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## **Abstract**

Little is known about infectious disease and parasites in the prehistoric inhabitants of the islands of the Aegean, in contrast to later time periods. It is only with the development of Greek medical texts in the 5<sup>th</sup> and 4<sup>th</sup> centuries BC we start to find evidence for the diseases that affected the population of region. Foremost amongst these authors was the medical practitioner Hippocrates, who lived on the island of Kos. The descriptions of the many diseases he and his students encountered were recorded in their medical texts in the 4<sup>th</sup> and 3<sup>rd</sup> centuries BC, known as the Hippocratic Corpus. These important texts provided the core philosophy underpinning medical theories in Europe and the Arab world for the following 2,000 years. Past research to determine which species of intestinal parasitic worms were described in the Hippocratic Corpus has suggested they indicate roundworm, pinworm and *Taenia* tapeworm. However, until now, there has been no archaeological evidence for which species of helminths were present in ancient Greece. In this study, we analysed soil sediment adherent to the sacrum and iliac bones of the pelvis of 25 burials dating from the Neolithic to Byzantine period on the Greek island of Kea, not far from Kos. Four individuals (16%) were positive for the eggs of intestinal helminths, dating from the Neolithic (4<sup>th</sup> millennium BC), Late Bronze Age, and the Roman Period. The species identified were whipworm (*Trichuris trichiura*) and roundworm (*Ascaris lumbricoides*). We consider reasons as to why fewer species of parasite appear to have been present on Kea than was the case for northern Europe at the same time period. This study of ancient parasites shows how we can combine archaeology with history of medicine to better understand the discoveries of key early scientists and medical practitioners.

## 1. Introduction

Hippocrates was a medical practitioner who lived in the 5<sup>th</sup>-4<sup>th</sup> century BC on the Greek island of Kos, in the Aegean Sea. The medical texts of Hippocrates and his students are important as they described humoral theory to explain the cause of disease for the first time. It was thought that when the four humours (black bile, yellow bile, blood and phlegm) were in balance a person would be healthy, but when out of balance or corrupted then sickness would result (Horden and Hsu 2013; Nutton 2013). These texts, known as the Hippocratic Corpus, were passed on and translated by the Romans, Arabs and the people of medieval Europe. Indeed, humoral theory became the accepted explanation for ill health and influenced medicine for the next two thousand years in Europe and the Mediterranean region (Cantor 2001; Mann 2012; Totelin 2011).

Modern researchers have studied the descriptions of many different diseases recorded in the Hippocratic Corpus to try and identify which conditions Hippocrates and his students encountered (Pappas et al. 2007; Sajadi et al. 2011; Tefekli and Cezayirli 2013). A number of these descriptions were those of intestinal parasitic worms. The Greek texts used three terms when describing helminths. *Helmins strongyle* was a large round worm, *Helmins plateia* was a flat worm, while *Ascaris* was a small round worm. These three terms have been interpreted as referring to roundworm (*Ascaris lumbricoides*), tapeworm segments (perhaps *Taenia* sp.), and pinworm (*Enterobius vermicularis*) (Trompoukis et al. 2007). However, it is known that interpreting ancient medical texts to make a modern diagnosis is not a simple process, as there are many confounding factors that may potentially lead to an error in interpretation (Mitchell 2011).

Until now there has been no archaeological study of the intestinal helminths present in the Greek islands at the time of Hippocrates, with which we might compare this written evidence. This is in contrast with the evidence that is available for disease found in human skeletal remains in Greece from the Neolithic onwards (Bourbou, 2003; Charlier and Tsigonaki, 2011; Papathanasiou et al. 2000). Such a study of intestinal helminths would allow us to be much more confident as to the species described by Hippocrates 2,500 years ago. It could also potentially give us an indication of when intestinal parasites were first brought to the island, and how the species of parasite

infecting the population might have changed over the millennia following settlement of the islands.

