tip of a wooden applicator stick. It is important to note that the Gum Dammar should be on the thinner side, roughly the consistency of olive oil, to make mounting archaeological mite specimens easier and less destructive. The mite specimen was pipetted out of the xylene using a micropipette and was injected into the Gum Dammar droplet on the microscope slide. An insect pin was then used to push the mite further down into the Gum Dammar and position the mite with its ventral side exposed. A round coverslip was then carefully placed over the mite. Finally, the slides were dried on a slide warmer, set between

32.5°F and 35.5°F, for five to eight days. Only 25 mite specimens were mounted using the Gum Dammar mounting medium.

Mounting in Hoyer's

When mounting mite specimens in Hoyer's mounting medium, the use of lactophenol cotton blue is not recommended. This is because the main ingredient of the Hoyer's mounting medium, Chloral Hydrate, is acidic. When introduced to an acidic environment, such as Hoyer's, the lactophenol cotton blue stain becomes mobile, causing it to leach out of the specimens. The mite specimens, however, should still be stored in a microcentrifuge tube with a small amount of 95% ethanol until they are ready to be mounted.

To mount archaeological mite specimens in the Hoyer's mounting medium, the contents of the microcentrifuge tube were transferred to a spot plate. The mite specimen was then located using a dissecting microscope. One to two drops of the Hoyer's mounting medium were added to a 75-x-25-x-1mm microscope slide. The mite specimen was pipetted out of the 95% ethanol using a micropipette and was injected into the Hoyer's droplet on the microscope slide. An insect pin was then used to push the mite further down into the Hoyer's and position the mite with its ventral side exposed. A round coverslip was then carefully placed over the mite and the slides were left to dry on a slide warmer, set between 32.5°F and 35.5°F, for five to eight days. In total, 100 mite specimens were mounted using the Hoyer's Mounting Medium.

Photographing the Mite Specimens

After the 225 mite specimens were mounted, a subset of mites from each of the mounting medium were photographed, with 37 mites being photographed for the PVA and Hoyer's mounting medium, and 11 being photographed for the Gum Dammar mounting medium. Pictures

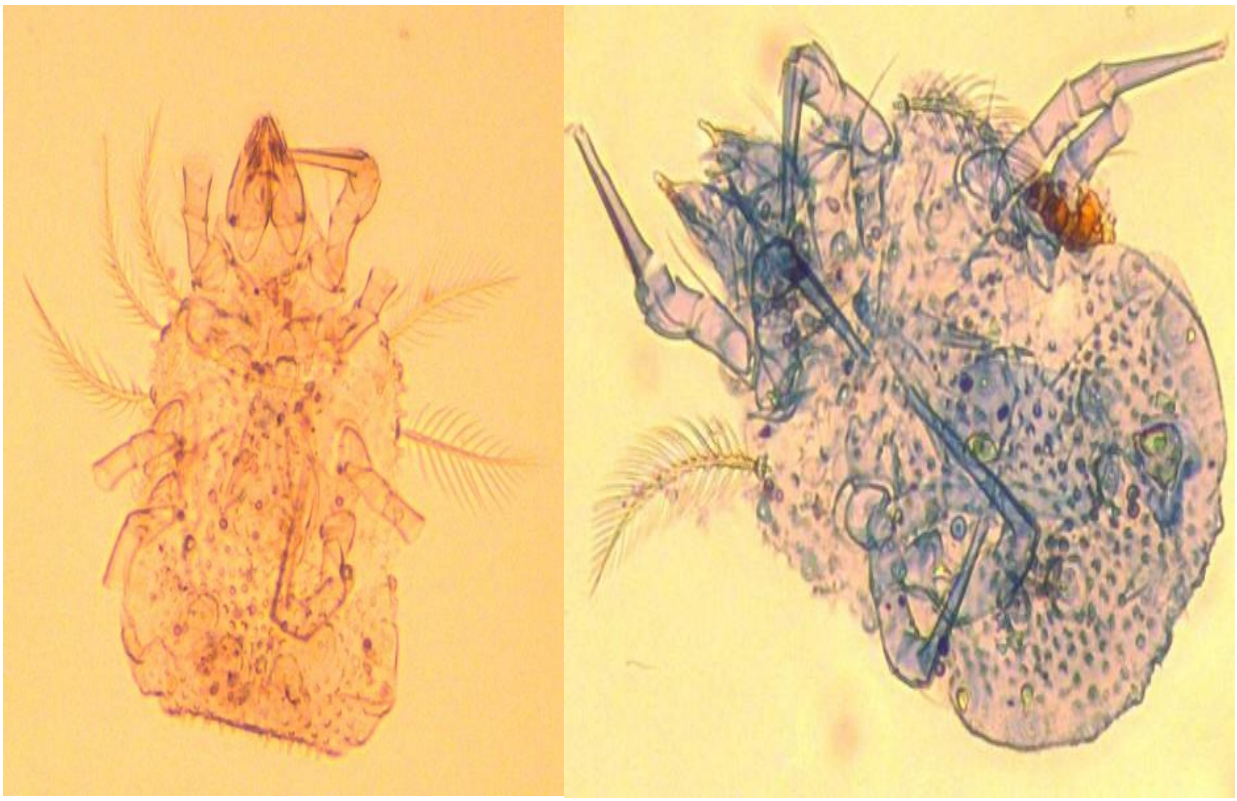
of the mites were captured using a Nikon Eclipse microscope equipped with an Infinity Image Capture system. An overall photograph of each mite specimen was taken using the 10x objective on the microscope. For larger mites, a second close-up photograph was taken using the 40x objective.

Results

The three mounting methods described in this chapter were utilized to mount 225 individual mite specimens. By mounting these individual specimens, taxonomically important morphological and histological features were able to be observed. Analysis of these features confirmed that at least six to seven different mite species were represented in the sample. Four of the species were able to be positively identified to the family level. The other two to three species, however, could not be immediately identified and further work is needed to determine their taxonomic classification.

The first type of mite specimen observed belonged to the family Glycyphagidae. Glycyphagidae is the largest in the superfamily Glycyphagoidea, which consists of 41 genera and 192 species (Krantz and Walter, 2009). Mites in this family are known to occupy the nests of vertebrates, with specific species preferring the nests of rodents, insectivores, and Didelphimorph marsupials. Some species in this family have also been found to colonize human domiciles, where they make up a significant part of the acarofauna of both house dust and stored food products (ibid). Glycophagidae often exhibit a variety of body morphologies and setal forms. However, virtually all mites within the family Glycyphagidae will display a pattern of external ridges on the venter surface of the subcapitulum (ibid). Other characteristics that are generally shared among Glycyphagidae include a rounded, soft body; extended and barbed idiosomatic

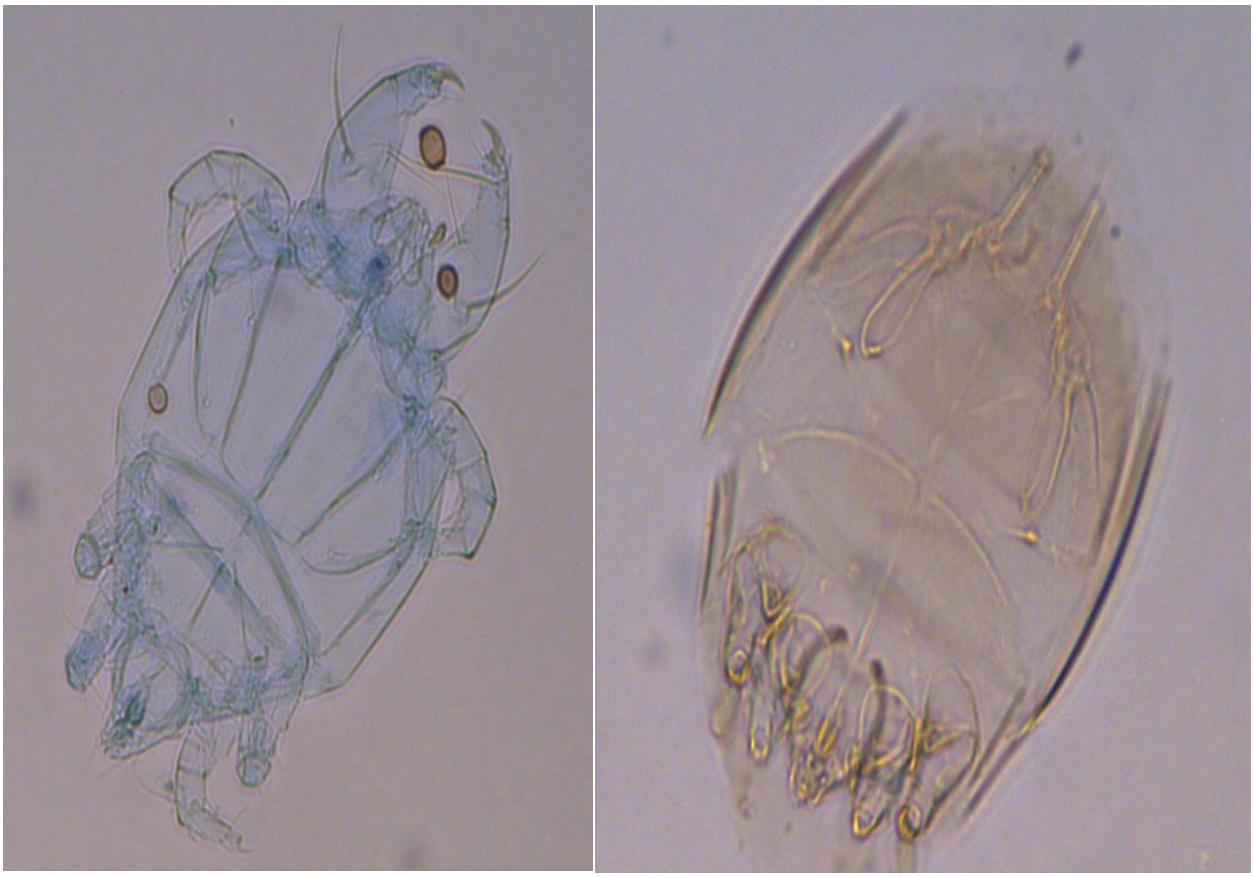
setae; a cuticle that is covered with pointed microtrichia or lobed protrusions; elongated tarsi; and a shield-like, reduced, or absent prodorsal sclerotization (ibid).



