

ly compromised preservation. Future research should prioritize sampling from contexts with optimal preservation potential, apply advanced analytical methods, and integrate multidisciplinary approaches combining bioarchaeology, soil science, and paleoparasitology. These approaches will enhance our understanding of ancient health, hygiene, diet, and human-environment interactions in northern Iran, and contribute to reconstructing the epidemiology of parasitic infections in past populations.

**Keywords:** Shahne-Pushteh, Bioarcheology, Paleoparasitology, Microscopy, Parasite, Iron Age.

gration of humans, animals, and associated pathogens between Asia, Europe, and Africa. Consequently, understanding the distribution of parasites in past populations provides unique insights into health, hygiene, diet, and human-environment interactions. Notable paleoparasitological discoveries in Iran have predominantly come from high-preservation contexts, such as the Chehrabad salt mine, where well-preserved human mummies and associated biological remains have yielded valuable information on intestinal parasite (Nezamabadi et al., 2011).

The site of Shahne-Pushteh, located in Mazandaran Province, represents an Iron Age cemetery with human burials (Soltysiak et al., 2019). During the 2019–2020 excavation campaigns, a total of sixteen trenches were systematically opened (Agha Aligol et al., 2022). That offered the opportunity to study soil sediments from the immediate vicinity of skeletal remains. The primary objective of this study was to assess potential evidence of parasitic presence and evaluate their utility as indicators of health, hygiene, and dietary practices among the Iron Age population. Soil samples were subjected to microscopic analysis following established paleoparasitological protocols, with a focus on detecting nematodes, mites, palynomorphs, and other micro-remains. By combining these analyses with an understanding of environmental and taphonomic factors, this research provides a comprehensive view of parasite preservation and its implications for reconstructing ancient human lifeways in northern Iran.

### Materials and Methods:

Nineteen soil sediment samples were collected from various skeletal elements of Iron Age burials at Shahne-Pushteh for microscopic paleoparasitological analysis. Sampling focused on the abdominal region (pelvis, lower lumbar vertebrae, inner surfaces of ribs) using sterile disposable tools, and each sample weighed approximately 100 g. Samples were stored in sterile zip-lock bags to prevent contamination.

For laboratory analysis, 5 g of each sediment sample was weighed and transferred into Erlenmeyer flasks. Samples were hydrated with 100 mL of 0.5% trisodium phosphate (TSP) solution and an additional 100 mL of 5% glycerol solution was added to create a suspension. The samples were stored in a refrigerator for one week to ensure thorough hydration. After this period, the suspension was filtered, and aliquots were mounted on glass slides for microscopic examination. Twenty slides per sample were prepared and systematically analyzed under light microscopy.

### Results

Microscopic examination revealed traces of biological micro-remains in all nineteen samples. Observations in-

cluded nematode larvae, two distinct mite species, and a single palynomorph resembling an *Ascaris* sp. egg in morphology. Plant-derived remains, such as fibers, pollen grains, and spores, were also detected in all samples.

Palynomorphs, due to morphological similarities with parasite eggs, require careful differentiation using high-resolution microscopy and morphometric analysis. Measurements of all observed elements were recorded in micrometers. Although these palynomorphs shared size and shape characteristics with helminth eggs, none exhibited definitive morphological features of parasitic organisms. The presence of plant micro-remains also provides insight into the paleoenvironment, vegetation cover, and human-plant interactions in the region.

### Discussion

Environmental and biotic conditions at archaeological sites strongly influence the preservation of biological micro-remains. In northern Iran, particularly in Mazandaran Province, high humidity, abundant rainfall, and alkaline soil conditions contribute to rapid degradation of organic matter, including parasite eggs. Such taphonomic factors explain the limited recovery of intestinal parasite remains from Shahne-Pushteh. Comparatively, sites with high natural preservation, yield more abundant and diagnostically informative remains. The limited findings at Shahne-Pushteh, including nematode larvae, mites, and palynomorphs, nevertheless provide indirect evidence of human-environment-parasite interactions. The discovery of mite eggs and nematode larvae may reflect soil-dwelling organisms involved in decomposition processes and micro-environmental conditions of burials, rather than definitive intestinal parasitism. Palynomorphs resembling *Ascaris* sp. eggs underscore the importance of using complementary analytical techniques, such as scanning electron microscopy (SEM), chemical analysis, or ancient DNA extraction, to accurately differentiate parasite eggs from morphologically similar non-parasitic micro-remains. Additionally, taphonomic processes such as root intrusion, water infiltration, and soil alkalinity significantly affect the preservation potential of parasite eggs and other micro-remains.

### Conclusion:

Microscopic analysis of soil samples from Iron Age burials at Shahne-Pushteh revealed biological micro-remains, including plant fibers, pollen, spores, nematode larvae, mites, and a single palynomorph resembling an *Ascaris* sp. egg. While no definitive evidence of internal parasitic infection was identified, these findings provide valuable insights into taphonomic processes and the paleoenvironment of the site. The absence of confirmed parasite eggs does not indicate that the population was free from infection; rather, environmental and soil conditions like-



Microscopic Paleoparasitological Analysis of Iron Age Burials from Shahne-Pushteh, Iran

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Over the past two decades, paleoparasitological research in Iran has expanded significantly in both scale and scope. Despite this progress, investigations across the Iranian Plateau have faced ongoing challenges, and relatively few findings have been published. One major limiting factor is the extensive degradation of biological micro-remains caused by the region's prevailing climatic conditions, which are generally unfavorable for preservation at most archaeological sites. Notably, the most significant discoveries have come from exceptional preservation contexts, such as the Chehrabad salt mine in Zanjan, renowned for the discovery of the Salt Men mummies, has yielded a wealth of well-preserved biological materials. The high salinity and stable temperatures at the site have created ideal conditions for the survival of delicate micro-remains over millennia. These rare cases emphasize the importance of focusing excavations in environments more conducive to organic preservation. More recently, excavations at Shahne-Pushteh an Iron Age burial site revealed human skeletal remains, presenting a rare opportunity to analyze soil sediment samples microscopically from around the bones. The goal was to detect parasitic remains as

indicators of health, hygiene, and diet among the site's ancient population. Although no definitive internal parasite remains were found, several nematode larvae, mites, and a single palynomorph resembling an *Ascaris* sp. egg were observed. While not conclusive evidence of intestinal parasitism, these findings highlight the taphonomic processes that affect micro-remains. They also underscore the crucial role of environmental and soil conditions in the preservation of biological material in archaeological contexts.

**Introduction**

Paleoparasitology, or the study of ancient parasites, is a specialized branch of bioarchaeology that reconstructs the historical interactions between humans, animals, and parasitic organisms through the analysis of preserved biological remains (Le Bailly et al., 2021). Over the last two decades, paleoparasitological research in Iran has accelerated, yet the expansion of these studies across the Iranian Plateau remains limited due to environmental and taphonomic constraints that compromise the preservation of micro-remains. The Iranian Plateau has long been a key corridor connecting ancient civilizations, facilitating the mi-