## **2. Material and Methods**

The samples under study originate from the island of Kea, which is situated in the Cyclades islands in the Aegean Sea. Human skeletal remains were excavated from two sites on the island, namely Kephala and Ayia Irini (Figure 1). Both excavations started in 1960 and ended in the early 1970s.

The Neolithic settlement of Kephala was situated on a promontory of the north-western coast of the island. Forty graves were excavated. The material culture of the occupation levels at Kephala can be dated to the 4<sup>th</sup> millennium BC. Kephala was inhabited for less than two centuries and was never re-inhabited after its abandonment in the Neolithic (Coleman 1977).

Ayia Irini is situated north of the port of Ayios Nikolaos in the north-west coast of the island. Most graves (25 individuals) in Ayia Irini date from Middle Bronze Age (2,000/1900-1550 BC), but graves dating from the later Bronze Age (1550-1100 BC), Roman period (146 BC-330 AD), and Byzantine period (330 AD – 1207 AD) were also excavated (Caskey 1962; Overbeck 1989; Wilson 1999).

For this study sediment was collected from the anterior aspect of unwashed sacral bones and internal aspect of the iliac bones of the pelvis from the skeletal remains, along with control samples from the unwashed crania and/or feet (Reinhard et al. 1986; Fugassa et al. 2008). Nine Neolithic burials from Kephala were sampled, as were sixteen individuals from Ayia Irini dating from the Chalcolithic (1), Bronze Age (2), Roman (10) and Byzantine (3) periods.

Each sample was processed by disaggregation in 0.5% trisodium phosphate, and the solution then passed through a series of microsieves with mesh sizes 300, 160, and 20 µm. Sediment particles trapped on the 20 µm mesh will contain any parasite eggs present. This sediment was mixed with glycerol and viewed with digital light microscopy (Anastasiou and Mitchell 2013). We used an Olympus BX40F microscope with GXCAM-9 digital camera and GXCapture version 7.1.0.0 software by GT Vision. Twenty slides were viewed for each sample. Eggs were identified by their shape, colour, dimensions and special characteristics. Disaggregated sediment was also analysed with

Enzyme linked immunosorbent assay (ELISA) in order to detect evidence for protozoal parasites that may cause dysentery, namely *Entamoeba histolytica*, *Giardia duodenalis*, and *Cryptosporidium parvum*. The ELISA kits used were factory made by Techlab<sup>®</sup>, Blacksburg, USA.

We attempted to further narrow down the date of the Neolithic burials using AMS radiocarbon dating at the University of Oxford, UK. However, samples of bone from these burials did not have sufficient collagen preserved to allow successful radiocarbon dating.

### **3.Results**

Twenty-five burials were analysed, with four (16%) yielding positive results for parasite eggs (Table 1). Of the nine Neolithic period burials from Kephala, one individual (grave 37) was positive for whipworm (*Trichuris trichiura*). Of the sixteen burials from Ayia Irini, one Late Bronze Age individual (grave 44) was positive for roundworm (*Ascaris lumbricoides*), one Roman period burial (grave 4) was positive for whipworm, and a further Roman Period burial (grave 5) was positive for roundworm (Figure 2-5). While we appreciate that human roundworm (*A. lumbricoides*) and pig roundworm (*A. suis*) are extremely similar at a genetic level, and may be indistinguishable, we have used the label *A. lumbricoides* as the eggs were recovered from the human pelvis.

Roundworm eggs were identified by their oval shape, thick wall with clear inner and outer surfaces, and dimensions falling within the expected range of 45-75 µm long and 35-50 µm wide (Garcia, 2009: 442). Each roundworm egg was decorticated, with loss of the outer mammillated coat in the soil due to taphonomic processes. This is common in ancient archaeological samples.