Figures 4 and 5. Two examples of Glycyphagidae mite specimens.

The second type of mite specimen observed belonged to the family Tarsonemidae. Tarsonemidae is a large family that consists of approximately 40 genera and around 530 species (ibid). Tarsonemidae mites are typically associated with the infestation of certain types of produce, such as strawberries, pineapples, mushrooms, and other fungi (Baker and Wharton 1952, Ewing 1922; Jeppson et al., 1975; Smith *et al*.., 2021), but have also been known to parasitize insects, other mites, small and large animals, and even humans (Jeppson et al., 1975; Krantz and Walter, 2009; Smith *et al*., 2021). Tarsonemid mites are generally very small ranging anywhere from 100 to 300μm, with males being much smaller than the females. Females of the

Tarsonemidae family tend to be more oval in body-shape, while the males tend to be rounder. The bodies of Tarsonemidae mites are also transected by a suture, which divides the anterior two pairs of legs and the posterior two pairs of legs (Smith *et al*., 2021). Male Tarsonemidae mites are primarily identified by the characteristics of the posterior legs due to the variability of these features. The fourth pair of legs in this species are considered accessory copulatory appendages due to their use in the mite's mating and premating behaviours (ibid). These legs are generally separated into 4 segments, although fusion of the tibia and tarsus can occur, giving the appearance of 3 segmentations. Claws located at the end of the tarsus are also observed on the fourth pair of legs. These claws can range from large and pronounced to relatively non-existent. The femur may also vary with the species as spurs and other modifications may be present to help with mating (ibid).



Figures 6 and 7. Male (right) and female Tarsonemidae specimens.

The third type of mite specimen observed belonged to the family Acaridae. Acaridae is one of the largest families in the Astigmatina cohort, with 80 genera and more than 500 species (Krantz and Walter, 2009). Due to the size of this family, Acaridae are highly ecologically diverse. Early derivative taxa were largely associated with the nests of vertebrates (ibid). However, species belonging to the Acaridae family have now been widely associated with stored food products, such as grain, flour, cheese, hay, and straw; insects, such as bees, ants, and termites; plants; peri-domestic situations; and grassland litter (ibid). The identification of Acaridae can be somewhat difficult as this family exhibits a wide diversity in their morphologies. The morphology of most species in the Acaridae family is relatively modest, but some taxa do display varying and more ornate morphologies (ibid). In general, the bodies of

Acaridae are smooth and soft. The bodies of some taxa, however, are highly sclerotized or embellished with cuticular tubercles (ibid). Setae located on the idiosoma can be long or short and simple, weakly barbed, or strongly barbed (ibid). The legs of Acaridae can also range from very short, as is seen in burrowing taxa, or rather long, as is seen in aquatic taxa (ibid).

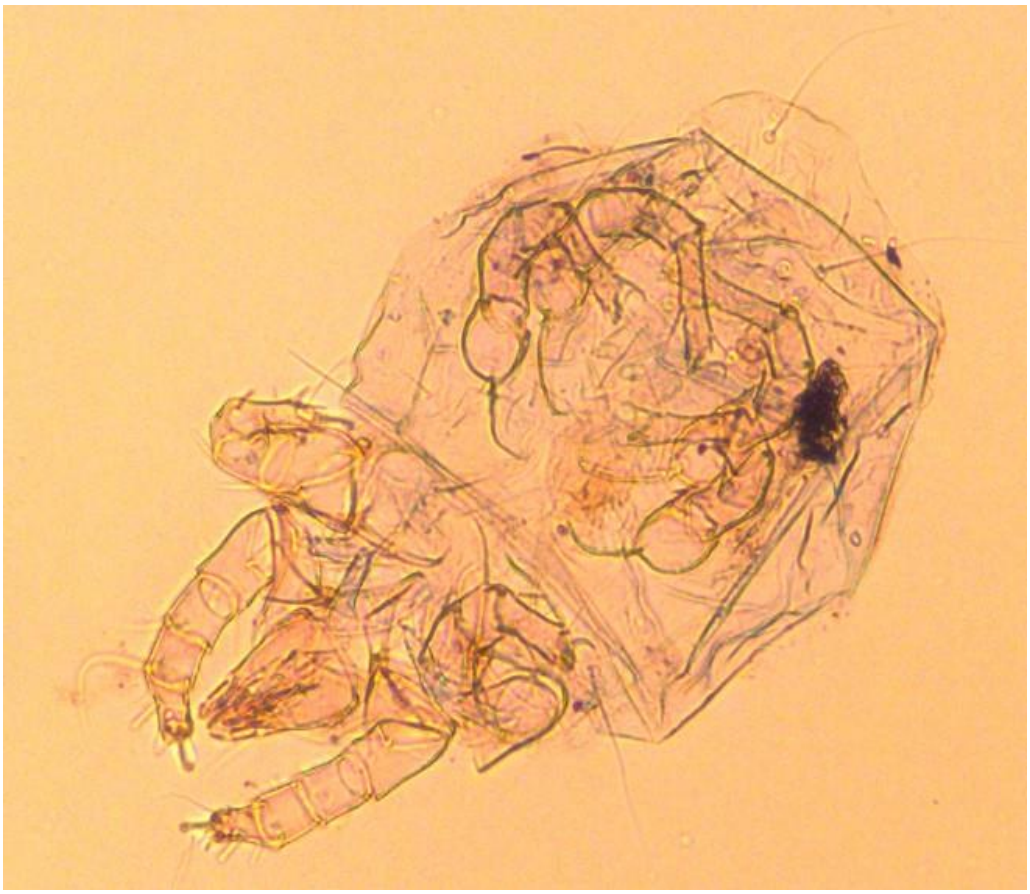
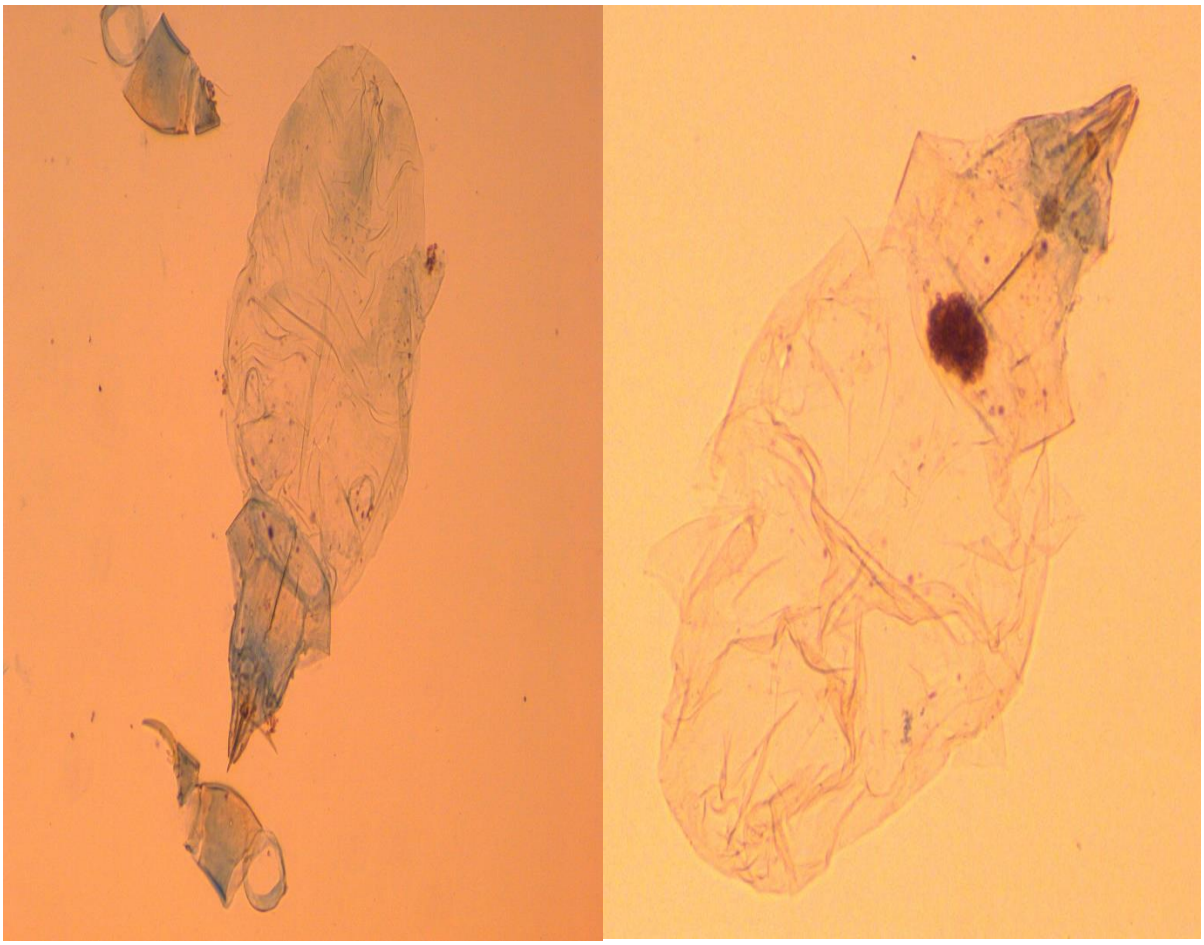


Figure 7. An example of an Acaridae mite specimen.

