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Ultrastructural Study of an Insect Recovered from a Joseon-era Tomb

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Abstract: In Korea, research on Joseon-period mummies has provided valuable biological insights into people from centuries ago. However, no reports have yet documented the recovery of ancient insect remains from these mummies. This study is significant as the first report on ancient *Dermestes* in South Korea. During our investigation of a 15th to 17th century Joseon mummy, we discovered beetle remains (genus *Dermestes*) embedded within the fabric wrapping the mummified body. Our macroscopic and electron microscopic analyses reveal near-perfect preservation of the beetle's head, thorax, and abdomen. This report highlights the exceptional morphological preservation of insect remains and explores the potential for such specimens to serve as subjects for future biological research.

Keywords: Joseon period; Mummy; Electron Microscope; Genus *Dermestes*

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Introduction

Scientific reports have documented the recovery of ancient insect remains from long-established archaeological sites. These studies provide valuable insights into the biological aspects of insects in evolutionary history (Kenward 1978; Panagiotakopulu 2001; Smith *et al.* 2021; Tuccia *et al.* 2022).

Over time, most ancient insect remains typically decompose and vanish. However, some insect remains retrieved from ancient mummies have been exceptionally well-preserved, making them ideal subjects for biological studies (Smith *et al.* 2021).

In Korea, research on mummies from the Joseon period has yielded abundant biological data about the people who lived several centuries ago. However, there have been no reports of ancient insect remains recovered from Joseon mummies, which means that the study of ancient entomology in Korea lags behind the current trends in other countries. In this regard, our current study holds academic significance as the first report on ancient *Dermestes* conducted in South Korea.

During our investigation of a centuries-old Joseon mummy, we discovered the remains of a beetle, which was found between fabrics that had been wrapped around the mummified body. Our study aims to assess how well the insect remains were preserved morphologically and to explore whether such insect samples could serve as a valuable resource for future biological research.

Case Report

Between 2018 and 2020, archaeological excavations were conducted at the Wonju Won Clan cemetery by the Nuri Institute for Archaeology (Gongju, South Korea). The excavation was part of a project to relocate military units to Manjong-ri, Wonju-si, Gangwon-do, and a cultural property investigation was carried out in compliance with relevant laws. During the investigation, a Hoegwakmyo, a Joseon period grave reinforced with a lime-soil mixture around the coffin, was uncovered. The grave's interior was perfectly sealed by the lime-soil barrier and remained intact until its recovery (Figure 1A and 1B).

When the hardened lime-soil barrier was breached and the coffin was opened, we discovered that both the corpse and other cultural relics inside the coffin were well preserved. The mummified body was tightly wrapped in undisturbed clothing and fabrics (Figure 1C). The mummy bundle was transported to Eulji University, where dress historians and anthropologists conducted further investigations.

For anthropological research, the fabric was carefully removed from the mummy in a controlled lab environment to minimize contamination. All participating researchers wore sterilized clothing and used disposable tools to prevent sample contamination (Figure 1D). The mummy was initially wrapped in a large blanket secured with several strings. Upon releasing the strings and opening the blanket, several additional layers of cloth were revealed. During the disassembly process, insect carcasses were identified and collected (Figure 1E and 1F).

Scanning electron microscopy (SEM) of the mummified insect sample was conducted using the methods outlined by Hayat (1970) and Bozzola and Russell (1992). The sample was pre-fixed in a 2% paraformaldehyde and 2.5% glutaraldehyde solution, post-fixed in 1% (w/v) osmium tetroxide, and then treated in a graded ethanol series and isoamyl acetate. The sample was dried in a critical point dryer (SCP-2, Hitachi, Japan) and coated with platinum-palladium using an ion coater (E-1030, Hitachi, Japan). The final sample was observed under a S-4700 SEM (Hitachi, Japan) (Lim *et al.* 2008).