Whipworm eggs were identified by their lemon shape, locations for polar plugs, clear inner and outer surfaced to their wall, and smooth surface. This differentiates them from *Capillaria spp.*, which have a reticulated pattern on their surface. They all fell within the expected dimensions for human whipworm. Whipworm with polar plugs intact have dimensions 50-54 µm long by 20-23 µm wide (Garcia, 2009: 442), while

archaeological eggs that have lost their polar plugs due to taphonomic processes in the soil have the same width but are slightly shorter in length.

Control samples taken from the sediment inside the skull or feet was negative for parasite eggs in each case, indicating that the soil was not affected by the generalised contamination by human faeces from later populations. Each sample was negative when tested for *Entamoeba*, *Giardia* and *Cryptosporidium* using the Techlab<sup>®</sup> ELISA test kits.

#### **4. Discussion**

This study provides the first archaeological evidence for intestinal helminths in ancient Greece. While many latrines have been excavated in ancient Greece (Antoniou and Angelakis 2015), those excavating them have not generally taken samples for parasite analysis. Until now, the only past study of ancient parasites in the region has been the finding of the protozoal parasite *Entamoeba histolytica* (which can cause dysentery) from Kouphovouno (5,000-2,000 BC) (Le Bailly and Bouchet 2006). We have shown that both whipworm and roundworm were endemic on the island of Kea in prehistoric times, with whipworm found during the 4<sup>th</sup> millennium BC.

##### *5.1 How common were intestinal parasites?*

We found that 16% of individuals over this 4,000-year period were infected with intestinal parasites. As this is a very long period of time, this figure should not be used to indicate the proportion of the population that may have been infected at particular moment. This figure may indicate that most individuals were not infected with parasites, but we must also consider the possibility that the true prevalence was originally higher. It is possible that over the thousands of years since burial the rains had washed away many of the original parasite eggs, so that the evidence for other cases has not survived. The relatively low egg concentrations in the individuals that were positive (4-8 eggs in 20 slides) would suggest that many of the original eggs have been lost over the millennia. We did not attempt to calculate the number of eggs per gram of soil as the samples were small (scraped from the surface of the bone), and such a figure using pelvic sediment would not give any indication of the original degree of parasite infection. Egg counts per gram are much more meaningful when studying coprolites than pelvic soil from burials,

as faecal material in pelvic soil is diluted out by sediment from the ground. Our main aim here has been to determine the species of parasite present.

### 5.2 *Species of parasites present on the island*

It is interesting to find that only whipworm and roundworm were present in these populations over a 4,000-year period. In northern Europe during in the Neolithic, Bronze Age, Iron Age and Roman periods there is evidence for a large range of intestinal parasites including beef/pork tapeworm (*Taenia* sp.), bile duct fluke (*Opisthorchis* sp.), *Capillaria* sp., *Entamoeba histolytica*, *Fasciola* liver fluke (*Fasciola hepatica*), fish tapeworm (*Diphyllobothrium* sp.), giant kidney worm (Dioctophymidae), hookworm (Ankylostomids), lancet liver fluke (*Dicrocoelium* sp.), as well as roundworm and whipworm (Anastasiou 2015; Mitchell 2017). The only other Mediterranean island to have undergone ancient parasite analysis is that of Cyprus, where pelvic soil from the population at 8,300-6,000 BC was found to contain the eggs of roundworm, whipworm, *Taenia* tapeworm, and *Fasciola* liver fluke (Harter-Lailheugue et al. 2005). Again, this was a broader range of parasite species than was found at Kea. In Roman period Sagalassos, in nearby Turkey, roundworm and *Giardia duodenalis* (which causes dysentery) was found (Williams et al, 2017).