The fourth type of mite specimen observed belonged to the family Cheyletidae. The Cheyletidae family is composed of roughly 74 genera and over 370 species. Cheyletidae are highly ecologically diverse, occupying a wide variety of habitats, including vegetation, soil, grain storages, debris, tree bark, and litter (ibid). Cheyletids have also been associated with

caves; birds and bird nests; insects and other arthropods, including millipedes, bees, flies, and beetles; scorpions; lizards; and mammals (ibid). Most of the genera and species (approximately three-fourths) that make up the Cheyletidae family are considered predators (ibid). These predatory genera and species frequently feed on small arthropods and other mites that occupy stored grain products. This includes mites of the family Glycyphagidae, which were also recovered from the mummified tissue samples. The discovery of these two mites potentially indicates that there was enough of a community of mites occupying the mummified tissue to maintain and sustain predator/prey interactions within the population. Cheyletidae are soft-bodied mites that typically have weak dorsal shields (ibid). However, the gnathosoma of these mites are well-defined with cheliceral stylophores that can either be free from the subcapitulum or fused to the gnathosomatic capsule (ibid). The movable portions of the cheliceral digits can be styliform, needle like, or retractable (ibid). The palpi of Cheyletids can be variously developed but are typically reduced with a fused femorogenu and tibiotarsus (ibid). Alternately, the palpi can also be large and telescoped, occasionally with a distinct tibial claw (ibid).



Figures 8 and 9. Two examples of Cheyletidae mite specimens.

Discussion

For this experiment, two criteria were utilized to compare the PVA, Gum Dammar, and Hoyer's mounting mediums. The first criteria used was the efficiency and convenience of the mounting method employed for each of the different mediums. Considerations for this criterion included the amount of handling, the equipment needed, and the time required to mount the mite specimens. Ease of use when working with the mounting medium was also considered as part of this criteria; this included the ease of applying the mounting medium and whether the viscosity of the medium needed to be regularly adjusted. Finally, the second criteria used was how defined and clear the mite specimens appeared under both the microscope and in photographs taken

using the microscope. For this criterion, key external morphological and histological features, such as the gnathosoma (including the chelicerae and palps, if present), idiosoma (including anal and genital valves/apertures and various shields located on the venter, if present), and specialized structures (including setae and other unique or defining characteristics), were observed under a microscope and in photographs, with the clarity and definition of each of the structures listed being evaluated.

Efficiency and Convenience

As Singer (1967) indicated it is imperative that acarine material not be subjected to excessive or unnecessary handling, as it may damage or destroy morphological and histological structures that are taxonomically important. This is especially true of acarine material recovered from archaeological contexts given that this material is oftentimes more fragile than fresh acarine material due to its age. For this reason, aqueous mediums are generally favoured over non-aqueous mediums because the mite specimens can be transferred directly from a buffer solution, such as ethanol, into the mounting medium (ibid). Thus, cutting down on or eliminating the need for excess manipulation of the specimen. The results of the mounting experiment demonstrated that aqueous mediums were, in fact, preferable to non-aqueous mediums. The methods utilized to mount mite specimens in PVA or Hoyer's mounting mediums are more streamlined and involve less handling than the mounting method for Gum Dammar. The mite specimens can be moved directly from the ethanol buffer into the PVA medium or Hoyer's medium, whereas moving mite specimens from ethanol into Gum Dammar requires three additional processing steps and two additional buffer solutions. The additional processing steps, which involve centrifuging the specimen, create the potential for the mite to become more brittle and fragile. Portions of the idiosoma, gnathosoma, and/or specialized structures of mite specimens in this state are more

easily damaged or destroyed while positioning the mite or even when sealing the microscope slide. Of course, that is not to say mite specimens cannot be damaged or destroyed when utilizing the PVA or Hoyer's mounting methods. Mites mounted using the PVA method are particularly susceptible to developing creases in their idiosoma, withering of appendages (ibid), or being excessively flattened during drying (ibid). The Hoyer's method, however, appears the least likely to result in damage or destruction to the mite specimen during mounting, although creasing can still sometimes occur. In addition to being overly laborious, there is also a greater potential for the mite specimen to be lost when using the mounting method for Gum Dammar since the ethanol buffer must be poured off three times before the xylene can be added.

In addition to considering the convenience and efficiency of the mounting method, the ease of use of the mounting medium must also be considered. In large part, all the mounting mediums were relatively easy to extract from their receptacles and apply to the microscope slides. The viscosity of the Gum Dammar needed to be adjusted with a few drops of xylene before it was suitable for use with the archaeological mite specimens. However, this adjustment was not difficult or inconvenient. The PVA and Hoyer's mediums did not require any adjustments. It should be noted that the viscosity of the mounting medium can drastically influence the clarity and definition of mite specimens mounted on microscope slides.

The method employed to mount mite specimens for analyses should also take as little time as possible (ibid). Again, when comparing the three methods, the PVA and Hoyer's mounting methods take significantly less time than the Gum Dammar method. This is due to the fact that the PVA and Hoyer's methods do not require the specimen to be centrifuged, nor do they require extra time for a new buffer solution to permeate the specimen. Once the mite has been stained it takes, on average, approximately 2 to 8 minutes from start to finish to mount a

single mite specimen in PVA or Hoyer's. With Gum Dammar, however, once the mite has been stained it must go through a modified rehydration series and be allowed to sit in xylene for approximately 30 minutes. The mite specimen can then be transferred to a microscope slide, which takes approximately 2 to 8 minutes per mite.

Finally, it is also important that the mounting technique require a minimal amount of basic equipment (ibid). Overall, all three methods described in this chapter utilize a similar set of basic laboratory equipment, such as spot plates, insect pins, pipettes, etc. The one exception, of course, is the Gum Dammar method, which requires a microcentrifuge. Moreover, the number of necessary buffer solutions should also be taken into consideration. The Gum Dammar method requires 95% ethanol, 100% ethanol, and xylene, while the PVA and Hoyer's method only require 95% ethanol.

Definition and Clarity

For mite specimens mounted in PVA, the idiosoma and specialized structures are generally clear and well-defined when looked at under a microscope or when photographed. If, however, the mite has developed creasing or withering during the mounting process this can sometimes affect its clarity and definition and make certain morphological and histological features (such as anal and genital valves/apertures, shields, or setae) hard to discern. Unlike the idiosoma and specialized structures, all parts of the gnathosoma are difficult to identify. The overall structure of the gnathosoma itself is easily recognizable. However, individual components, such as the chelicerae and palps, are often obscured and difficult to distinguish between in most of the mite specimens.

Figure 10. Example of unidentified mite specimen mounted in PVA.

