



# Morphological studies of fly puparia (Diptera: Calliphoridae) in the soil from a Joseon Dynasty grave in Korea



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## Abstract

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Archaeoentomological investigations were conducted on soil contents from a grave belonging to the Joseon Dynasty as part of the Urban Environment Maintenance Project (UEMP) in Cheongjin 12–16 dong (districts), Jongno-gu, Seoul, Korea, from December 01, 2008 to February 19, 2011. A total of 28 insect puparia with hard shells of the common green bottle fly *Lucilia sericata* were identified in the soil. Evidence suggested that the corpse was placed outside for some days instead of being buried immediately after death. This is the first report of fly puparia in soil samples from a tomb of the Joseon Dynasty during 16–17 AD in Korea. Our findings may help determine the timeframe of burial and offer archaeological insights into the funerary customs of the period.

## Citation

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Remnants found in ancient tombs often comprise human and/or animal skeletal remains, burial artifacts, foods, and arthropod fragments. Investigations into arthropod fragments, including insects, in archaeological contexts can provide significant data, enabling the reconstruction of historical events, climates, and environments [1].

Archaeoentomological remains of puparia attached to corpses have been excavated from catacombs in Europe [2], mummies in Egypt [3], and tombs in Central and South America [4,5] and Japan [6]. Arthropod artifacts from tombs, such as beetle pieces, insect fragments (bodies and eggshells), and fly puparia, are covered with hard shells, which act as important archaeological and forensic indicators because their development can help determine the time of death and provide archaeological evidence [7].

In Korea, two types of insect artifacts have been found in tombs; those attached to the corpse and those associated with ornaments and clothing placed in the coffin. The insects identified in connection with these tomb remains primarily belong to the Diptera (flies), Phthiraptera (lice), and Coleoptera (beetles) orders [6,8-11].

The first report on fly puparia attached to a corpse in Korea described the presence of a dozen puparia of blowflies in the gilt-bronze shoes from the Mahan Confederacy (1 BC–5 AD) tomb in Naju Jungchon, Jeollanam-do (Naju City Native Cultural Heritage No. 13) in 2014 [6]. Excavation of these artifacts is of considerable significance in relation to the funeral culture. Traditionally, funeral customs in Korea have primarily involved burial prac-

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**Conflict of interest**

The authors declare no conflict of interest.

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tices and bin-jang, wherein a corpse was kept intact in a mortuary until the mourning was completed. These funeral ceremonies were practiced from the Baekje (18 BC–660 AD) and Silla (57 BC–935 AD) kingdoms to the Joseon Dynasty, also known as the Yi Dynasty (1392–1910) [12].

Body lice in coffins were first reported in 2006 from 19 egg-lice shells in clothes from Jeju Ko's grave in Geumsan, Chungcheongnam-do. These species were identified as *Pediculus humanus humanus*, also known as *Pediculus humanus corporis*, from the mummy quilts of the Joseon Dynasty 400 years ago, indicating that the corpses were placed in the coffins with their clothes on [8].

In addition, the elytra wings of Korean jewel beetles (*Chrysochroa coreana*, Coleoptera) in tomb remains have been reported in the Hwangnamdaechong tomb (early 5th century AD), Hwangdong no. 100 tomb (after the 5th century AD), Jjoksaem no. 44 (after the 5th century AD), Geumgwanchong tomb (6th century AD), and Gyerim no. 14 tomb (6th century AD) in Gyeongju, Gyeongsangbuk-do, and Jinpari no. 7 tomb in Pyongyang [9–11]. These artifacts show that the funeral tradition of placing souvenirs in the tomb existed in the royal families of Silla and Goguryeo.

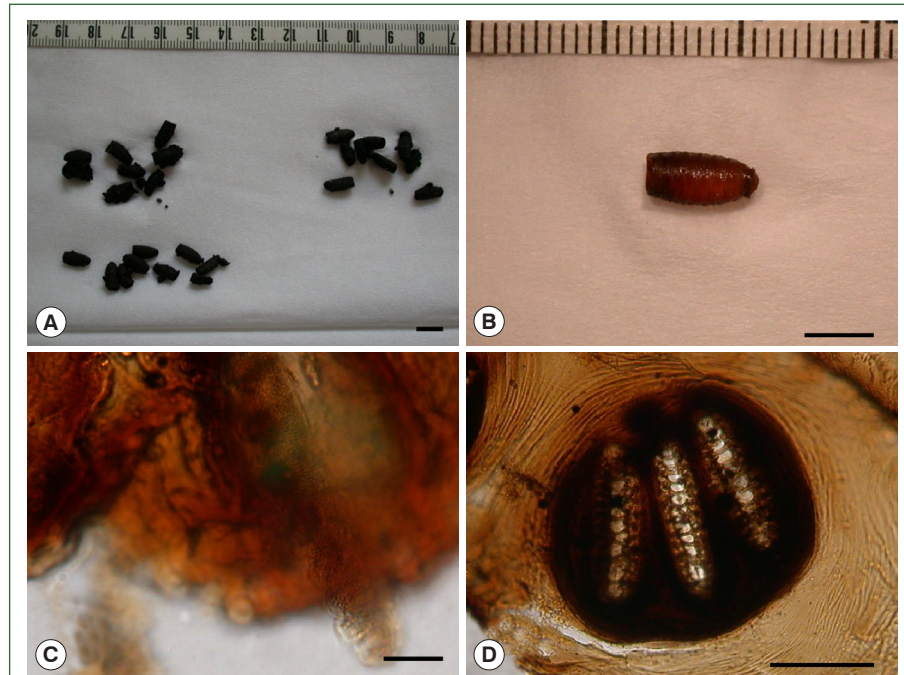
This study aimed to identify the fly puparia species excavated from a Joseon Dynasty grave. Archaeoentomological investigations of soil samples from the grave were conducted as part of the Urban Environment Maintenance Project (UEMP) in the Jongno-gu, Cheongjin 12–16 districts (37°34'15 "N, 126°58'49 "E) of Seoul from December 01, 2008 to February 19, 2011. Archaeologists presumed that this excavation site might have been a residence during the reign of the Joseon Dynasty between 1,600 and 1,700 AD. The artifacts were discovered at a depth of 1.5 meters below the ground surface [13].