One possibility is that taphonomic processes over archaeological time at Kea led to the breakdown of less robust parasite eggs in the soil, only leaving the tough eggs of whipworm and roundworm. While this could potentially have been the case for the fragile eggs of species such as hookworm and pinworm, we would not expect the tough eggs of *Taenia* tapeworm and fish tapeworm to have been destroyed by soil microorganisms if whipworm and roundworm survived. Whipworm and roundworm are geohelminths, spread by the contamination of hands, water or food with human faeces. These two species are thought to have infected humans throughout our evolution (Mitchell 2013), so could potentially have been brought to Kea by the earliest settlers. They have been noted to be more common in sedentary farming populations that do not have good sanitation and who fertilise their crops with human faecal waste (Reinhard and Pucu de Araújo 2014; Mitchell 2015). The absence of species found in other parts of Europe during antiquity is likely to reflect the environment and lifestyle of people living on Kea. Many parasites have a complex life cycle that limits their endemic region to

those with suitable geography, the presence of specific wild animals that act as intermediate hosts, and certain cultural practices within the human population (Garcia 2009; Gunn and Pitt 2012). In the absence of a network of perennial freshwater lakes and rivers, a rather limited range of wild animals hunted for food, and cooking of meat, may explain why many of the parasite species of Neolithic and Bronze Age Europe were absent from Kea.

### 5.3 *Hippocrates and intestinal parasites*

Previous research has looked for descriptions of parasites in the medical writings of Hippocrates and his students, dating from the 4<sup>th</sup> and 3<sup>rd</sup> centuries BC. Works in the Hippocratic Corpus that mention intestinal helminths most frequently were ‘Epidemics’, ‘Prognostics’, and the ‘Coan Prognoses’ (Trompukis et al. 2007). The large round worm ‘*Helmins strongyle*’ was suggested to be roundworm (*Ascaris lumbricoides*), the small round worm ‘*Ascaris*’ that sometimes causes itching was proposed to be pinworm (*Enterobius vermicularis*) and the flat worm ‘*Helmins plateia*’ interpreted to be tapeworm segments (*Taenia* sp.) (Trompoukis et al. 2007). Roundworms are about 30cm long and pinworms are about 1cm long, and pinworms do cause itching around the anus at night, so such a proposal sounds plausible based on this textual evidence alone.

Our study of the population in Kea shows that roundworm was indeed present in the Greek Islands of the Aegean, so it is quite likely that the term ‘*Helmins strongyle*’ in the Hippocratic texts does refer to roundworm (*A. lumbricoides*). However, we did not find evidence for pinworm (*E. vermicularis*) or *Taenia* tapeworms. We did find evidence for whipworm, which is a small round worm, 3-5cm long. Hence it is possible that the Hippocratic texts used the term ‘*Ascaris*’ to apply to both whipworm and pinworm. Since pinworm eggs are fragile, they may theoretically have been present but not survived thousands of years in the soil. We have yet to find any evidence for worms that are flat in shape, as referred to in the Hippocratic corpus as ‘*Helmins plateia*’. Beef tapeworm (*Taenia saginata*), pork tapeworm (*T. solium*) and fish tapeworm (*Diphyllobothrium* sp.) have been found in Europe and the Mediterranean region in archaeological samples (Anastasiou 2015), so it is quite possible that Hippocratic descriptions using the term ‘*Helmins plateia*’ were referring to one of these species.



## 5. Conclusion

In this study we have searched for parasites in the populations of the island of Kea over a 4,000-year period. We have shown that parasitic worms were endemic on the island from the Neolithic right through to the Roman period. Interestingly, it was discovered that this Greek island did not have the same range of parasites as were found in Neolithic and Bronze Age populations of mainland Europe. Only whipworm and roundworm were present over this 4,000-year period. We have suggested that this may reflect an environment that did not suit the range of zoonotic parasites found in the well-watered regions of northern Europe.

Whipworm was found from the Neolithic onwards, while roundworm was found only from the Bronze Age onwards. While it is possible that roundworm may have been introduced to the island at a later date than whipworm, our limited sample size means that we cannot exclude the presence of roundworm at earlier periods than the Bronze Age.