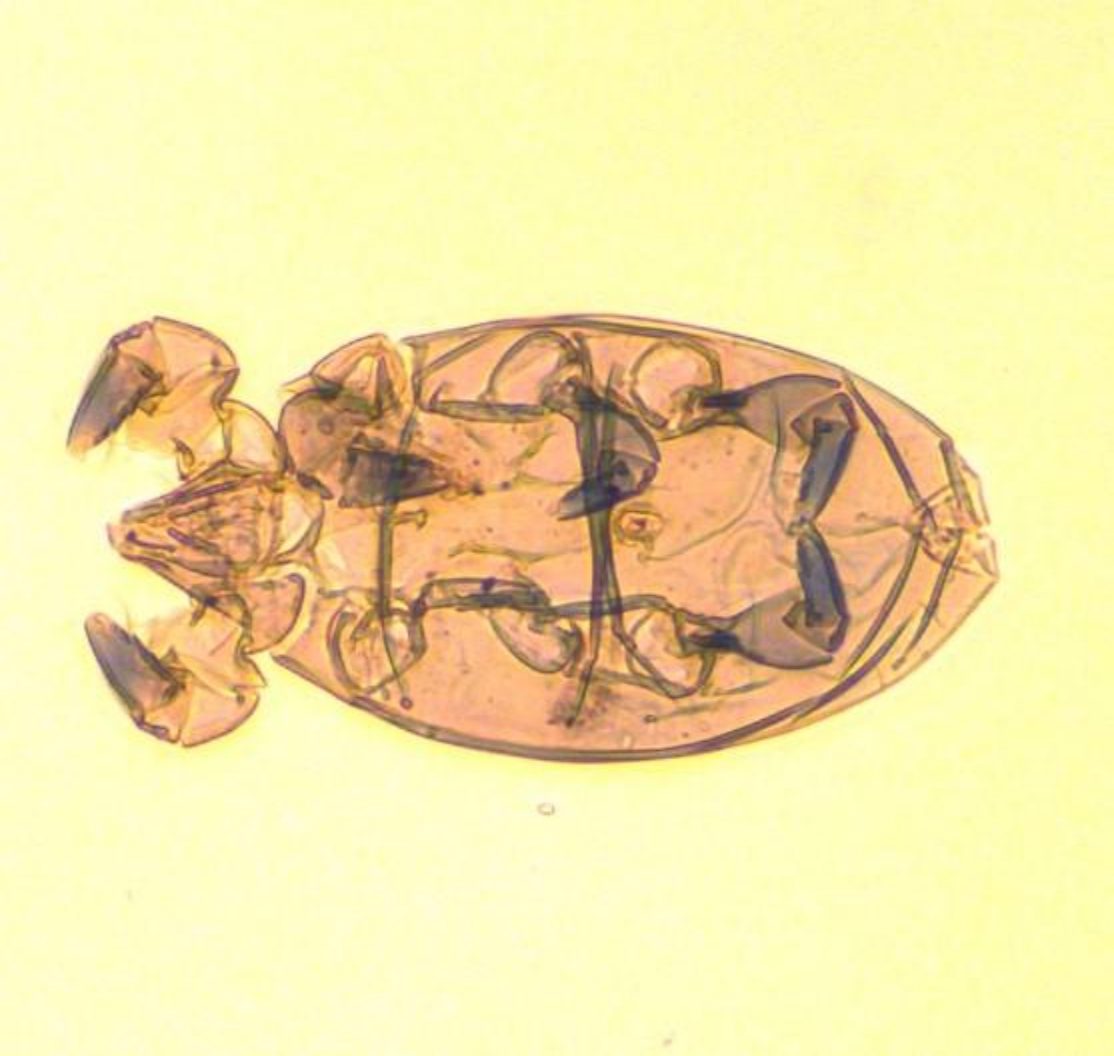


Figure 11. Example of unidentified mite specimen mounted in PVA.

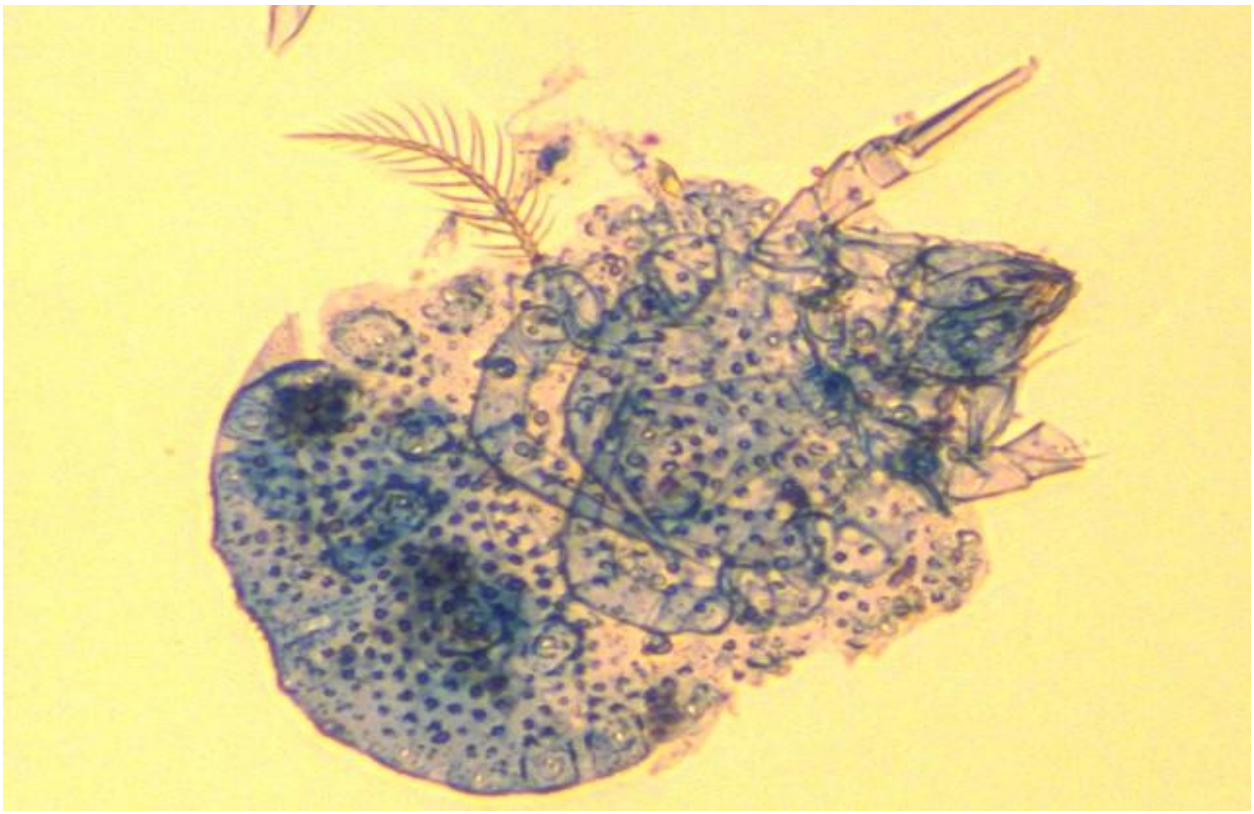


Figure 12. Example of a Glycyphagidae mite specimen mounted in PVA.

The clarity and definition of mites mounted in Gum Dammar is extremely unpredictable and inconsistent. In general, the idiosoma of smaller mites were clear and well-defined when placed under the microscope or when photographed. The idiosoma of bigger mites, however, became unfocused and indistinct, especially when photographed. Similarly, the clarity and definition of the gnathosoma widely varied between mite specimens. This is not connected to the size of the mite, but it is not clear why the definition and clarity of the gnathosoma varies so drastically between these mite specimens. In some of the specimens, all portions of the gnathosoma are indistinct or distorted. For other specimens, only one portion of the gnathosoma, either the palps or the chelicerae, are obscured, and for others both the palps and chelicerae are relatively clear but may or may not be particularly well-defined.

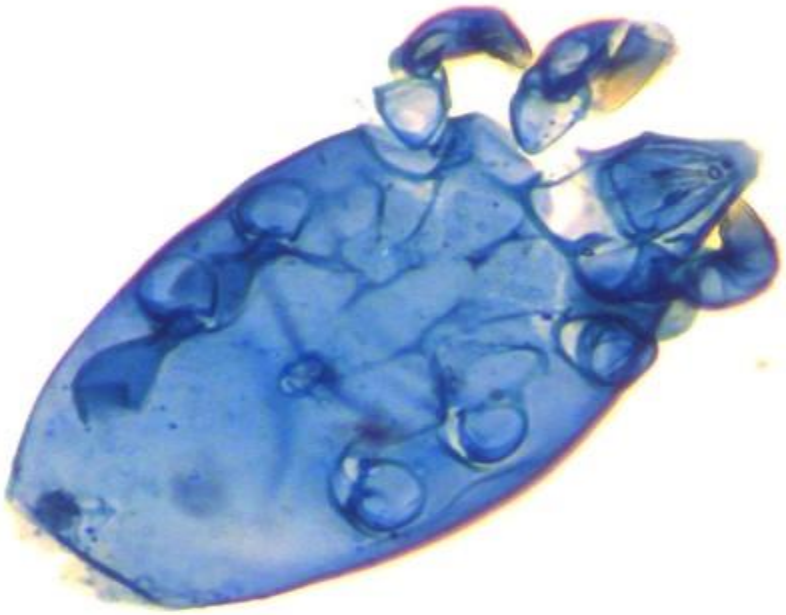


Figure 13. Example of unidentified mite specimen mounted in Gum Dammar.