The excavated artifacts included human remains, such as the skull, ribs, arm bones, hips, and pelvis (Fig. 1), and 28 specimen artifacts from insect puparia (Fig. 2A). The puparia were oxidized, hard, and dark in color. The average length and width of the 28 puparia, measured with a ruler (Vernier Caliper, Mitutoyo, Japan), were 6 mm and 3 mm, respectively.

Each specimen was preserved in 70% ethanol until use. The puparia were placed in 10% KOH solution for 5–7 days and fixed using a dehydration process to make them transparent for microscopic identification. Subsequently, they were identified using the morphological keys described in a previous study [14] and the distinct characteristic form of the third



**Fig. 1.** Photographs of the site in Cheongjin 12–16 districts in Seoul. (A) Aerial photographs of the excavation site (red circle: excavated site). (B) Excavated human skeletal artifacts (yellow arrow). Scale bar = 10 cm.



**Fig. 2.** Microscopic examination of the excavated puparia. (A) Puparia specimens were collected from the soil of a grave. Scale bar = 0.5 cm. (B) Dorsal view of puparia soaked in 10% KOH for 5 days. Scale bar = 0.5 cm. (C) Micrograph of the anterior spiracle. Scale bar = 200  $\mu$ m. (D) Micrograph of the posterior spiracle. Scale bar = 200  $\mu$ m.

instar larvae with anterior and posterior spiracles in the pupae shell. Based on the microscopic observations, the puparium, which appeared hard and brown (Fig. 2B), was identified as *Lucilia sericata*. The anterior spiracles of the puparia had eight fan-shaped lobes on the distal edges (Fig. 2C), and the posterior spiracles were elongated with three slits in the spiracular plate (Fig. 2D).

As shown in Fig. 2, the presence of puparia on the corpse in the surrounding soil suggested they had metamorphosed before or after burying it. In addition, the funeral ceremony and customs of the Joseon Dynasty suggest that the body was placed outside for several days instead of being buried immediately after death; moreover, they were probably buried 5–7 days after death. The duration of the funeral ceremony and customs of the Joseon Dynasty varied depending on the family and official rank [12]. These funeral customs made it easier for the *L. sericata* flies to lay their eggs and proliferate in the corpses.

The results of the present study and previous research [15,16] suggest that the developmental state of the puparia was formed between 3 and 7 days. Identifying the fly in these studies can provide information about the archaeological investigations.

*L. sericata* is widely distributed in Korea [17]; hence, it is a forensic indicator and an important ecological species associated with health problems in the country [15,16]. The first recorded discovery of fly puparia in Korea was made in 2014 when they were found in a corpse that had undergone bin-jang in a Naju Jungchon tomb in Jeollanam-do [6]. The puparia were well-preserved in their original form despite having been buried for 1,500 years. However, in other countries, the excavation of puparia artifacts has been interpreted differ-

ently. For example, the presence of fly puparia in Egyptian mummies is attributed to the use of fly maggots during artificial mummification [3], whereas, in coffins in the basements of European churches and tomb artifacts in Central and South America, they may be formed during storage [2-5].

The puparium (body or eggshell) of Diptera is a hard shell that can be preserved for a long time in its natural state. Puparia and insect materials are the only available archaeoentomological evidence. Arthropod shells are a relatively recent addition to the archaeological toolkit, and they can help assess past environments, the biogeography of various arthropod species, and the funeral and burial customs of different groups over time [18]. Arthropod specimens, particularly puparia, from tomb remains are old, oxidized, and have irregular shapes; they must be chemically treated to determine their characteristics [1,10].

In old Diptera puparia, identification is difficult because of the lack of identification keys and the presence of dust, soil, dry decomposition fluids, and bacteria on their surfaces, which mask their diagnostic characteristics. The quality of the sample can be enhanced by cleaning with warm water and soap, sonication, and treatment with a sodium hydroxide solution [19]. Various foreign substances and bronze rust were removed from the remains of the Korean jewel beetle through chemical cleaning, and the condition of the surface of the wing decoration was improved [10,20]. These methods can also be used for other insect artifacts in tombs.

Insect shells and beetle wings associated with funeral cultures have been discovered, and one case of fly pupae, one case of body lice, and seven cases of beetles have been reported in Korea [6,8-11]. These remains serve as archaeoentomological evidence, which can help determine the time of death of individuals and understand the past funeral cultures and burial customs in Korea.

In conclusion, this is the first report of fly puparia in the soil of a grave of the Joseon Dynasty. The findings of this study suggest that *L. sericata* is an archaeological indicator species. Studying these artifacts may provide us with insights into the life of Korean people in the past, particularly those with no written records. Furthermore, they can be used to assess past environments and human activities.

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