We have used our positive results to interpret the medical texts of the Hippocratic Corpus, which originated on the nearby island of Kos in the 4<sup>th</sup> and 3<sup>rd</sup> centuries BC. We agree with past suggestions that Hippocrates and his students did most likely see examples of roundworm, and described these as '*Helmins strongyle*'. The other suggested diagnoses of *Taenia* tapeworms and pinworm sound plausible from the textual evidence, but were not identified in the archaeological samples at Kea. However, our identification of whipworm means that the small worms described by Hippocrates as '*Ascaris*' may well have included whipworm.

## **Acknowledgements**

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### **Figure Captions:**

Figure 1: Map showing location of the island of Kea (Keos), and the sites of Kephala and Ayia Irini. Reproduced with the permission of Cincinnati.

Figure 2: Whipworm egg from Neolithic burial at Kephala (4<sup>th</sup> millennium BC). Dimensions 50 x 23  $\mu\text{m}$ . Black bar indicates 20  $\mu\text{m}$ .

Figure 3: Decorticated roundworm egg from Late Bronze Age burial at Ayia Irini (1600-1100 BC). Dimensions 65 x 45  $\mu\text{m}$ . The outer mammillated coat has been lost due to taphonomic processes in the soil, which is common in ancient samples. Black bar indicates 20  $\mu\text{m}$ .

Figure 4: Whipworm egg from 2<sup>nd</sup>-3<sup>rd</sup> century AD Roman period burial (grave 4) at Ayia Irini. Dimensions 49 x 22  $\mu\text{m}$ . Black bar indicates 20  $\mu\text{m}$ .

Figure 5: Decorticated roundworm egg from 2<sup>nd</sup>-3<sup>rd</sup> century AD Roman period burial (grave 5) at Ayia Irini. Dimensions 62 x 44  $\mu\text{m}$ . Black bar indicates 20  $\mu\text{m}$ .

**Tables:**

Table 1: Parasite eggs identified in the burials on the island of Kea

<b>Time Period</b>	<b>Site</b>	<b>Parasite Egg Species</b>	<b>Egg Dimensions (<math>\mu\text{m}</math>)</b>	<b>Number in 20 slides</b>
Neolithic (4 <sup>th</sup> millennium BC)	Kephala Grave 37	Whipworm ( <i>T. trichiura</i> )	Length: mean 49.4 SD 1.2 Width: mean 23.8 SD 1.2	6
Late Bronze Age (1600-1100 BC)	Ayia Irini Grave 44	Roundworm ( <i>A. lumbricoides</i> )	Length: mean 64.8 SD 2.5 Width: mean 44.6 SD 1.2	6
Roman Period (2 <sup>nd</sup> -3 <sup>rd</sup> century AD)	Ayia Irini Grave 4	Whipworm ( <i>T. trichiura</i> )	Length: mean 49.0 SD 0.9 Width: mean 22.5 SD 0.6	4
Roman Period (2 <sup>nd</sup> -3 <sup>rd</sup> century AD)	Ayia Irini Grave 5	Roundworm ( <i>A. lumbricoides</i> )	Length: mean 62.2 SD 1.5 Width: mean 43.9 SD 20.8	8



Figure 1: Map showing location of the island of Kea (Keos), and the sites of Kephala and Ayia Irini.