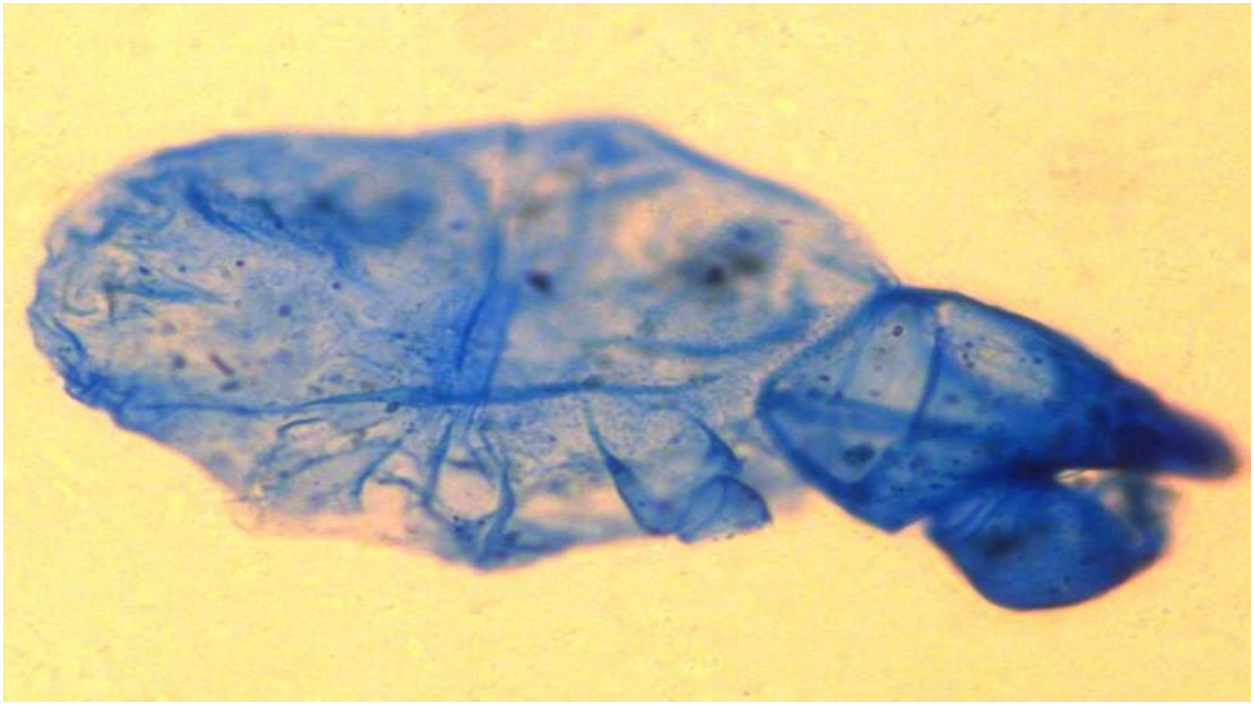


Figure 14. Example of a Cheyletidae mite specimen mounted in Gum Dammar.

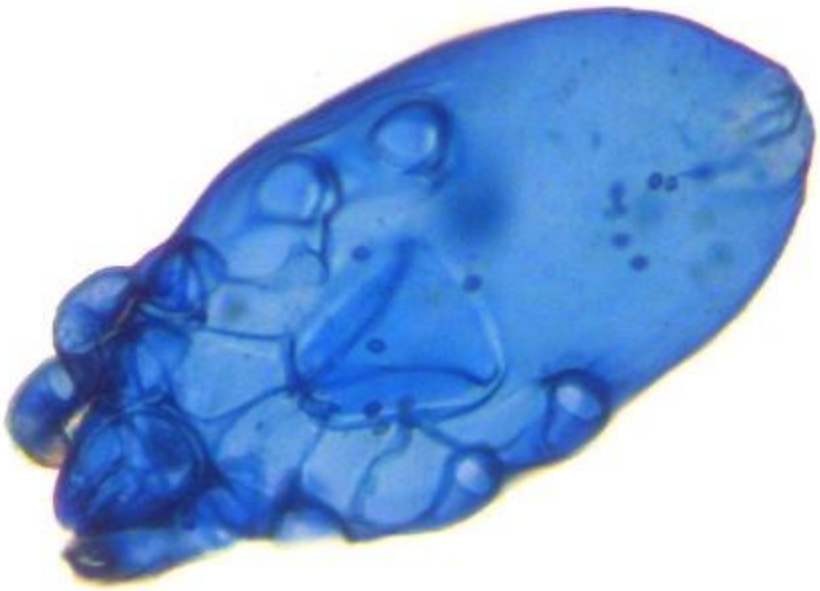


Figure 15. Example of unidentified mite mounted in Gum Dammar.

Overall, Hoyer's produced the clearest mite specimens. The idiosoma and specialized structures of most of the mites mounted in Hoyer's were clear and well-defined. Likewise, all portions of the gnathosoma were clear. However, the individual components of the gnathosoma, while distinct, were not well-defined in most specimens. As previously mentioned, it is not recommended that lactophenol cotton blue stain be used with mites that are mounted in Hoyer's mounting medium, as the stain tends to leach out of the mite specimens. Consequently, the absence of a staining agent causes mites mounted in Hoyer's to take on a more amber hue. For the most part, the lack of a staining agent does not impact definition or clarity in the majority of specimens. However, some specimens can appear translucent when looked at under a microscope or when photographed, which significantly reduces their clarity and definition. Similar issues were not observed in mites mounted in the Gum Dammar or PVA mounting mediums. Mites

mounted in the Gum Dammar retained the vivid blue colouring of the lactophenol cotton blue stain whereas mites mounted in the PVA did tend to lose some of the blue colouring, making them slightly more transparent than those mounted in Gum Dammar. The loss of the blue colouring, however, did not affect the definition or clarity of any of the mite specimens mounted in PVA.

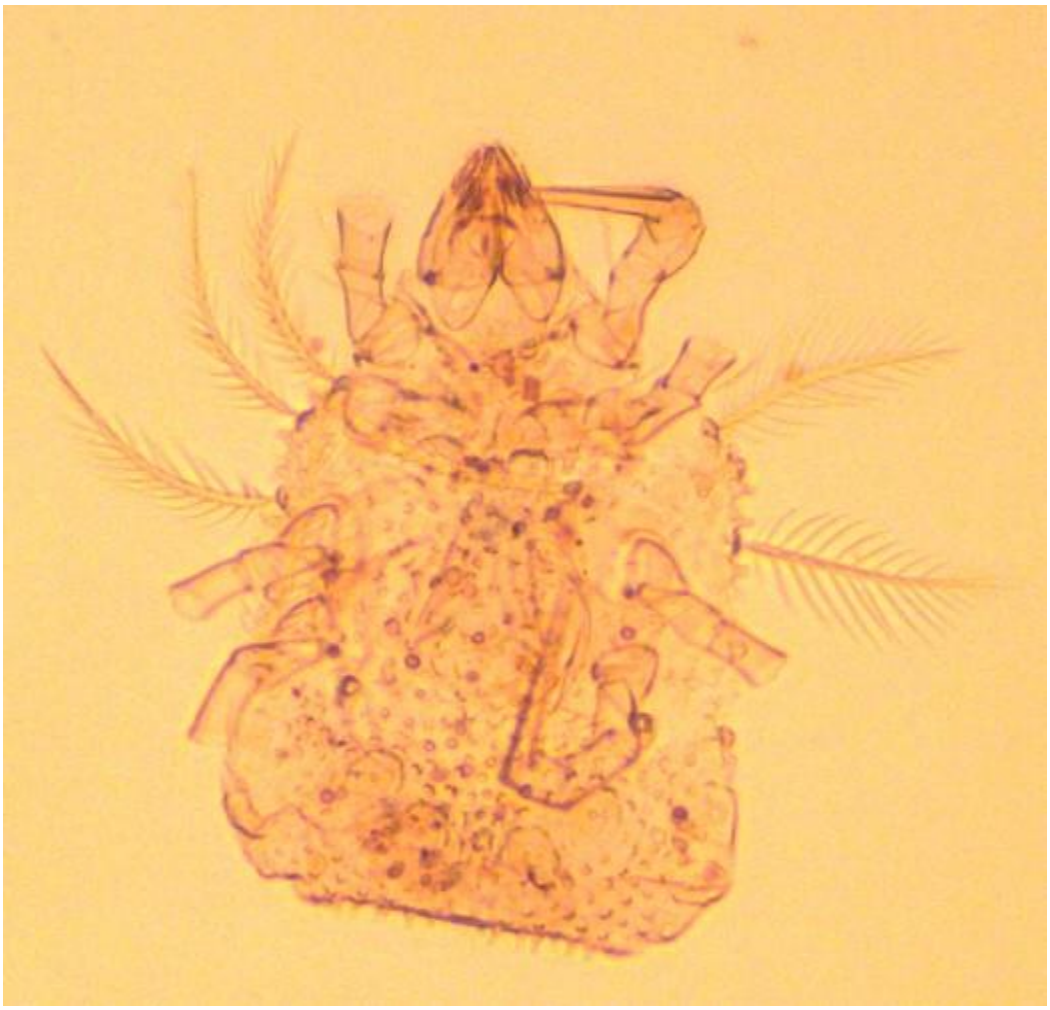


Figure 16. Example of a Glycyphagidae mite specimen mounted in Hoyer's.