Given the archaeological context of the discovery, we confidently speculate that the beetle dates to the Joseon Dynasty, as it was found while disassembling the tightly bound Joseon fabric around the mummified body. We believe the insect could not have infiltrated after the burial, as the lime-soil barrier around the coffin was too solid and lacked any gaps that would have allowed for insect intrusion. To confirm this, we conducted carbon dating analysis on the insect remains. The results indicated a date range of 1484 to 1644 cal CE (466-306 cal BP) with a 95.4% probability. This places the insect within the 15th to 17th century, which aligns well with the archaeologists' assessment that the grave was constructed during the Joseon Dynasty.

On macroscopic examination, the head, thorax, and abdomen were completely preserved. Eyes and mandible on the head, hard outer wings (elytra), prosternum, mesosternum, metasternum, abdominal sterna could be clearly visible; but antennae and legs were missing (Figure 2). In SEM images, we observed well-preserved compound eyes, vertex and frons in the head part (Figure 3A and 3B). In the hard outer wings and abdominal sterna, hairs could be clearly identified (Figure 3C to 3F).

Discussion

For decades, researchers have analyzed ancient insect specimens recovered through archaeological investigations, yielding remarkable insights that cannot be obtained through other methods. Hope (1842) was the first to report insect remains found in the gut of an ibis mummified by the ancient Egyptians. Huchet (1996) applied medicolegal entomology techniques to interpret insect remains from ancient and medieval graves. Fossilized beetles (order Coleoptera) were discovered at an archaeological site in Egypt (Panagiotakopulu and van der Veen 1997; Panagiotakopulu 1999). Smith *et al.* (2021) also explored ancient mites, which had previously been overlooked in scientific literature. These studies demonstrate that knowledge about historical climate, environment, diet, agricultural practices, living conditions, and hygiene can be effectively inferred (Tuccia *et al.* 2022).

Korea does not have a tradition of artificial mummification, but naturally formed mummies have been repeatedly reported in the country. The perfect sealing of graves and the anoxic conditions within may have played a crucial role in the mummification process (Shin *et al.* 2021). Mummies from the Joseon period exhibit exceptional preservation, as evidenced by anatomical, histological, and radiological studies. As a result, the value of Korean mummies has been increasingly recognized from a scientific perspective (Shin *et al.* 2021; Kim *et al.* 2021). These mummies serve as invaluable repositories of scientific information, providing insights into the biomedical and socio-cultural aspects of ancient peoples, as well as their diseases, in rich detail.

In this study, we recovered insect remains during the investigation of a Joseon period mummy. The remains were found within the layers of fabric that were wrapped around the mummy's body. In Joseon period funerary practices, the corpse was typically wrapped in multiple layers of clothing and fabrics, which were tightly bound before being placed in a coffin. Therefore, when a mummy is discovered during archaeological excavation, it is necessary to carefully remove the fabrics encasing the body. The insect remains in this study were found between these fabric layers during this process.

Based on its morphological characteristics, the insect is likely a species from the genus *Dermestes* (Peacock 1993). The Dermestidae family comprises approximately 1,300 species and subspecies (Hava and Kadej 2006). These insects vary in size and are typically covered with dense short hairs or scales (Kumara *et al.* 2009). In forensic science, Dermestidae are commonly used to estimate the post-mortem interval. In humid environments, fly larvae contribute significantly to the decay of deceased bodies. However, in the case of severely dried or mummified corpses, the environment is often too dry for flies to thrive. In such cases, Dermestidae often play a crucial role in skeletonizing the dried remains, as they are highly adapted to feed on such corpses due to their strong mouthparts (Kulshrestha and Satpathy 2001; Schroeder *et al.* 2002). As observed in this study, the preservation of the insect recovered from the Joseon mummy is exceptional.