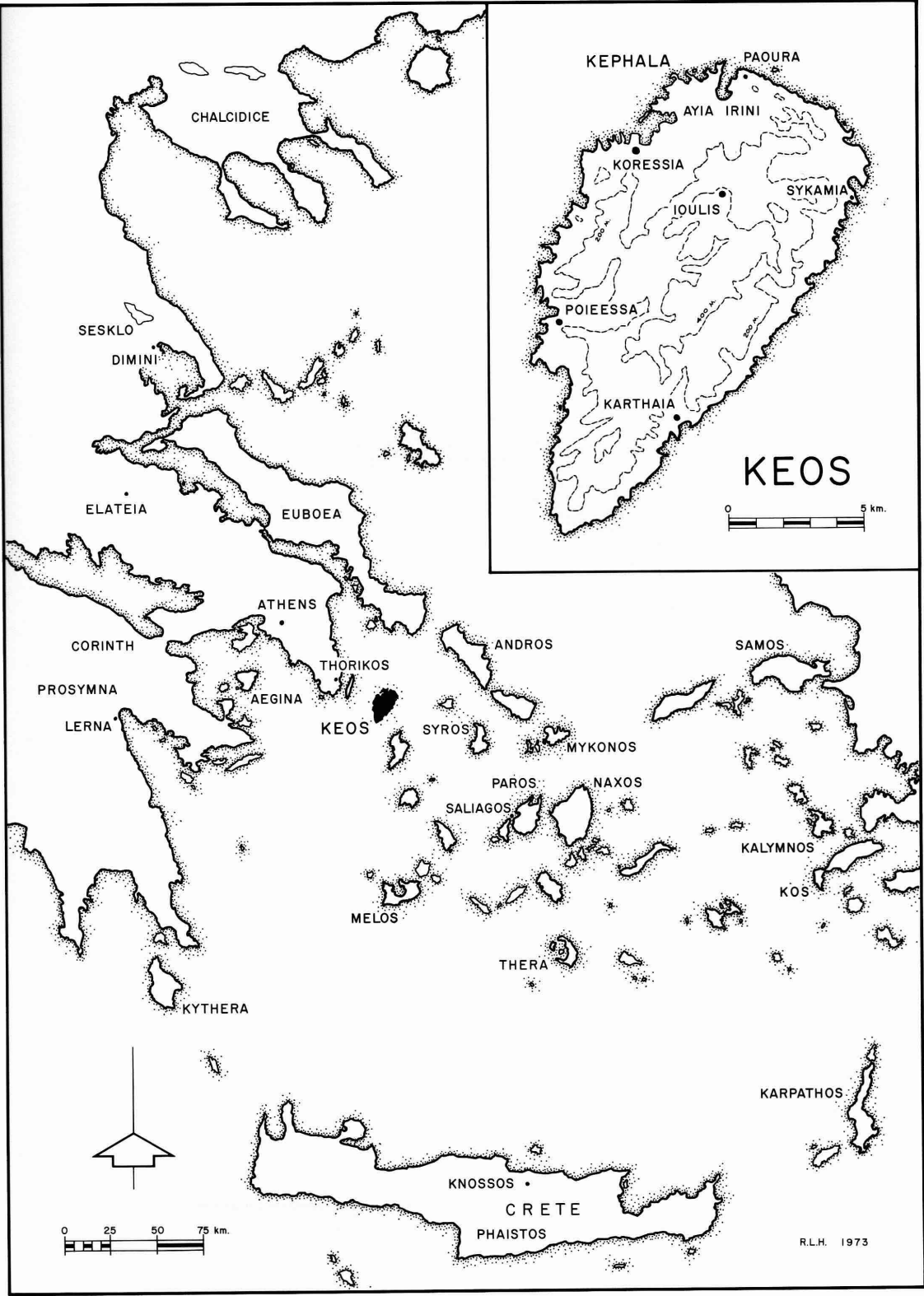


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Figure 3: Decorticated roundworm egg from Late Bronze Age burial at Ayia Irini (1600-1100 BC). Dimensions 65 x 45  $\mu\text{m}$ . The outer mammillated coat has been lost due to taphonomic processes in the soil, which is common in ancient samples. Black bar indicates 20  $\mu\text{m}$ .