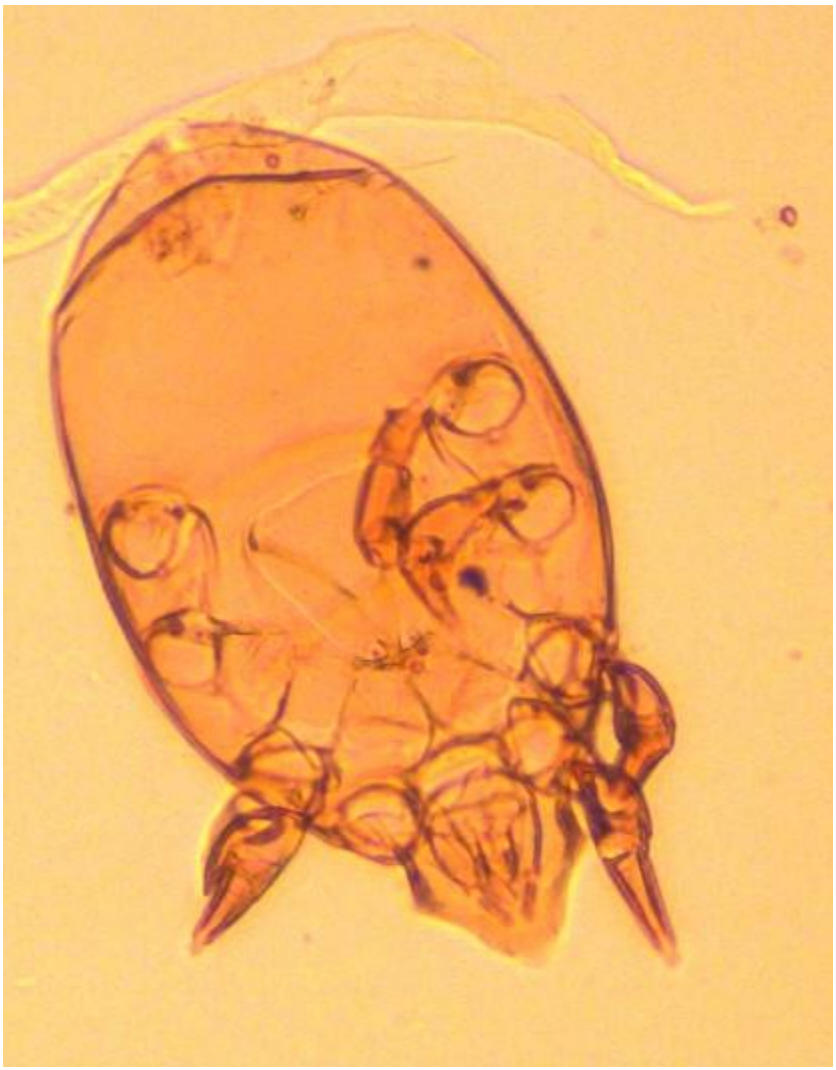


Figure 17. Example of unidentified mite specimen mounted in Hoyer's.

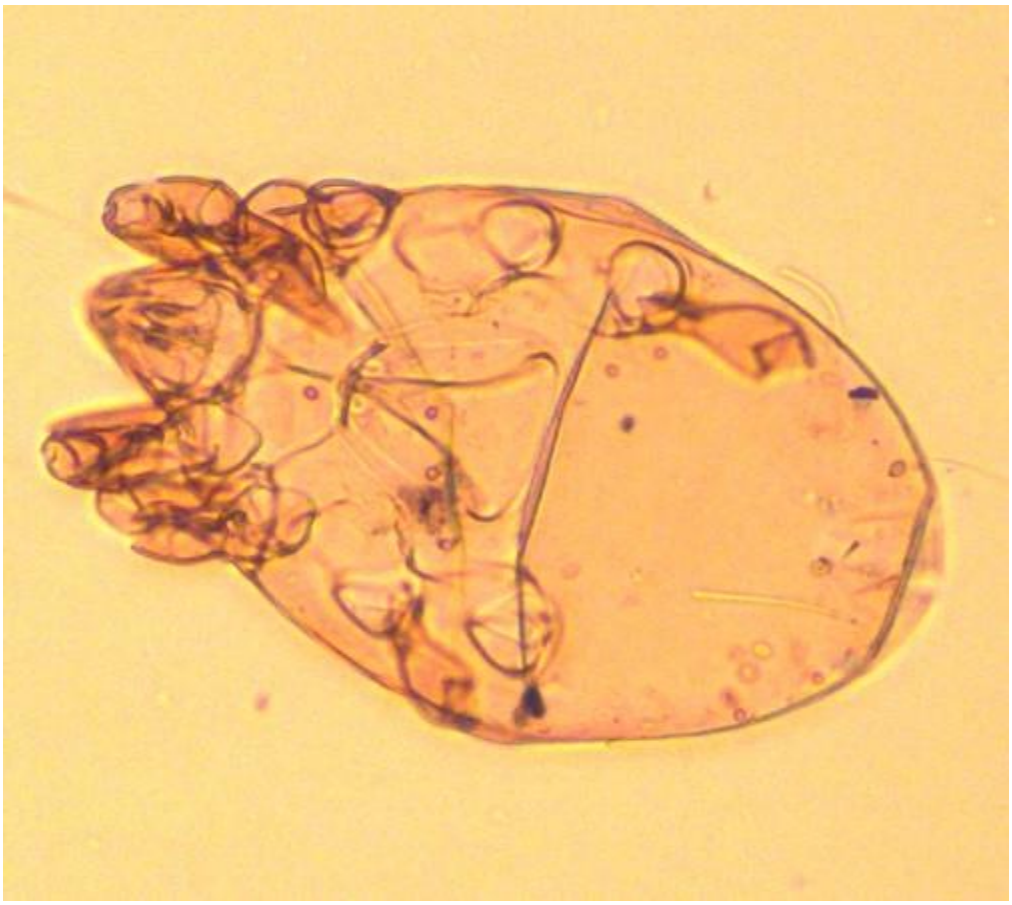


Figure 18. Example of unidentified mite specimen mounted in Hoyer's.

Conclusion

Despite decades of growing interest in archaeoacarology and intermittent championing of the prospective archaeological value of mites, archaeologists still rarely utilize mites in their analyses of archaeological sites. Two reasons that are often cited for this lack of incorporation of mites into archaeological analyses are the difficulty of specimen identification and the absence of methods available to archaeologists that can assist in making specimen identification easier. The purpose of this chapter was twofold. First, this chapter sought to present three mounting methods that can reduce various barriers often encountered when trying to identify archaeological mite specimens. Second, this chapter provided a short comparison of the three methods to help

archaeologists interested in pursuing the identification of archaeological mite specimens in better understanding advantages and disadvantages associated with each method.

As demonstrated, each of the methods described in this chapter produces its own set of benefits and challenges. The PVA method is efficient and convenient, but mites mounted in PVA are often susceptible to developing creases in their idiosoma, withering of appendages, or being excessively flattened during drying. The clarity and definition of mites mounted in PVA is variable, with the idiosoma and specialized structures generally being clear and well-defined while components of the gnathosoma are obscured and indiscernible. The Gum Dammar method is both inconvenient and time-consuming, and the clarity and definition of mites mounted in Gum Dammar is unpredictable and inconsistent. The Hoyer's method is also convenient and efficient and tends to produce the clearest mite specimens when looked at under a microscope or when photographed. However, the Hoyer's method can also sometimes produce creases in the idiosoma of the mites and the absence of a suitable staining agent can result in reduced visibility and definition of the mite specimens. Regardless of its flaws, the Hoyer's method provides the most practical way for archaeologists to better visualize taxonomically important structures that can aid in the identification of mites recovered from archaeological sites. Establishing the practicality of these methods, however, is only the first step to breaking down the significant barriers that are often encountered when trying to identify archaeological mite specimens. The next step will be for future researchers in the field of archaeoacarology to focus on creating more user-friendly dichotomous keys. The creation of such keys will make it easier for archaeologists, who may be unfamiliar with mite taxonomy and anatomy, to identify mite specimens when they are recovered from archaeological assemblages.

CHAPTER 6: DISCUSSION AND CONCLUSION

Much like other arthropods, mites have been discovered in a wide variety of forensic and archaeological contexts featuring biological remains. Their accurate identification has assisted forensic scientists in estimating post-mortem interval, determining whether a body has been moved or relocated, and reinforced information about the types of arthropod decomposers that have visited the body since deposition. Similarly, mites have also assisted archaeologists by providing valuable insights into the taphonomic conditions associated with biological remains, the environmental and depositional contexts surrounding these remains, post-mortem burial rituals and environmental conditions, and the curatorial needs of biological remains housed in museum collections (*Morrow et al*., 2015). Consequently, this close association between mites and cadavers has led some researchers in the fields of archaeology and forensic science to intermittently advocate for the inclusion of mites in archaeological site analyses and forensic case studies. However, despite the potential value they hold for analysing archaeological assemblages and forensic cases, mites have rarely been utilized. Various reasons have been provided for the lack of inclusion of mites in archaeological and forensic analyses. Chief amongst these reasons is the lack of a systematic method for extracting mite specimens from recovered human remains, the absence of methods available to archaeologists and forensic scientists that can aid in specimen identification (Baker, 1990; Baker, 2009), and the difficulty of specimen identification (Baker, 2009). The goal of this thesis is to present a unified method for sampling, recovering, and mounting mite specimens that have been recovered from mummified human tissue to significantly reduce barriers that are often encountered by archaeologists and forensic scientists seeking to incorporate mites into their archaeological and forensic analyses.