During the Joseon period, deceased individuals were placed in a mortuary for several months prior to burial. In the mortuary, the corpse was tightly bound in layers of clothes and fabrics, secured with ropes. The insect in this study may have penetrated the mummy bundle or become trapped inside while the body was being wrapped. Given that the body remained in the mortuary for months before burial, it likely became sufficiently dry, creating an ideal environment for the beetle. *Dermestes* must



Figure 1: (A) Location of archaeological excavation in Wonju. (B) The Joseon tomb (VI-1) investigated in this study. (C) The mummy bundle tightly wrapped in undisturbed clothing and fabrics. (D) Dress historians removed the fabric from the mummy in a lab. (E) and (F) During the process, insect carcasses were identified and collected.

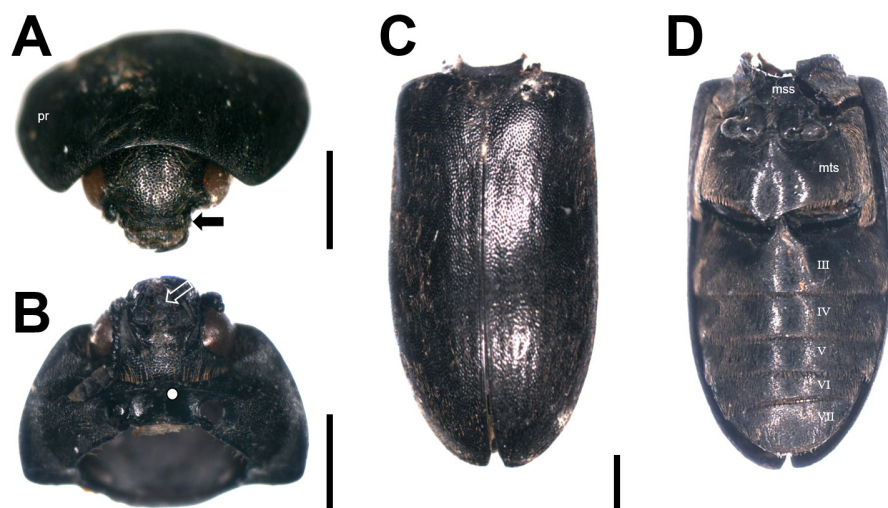


Figure 2: The insect of Joseon period examined macroscopically. (A) and (B) head and pronotum (pr). (A) Dorsal view. Solid arrow, maxilla. (B) Ventral view. Hollow arrow, mandible; white dot, prosternum. (C) Hard outer wings (elytra), dorsal view. (D) Ventral views of thorax and abdomen. Abdominal segments (sternites III to VII) are well-identified. mss, mesosternum; mts, metasternum; Scale bars = 1 mm for (A), (B); 2 mm for (C), (D)