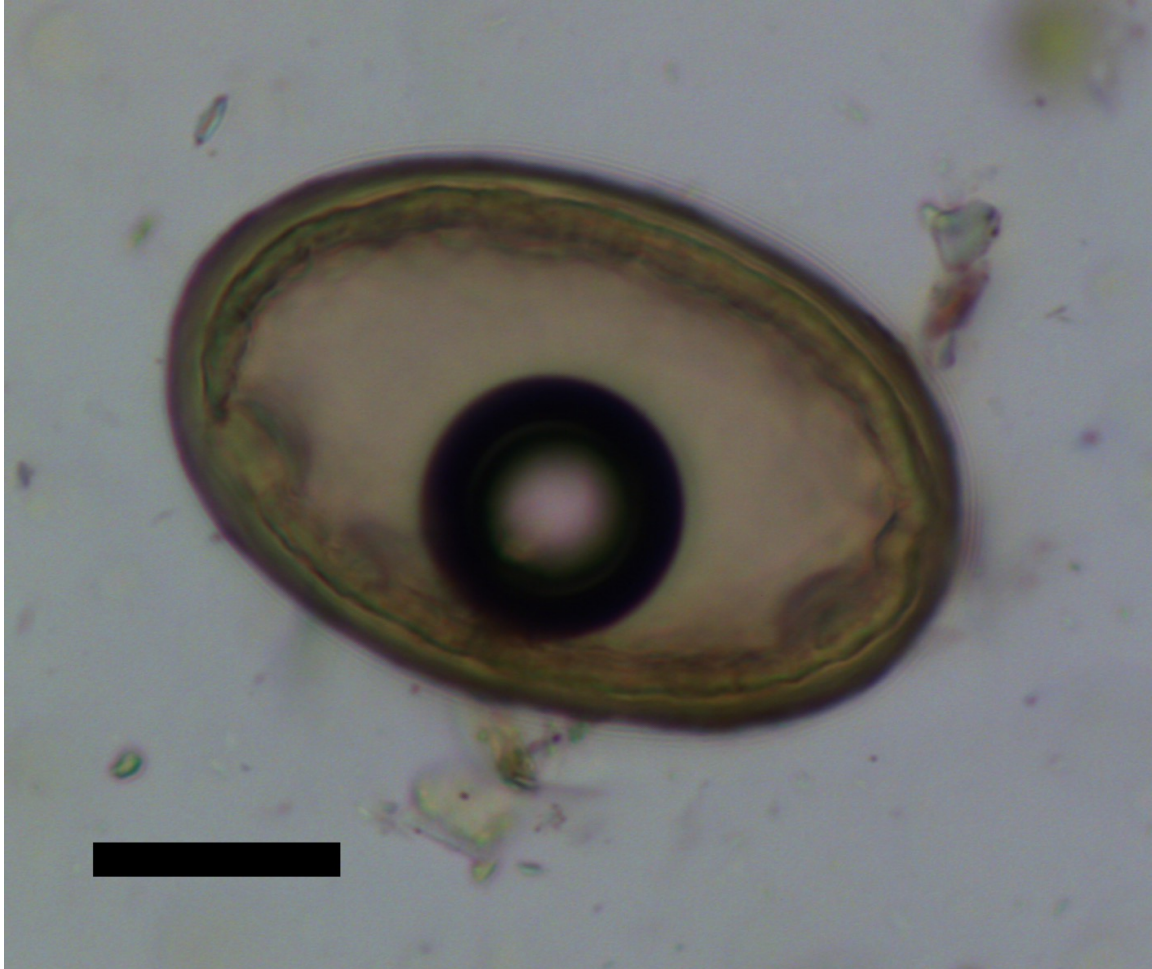


Figure 4: Whipworm egg from 2<sup>nd</sup>-3<sup>rd</sup> century AD Roman period burial (grave 4) at Ayia Irini. Dimensions 49 x 22µm. Black bar indicates 20 µm.