As demonstrated above, each of the methods presented in this thesis provides practical, efficient, and cost-effective techniques for extracting mites from mummified human tissue. Likewise, each of the methods serves to break down obstacles associated with specimen identification. The sampling method described in chapter 3 allows archaeologists and forensic scientists to test mummified human tissue for the presence of mites and serves as the first means of visualizing potential mite specimens that inhabited the tissue. This can allow researchers to make preliminary identifications of mite specimens based on their external morphology. The recovery method described in chapter 4 provides a utilitarian procedure for extracting individual mite specimens from mummified human tissue, affording researchers the opportunity to mount whole and fragmentary mites for microscopic analysis. Finally, chapter 5 presented three different mounting methods (PVA, Gum Dammar, and Hoyer's) for mite specimens. The mounting of mite specimens allows researchers to microscopically analyse and visualize taxonomically important gross morphological and histological features, which can aid in specimen identification.

Furthermore, the sampling and mounting methods managed to provide new insights into the mites associated with mummies housed within the crypts of the Dominican Church of the Holy Spirit. By employing the sampling method to observe mites present in the VD-2, VD-5, and VD-11 samples, it was determined that VD-11 appeared to contain the greatest abundance of mites, while mites were more sparsely dispersed in VD-2 and VD-5. The reason for the difference in abundance between the samples is unclear. However, it is possible that resources contained within the coffin microhabitat of VD-11 were more attractive than those contained in either VD-2 or VD-5. Surprisingly, there did not seem to be a substantial amount of diversity in mite species between the samples. At least three of the identified mite species, those belonging to

the Glycyphagidae, Tarsonemidae, and Acaridae families, were found in all three of the samples. The one exception were mites belonging to the family Cheyletidae, which were only found in the VD-11 sample. However, the absence of Cheyletidae mites may be attributable to fewer analyses being performed on VD-2 and VD-5. Finally, the presence of a predatory mite species, Cheyletidae, and its prey confirmed the community of mites that colonized VD-11 was sufficient to support and maintain both predators and prey, which indicates that this population of mites was established and stable enough to create sustainable trophic interactions.

This research project constitutes a pioneering study on the types of mites that are associated with corpses. Although the scope of this research was limited to mummified human tissue, the hope is that the methods presented in this thesis will provide a way forward for forensic scientists and archaeologists interested in incorporating mites into their analyses. This research, however, only constitutes the first step in breaking down the significant barriers that are often encountered when trying to identify archaeological and forensic mite specimens. More work is needed to fully make the analyses and identification of these specimens accessible to both archaeologists and forensic scientists. The next step will be for future researchers to focus on developing a processing and recovery method for human tissue recovered from cadavers in the fresh and active decay stages of decomposition, so that mite analyses can be more easily included in forensic investigations. Moreover, future research endeavours should also concentrate on the creation of more user-friendly dichotomous keys. The creation of such keys will make it easier for archaeologists and forensic scientists, who may be unfamiliar with mite taxonomy and anatomy, to identify mite specimens when they are recovered from archaeological assemblages and forensic scenes.

REFERENCES CITED

Baker, A. S. (1990). Two New Species of Lardoglyphus Oudemans (Acari: Lardoglyphidae) Found in the Gut Contents of Human Mummies. *Journal of Stored Products Research, 26*(3), 139-147. doi:10.1016/0022-474x(90)90014-j.

Baker, A. S. (2009). Acari in Archaeology. *Experimental and Applied Acarology, 49*(1-2), 147-160. doi:10.1007/s10493-009-9271-1.

Baker, E.W., & Wharton, G.W. (1952). An Introduction to Acarology (1st ed.). New York: Macmillan.

Baker, A.S., & Craven, J.C. (2003). Checklist of the Mites (Arachnida: Acari) Associated with Bats (Mammalia: Chiroptera) in the British Isles. *Systematic and Applied Acarology: Special Publication, 14.*

Barton, P. S., Weaver, H. J., & Manning, A. D. (2013). Contrasting Diversity Dynamics of Phoretic Mites and Beetles Associated with Vertebrate Carrion. *Experimental and Applied Acarology, 63*(1), 1-13. doi:10.1007/s10493-013-9758-7.\

Benecke, M. (2008). A Brief Survey of the History of Forensic Entomology. *Acta Biologica Benrodis, 14.*

Braig, H. R., & Perotti, M. A. (2009). Carcasses and Mites. *Experimental and Applied Acarology, 49,* 45-84.

Brouardel, P. (1879). De la détermination de l'époque de la naissance et de la mort d'un nouveau-née, faite à l'aide de la présence des acares et des chenilles d'aglosses dans cadavre momifié [Determination of the Time of Birth and of Death of a New-born Child, Made Using the Presence of Mites and Aglossa Caterpillars on the Mummified Corpse]. *Ann Hyg Publ Méd Lég* (série 3) 2.

Camacho, M., Araujo, A., Morrow, J., Buikstra, J., & Reinhard, K.J. (2018). Recovering Parasites from Mummies and Coprolites: An Epidemiological Approach. *Parasites & Vectors, 11*:248. 48. Doi:10.1186/s13071-018-2729-4.

Chaves da Rocha, C, Maues da Serra-Freire, N. (2014). Mites, Ticks, and Paleoparasitology. In: Ferreira, F.F., Reinhard, K.J., Araujo, A. (Eds) *Foundations of Paleoparasitology*. Editora Fiocruz, Rio de Janeiro, p 171-186.

Damann, F.E., Carter, D.O. (2014). Human Decomposition Ecology and Postmortem Microbiology. In: Pokines JT, Symes SA (Eds) *Manual of Forensic Taphonomy*. CRC Press, Boca Raton, p 37-50.

Denford, S., & Brock, J. (1978). Mites and their Potential Use in Archaeology. In 1209942251 901804611 D. R. Brothwell & 1209942252 901804611 K. D. Thomas (Eds.), *Research Problems in Zooarchaeology* (3rd ed., p. 77). London, UK: Institute of Archaeology.

Denmark, H.A. (2000). Cyclamen Mite, Phytonemus pallidus (Banks) (Arachnida: Acari: Tarsonemidae). PDF.

Durkin, E.S., Proctor, H., Luong, L.T. (2019). Life History of Macrocheles muscaedomesticae (Parasitiformes: Macrochelidae): New Insights on Life History and Evidence of Facultative Parasitism on Drosophila. *Experimental and Applied Acarology, 79*. Doi: 10.1007/s10493-019-00431-y.

Efremov, I. (1940). Taphonomy: New Branch of Paleontology. *American Geologist, 74*, 81-93. Retrieved March 24, 2021, from ae.makorzh.ru/science/taph.htm.

Ewing, H. E. (1922). Studies on The Taxonomy and Biology of the Tarsonemid Mites, Together with a Note on the Transformations of Acarapis (Tarsonemus) Woodi Rennie. (Acarina). *The Canadian Entomologist, 54*(5), 104–113. doi: 10.4039/ent54104-5.

Fain, A., & Domrow, R. (1980). A New Species of Myianoetus Oudemans (Acarina: Anoetidae) from a Ceratopogonid Fly in Australia. *The Australian Entomologist*, Mag 7.

Fain, A., Britt, D.P., Molyneux, D.H. (1980). Myianoetus copromyzae sp. nov. (Acari, Astigmata, Anoetidae) Phoretic on Copromyza atra (Meigen 1830) in Scotland. *Journal of Natural History, 14*.

Guerra, R.M.S.N.C., Gazeˆta, G.S., Amorim M., et al. (2003). Ecological analysis of Acari recovered from coprolites from archaeological site of Northeast Brazil. *Memorias Instituto Oswaldo Cruz 1*, 181-190. DOI: 10.1590/s0074-02762003000900027.

Haglund, W. D. (2014). Forensic Taphonomy. In *Forensic Science: An Introduction to Scientific and Investigative Techniques* (4th ed.). New York, NY: CRC Press. Retrieved November 20, 2018.

Holden, T.G., & Nunez, L. (1993). Analysis of the Gut Contents of Five Well-preserved Human Bodies from Tarapaca´, Northern Chile. *Journal of Archaeological Sciences, 20*. doi:10.1006/jasc.1993.1036.

Jeppson, L. R., Keifer, H. H., & Baker, E. W. (1975). Mites Injurious to Economic Plants. Berkeley, CA: University of California Press.

Joseph, I., Mathew, D., Sathyan, P., & Vargheese, G. (2011). The Use of Insects in Forensic Investigations: An Overview on the Scope of Forensic Entomology. *Journal of Forensic Dental Sciences, 3*(2), 89. doi:10.4103/0975-1475.92154

Kamaruzaman, N.A.C., Msan, P., Velasquez, Y., Gonzalez-Medina, A., Lindstrom, A., Braig, H.R., Perotti, M.A. (2018). Macrocheles species (Acari: Macrochelidae) associated with human corpses in Europe. *Experimental and Applied Acarology, 76.* Doi: 10.1007/s10493-018-0321-4.

Krantz, G.W. and Walter, D.E. (2009). The Manual of Acarology. Lubbock, Texas, USA. Proctor.

Medina, A.G., Herrera, L.G., Perotti, M.A., & Rios, G.J. (2013). Occurrence of Poecilochirus austroasiaticus (Acari: Parasitidae) in Forensic Autopsies and its Application on Postmortem Interval Estimation. *Experimental and Applied Acarology 59*, 3.

Megnin, J.P. (1887). La faune des tombeaux. *Comptes-rendus hebdomdaires des seances de l'Academie des Sciences*. Paris: G. Masson: Gauthier Villars.