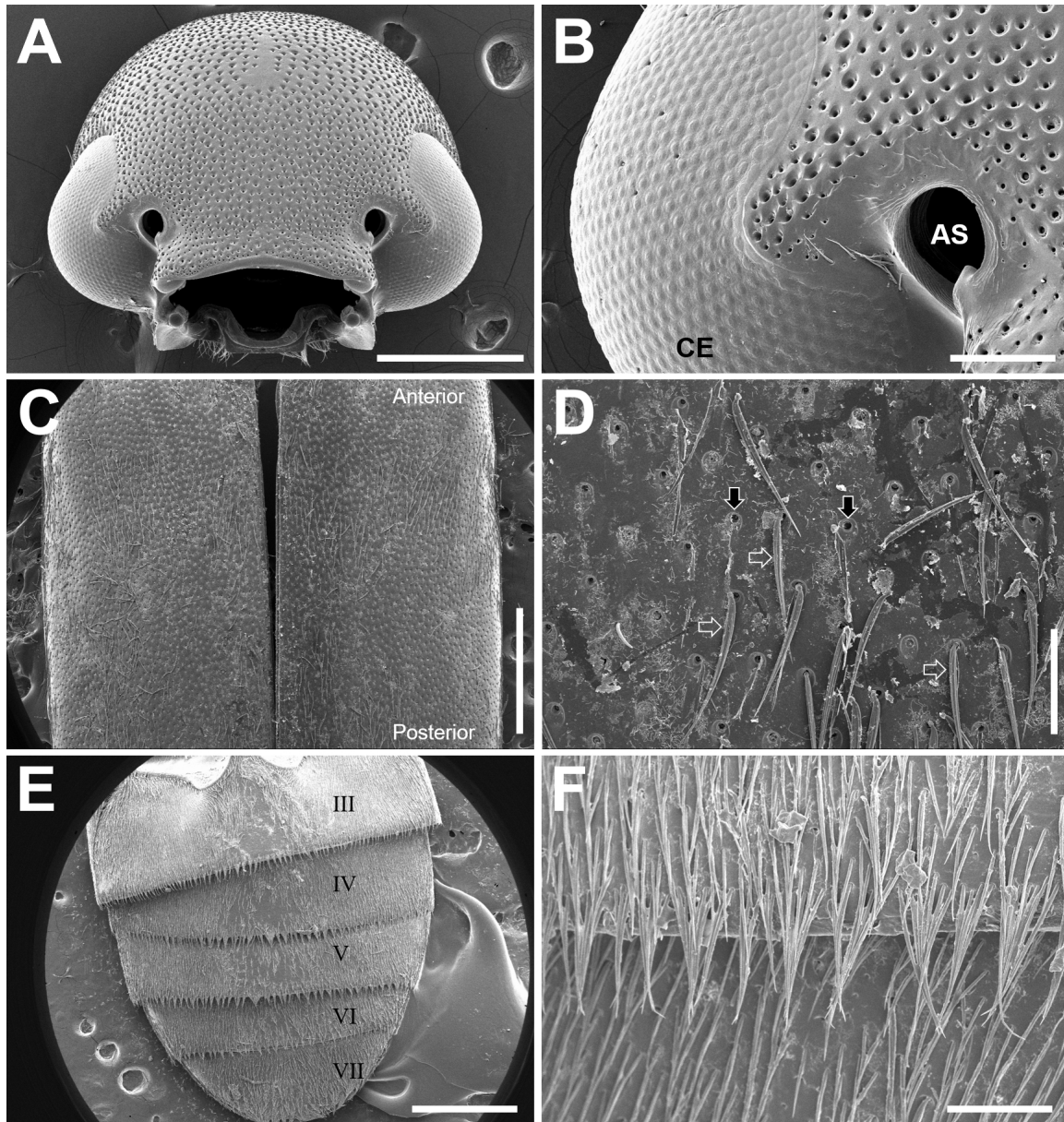


Figure 3: SEM images. (A) and (B) The head and well-preserved compound eyes (CE), vertex and frons. AS, antennal socket. **(C) and (D)** The hard outer wings (elytra). Hollow arrows, hairs; Solid arrows, the openings of hair. **(E)** The abdominal segments (sternites III to VIII) and **(F)** hairs. Scale bars = 500 μ m for (A); 100 μ m for (B), (D), (F); 1 mm for (C), (E).

have been alive and active inside the mummy bundle, feeding on the corpse for an extended period. However, once the funeral rites were completed and the coffin was buried, the beetle may have become trapped within the fabric, unable to escape due to the cement-like materials surrounding the coffin, ultimately mummifying within it.

Taken together, both macroscopic and electron microscopic observations of the head, thorax, and abdomen revealed near-perfect preservation of the ancient insect. While a complete understanding will only emerge through further research, this study opens the door to various pioneering investigations, including molecular biological analyses, of such insects. We believe these studies will offer valuable and detailed insights into the insect fauna of the Joseon Dynasty.

Authors' contributions

Conceptualization: CSO, DHS. Data acquisition: CSO, SM. Data analysis or interpretation: CSO, D-SL, DHS. Drafting of the manuscript: CSO, DHS. Correspondences: CSO, DHS. Approval of the final version of the manuscript: all authors

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Competing interests

The authors have no competing interests to declare.

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