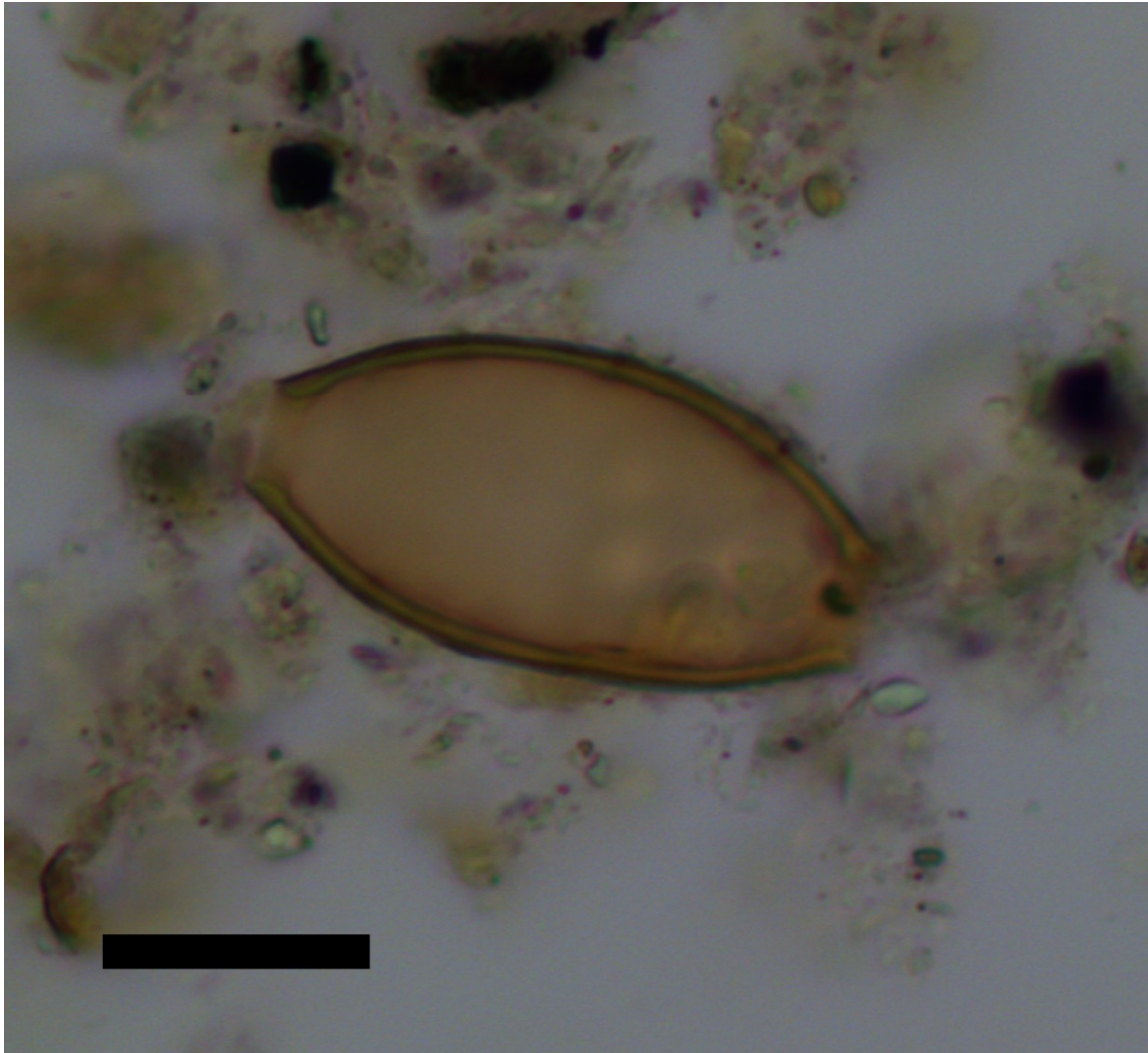


Figure 5: Decorticated roundworm egg from 2<sup>nd</sup>-3<sup>rd</sup> century AD Roman period burial (grave 5) at Ayia Irini. Dimensions 62 x 44  $\mu\text{m}$ . Black bar indicates 20  $\mu\text{m}$ .