Megnin, J.P. (1889). Entomologie appliquee a la medicine legale a propos de la these de M. George Yovanovitch. *Bulletin de la societe de Medicine legale de France* 21.

Megnin, J.P. (1894). *La Faune des Cadavres: Application de L'entomologie à la Médecine Légale*. Paris: G. Masson: Gauthier-Villars et fils.

Megnin, J.P. (1896). Note sur une collection d'insectes des cadavres interessants a connaitre au point de vue medico-legal, offerte au Museum. *Bulletin du Museum D'Histoire naturelle* 2. Paris: G. Masson: Gauthier-Villar et fils.

Miranda, R., & Bermudez, S. (2008). Mites (Arachnida: Acari) Associated with Calliphoridae Flies (Diptera: Oestroidea) at Three Localities in Panama. *Revista Colombiana Entomololologia 34*.

Morrow, J.J., Baldwin, D.A., Higley, L., Piombino-Mascali, D., Reinhard, K.J. (2015). Curatorial implications of Ophyra capensis (Order Diptera, Family Muscidae) puparia recovered from the body of the Blessed Antonio Patrizi, Monticiano, Italy (Middle Ages). *Journal of Forensic and Legal Medicine, 36*. Doi: 10.1016/j.jflm.2015.09.005.

Morrow, J.J., Myhra, A., Piombino-Mascali, D., Lippi, D., Roe, A., Higley, L., Reinhard, K.J. (2016). Archaeoentomological and archaeoacarological investigations of embalming jar contents from the San Lorenzo Basilica in Florence, Italy. *Journal of Archaeological Science, 10.*

Morrow, J.J., Taylor, L., Peck, L., Elowsky, C., Owens, L.S., Eeckhout, P., Reinhard, K.J. (2017). Pseudoscorpions of the family Cheiridiidae (Arachnida: Pseudoscorpiones) recovered from burial sediments at Pachacamac (500–1,500 CE), Perú. *The Journal of Arachnology, 45*.

Negm, M.W., & Alatawi, F.J. (2011). Four New Records of Mites (Acari: Astigmata) Phoretic on Insects in Riyadh, Saudi Arabia. *Journal of the Saudi Society of Agricultural Sciences 10*.

O'Connor, B.M. (2009). Astigmatid Mites (Acari: Sarcoptiformes) of Forensic Interest. *Experimental and Applied Acarology 49*. DOI 10.1007/s10493-009-9270-2.

O'Connor, B. M., Pimsler, M., Owings, C. G., & Tomberlin, J. K. (2015). Redescription of Myianoetus muscarum (Acari: Histiotomatidae) Associated with Human Remains in Texas, USA, with Designation of a Neophyte from Western Europe. *Journal of Medical Entomology,* 1-12. doi:10.1093/jme/tjv045.

Perotti, M.A. (1998). Moscas sinantrópicas (Diptera: Muscidae y Fanniidae) en producciones avícolas del sud este bonaerense [Synantropic Flies (Diptera: Muscidae y Fanniidae) Associated with Poultry Farms in South-eastern Buenos Aires Province]. *Natura Neotropicalis 29*.

Perotti, M.A. (2009). Mégnin Re-analysed: The Case of the Newborn Baby Girl, Paris, 1878. *Experimental and Applied Acarology 49*. doi: 10.1007/s10493-009-9279-6.

Perotti, M.A., & Braig, H.R. (2009). Phoretic Mites Associated with Animal and Human Decomposition. *Experimental and Applied Acarology 49*; 1-2. Doi: 10.1007/s10493-009-9280-0

Perotti, M.A., Goff, M.L., Baker, A.S., Turner, B.D., & Braig, H.R. (2009). Forensic Acarology: An Introduction. *Experimental and Applied Acarology 49*:3-13. doi:10.1007/s10493-009-9285-8

Perotti, M. A., Braig, H. R., & Goff, M. L. (2010). Phoretic Mites and Carcasses: Acari Transported by Organisms Associated with Animal and Human Decomposition. In 1250650925 926382245 J. Amendt, 1250650926 926382245 M. L. Goff, 1250650927 926382245 C. P. Campobasso, & 1250650928 926382245 M. Grassberger (Eds.), *Current Concepts in Forensic Entomology*. Dordrecht (Holland): Springer.

Pimsler, M.L., Owings, C.G., Sanford, M.R., O'Connor, B.M., Teel, P.D., et al. (2016). Association of Myianoetus muscarum (Acari: Histiostomatidae) With Synthesiomyia nudiseta (Wulp) (Diptera: Muscidae) on Human Remains. *Journal of Medical Entomology, 53* (2). DOI:10.1093/jme/tjv203.

Piombino-Mascali, D., Kozakaitė, J., Tamošiūnas, A., Valančius, R., Panzer, S., et al. (2014). Skeletal Pathological Conditions of Lithuanian Mummies. *Papers on Anthropology 23*:1. DOI:10.12697/poa.2014.23.1.10.

Piombino-Mascali, D., Urbanavičius, A., Daubaras, M., Kozakaitė, J., Miliauskienė, Z., & Jankauskas, R. (2015). The Lithuanian Mummy Project: A Historical Introduction. *Lietuvos archeologija 41*.

Piombino-Mascali, D., Jankauskas, R., Tamošiūnas, A., Valančius, R., Gill-Frerking, H., Spigelman, M., & Panzer, S. (2015). Evidence of Probable Tuberculosis in Lithuanian Mummies. *HOMO*, *66*(5), 420–431. https://doi.org/10.1016/j.jchb.2015.01.004.

Pokines, J. T. (2014). Introduction: Collection of Macroscopic Osseous Taphonomic Data and the Recognition of Taphonomic Suites of Charateristics. In 1250611216 926358071 J. T. Pokines & 1250611217 926358071 S. A. Symes (Eds.), *Manual of forensic taphonomy* (pp. 3-8). Boca Raton, FL: CRC Press.

Proctor, H.C. (2001). Extracting Aquatic Mites from Stream Substrates: A Comparison of Three Methods. *Experimental and Applied Acarology 25*:1. DOI: 10.1023/a:1010677700404.

Radovsky, F.J. (1970). Mites Associated with Coprolites and Mummified Human Remains in *Nevada. Contributions of the University of California Archaeological Research Facility*, 10.

Rasmy, A. H. (2008). Mites and Insects as Indicators of Physical Abuse. *Acarines: Journal of the Egyptian Society of Acarology, 2*(1), 1-2. doi:10.21608/ajesa.2008.4970

Rasmy, A.H. (2011). The Humans Lie but the Spiders Do not Lie: An Overview on Forensic Acarology. *Egyptian Journal of Forensic Sciences 1*:3-4. doi: 10.1016/j.ejfs.2011.07.001

Reinhard, K.J., Milanello do Amaral, M., Wall, N. (2017). Palynological Investigation of Mummified Human Remains. *Journal of Forensic Science*. doi: 10.1111/1556-4029.13463.

Russell, D.J., Schulz, M.M., O'Connor, B.M. (2004). Mass Occurrence of Astigmatid Mites on Human Remains. *Abh Ber Naturkundemus Gorlitz 76*:51–56.

Saloña-Bordas, M. I., & Perotti, M. A. (2014). First Contribution of Mites (Acari) to the Forensic Analysis of Hanged Corpses: A Case Study from Spain. *Forensic Science International, 244*. doi:10.1016/j.forsciint.2014.08.005

Schelvis, J. (1987). Some Aspects of Research on Mites (Acari) in Archaeological Samples. *Palaeohistoria, 29*, 211-218.

Schelvis, J. (1990a). Mites (Acari) in Archaeology. *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society, 1*, 90-95.

Schelvis, J. (1990b). Mites from medieval Oldeboorn. An Environmental Reconstruction. *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society, 1*, 96-97.

Schelvis, J. (1990c). The Reconstruction of Local Environments on the Basis of Remains of Oribatid Mites (ACARI; Oribatida). *Journal of Archaeological Science, 17*(5), 559-571. doi:10.1016/0305-4403(90)90036-5

Schelvis, J. (1992c). General Conclusions. In Schelvis, J. (ed) *Mites and Archaeozoology: General Methods: Applications to Dutch Sites*. Groningen: Rijksuniversiteit, Groningen.

Singer, G. (1967). A Comparison Between Different Mounting Techniques Commonly Employed in Acarology. Acarologia.

Smith, J., Gipson, L., Piombino-Mascali, D., & Jankauskas, R. (2021). Mummy Taphonomy and Arthropod Associates of Decay. *The Handbook of Mummy Studies*, 1-18. doi:10.1007/978-981-15-1614-6_40-2.

St. Fleur, N. (2017, June). The Mummies' Medical Secrets? They're Perfectly Preserved. *The New York Times*. https://www.nytimes.com/2017/06/02/science/mummies-smallpox-vilnius-lithuania-crypt.html#:~:text=Hundreds%20of%20skeletons%20have%20lain,organs%20still%20fill%20their%20insides.

Weintraub, K. (2015, April). Pictures: Centuries After Death, Mummies Still Telling Stories. *National Geographic*. https://www.nationalgeographic.com/culture/article/150416-mummy-pictures-crypt-death-health-medicine-lithuania.