Structure of the mouthparts and alimentary canal of *Eusomus ovulum* Germar, 1824 (Coleoptera: Curculionidae)

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**A R T I C L E  I N F O**

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**A B S T R A C T**

The structural morphology of mouthparts, the distribution of sensilla on the mouthparts, histology, and the morphology of the alimentary canal in adult *Eusomus ovulum* Germar, 1824 were examined under both light and scanning electron microscopy (SEM). The mouthparts are located at the tip of the short rostrum. The mandibles, the maxillae and the labial palpi comprise the moveable mouthparts. Also there are sensilla which act as chemoreceptors on the terminal segment of the snout.

**Introduction**

Curculionidae is one of the largest families of Coleoptera. Almost all species of Curculionidae are phytophagous according to Ross (1963). Larvae and adults feed on various plant organs: roots, stems, leaves and fruits. Some species can cause economic losses (Hoffmann, 1950; Mihajlova, 1978; Caldara, 1990). *Eusomus ovulum* uses many plants as hosts, and it has been reported to cause significant damage, especially of forage clover. Some host plants of *E. ovulum* are *Amygdalus communis* L., *Avena sativa* L., *Centaura sp., Medicago sativa* L., *Onobrychis sativa* Lam., and *Vicia sativa* L (Lodos et al., 2003; Bolu, 2016).

To provide insight into feeding mechanisms and to provide characters for use in phylogenetic and taxonomic studies, research on mouthpart morphology is needed. Studies on mouthpart morphology can also reveal previously unknown feeding habits of insects. While most beetles have mouthparts designed for chewing solid food, Curculionoidea have mouthparts at the end of a distinct snout for boring holes for egg-laying as well as for feeding (Moon, 2015; Chen et al., 2016). cardiovascular, respiratory and excretory systems, gut system, assimilation, and storage, digestion, absorption of food and water balance (Calder, 1989; Romoser and Stoffolano, 1998; Bu and Chen, 2009; Aldigail et al., 2013).

The foregut of coleopterans, generally tubular, is composed of a narrow pharynx opening into the tubular esophagus. It is narrow anteriorly but may be distended posteriorly to form a crop and proventriculus (Ekis et al., 2013).

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and Gupta, 1971; Sarwade and Bhawane, 2013; Sousa et al., 2013). In many insects, the crop acts as a storage organ (Crowson, 1981) whereas the muscular proventriculus is the site where food particles are broken or filtered to the midgut (Kissingner, 1963; Serrão, 2005, 2007).

The midgut is generally the longest part of the gut, and is occasionally referred to as the stomach or ventriculus. The midgut lacks a chitinous lining. Most digestion occurs in this region. Epithelial cells of the midgut produce and secrete digestive enzymes and then the resultant nutrients are reabsorbed (Gilmour, 1961; Dadd, 1970; Wigglesworth, 1972; Gullan and Cranston, 2005; Sarwade and Bhawane, 2013).

The hindgut, the final part of the digestive system, is subdivided into the pylorus, the ileum, followed by the colon and rectum and ending exteriorly with the anus. The hindgut is lined with a thin layer of cuticle (Sinha, 1958; Maddrell and Gardiner, 1980; Sarwade and Bhawane, 2013) with crucial roles in excretion and homeostasis (Garaya et al., 1992; Serrão et al., 2004; Santos et al., 2009). Malpighian tubules arise at the junction of midgut and hindgut. Adult Coleopterans have a modified arrangement of Malpighian tubules which are responsible for efficient dehydration of feces before their elimination (Gullan and Cranston, 2005). Gullan and Cranston (2005) stated that Coleopterans generally have a cryptonephric system in which the distal ends of these tubules are held in contact with the rectal wall by the perinephric membrane.

*E. ovulum* is an economically important insect, so it can prove useful in providing basic information about the morphology of its mouthparts and alimentary tract can prove useful in controlling this species.

Materials and methods

**Insect**

Adult males and females of *E. ovulum* were collected from trifolium fields in Kırşehir ( Özbağ), Turkey in May 2018. The weevils were placed in plastic containers with their food plant and were transported to the laboratory. In the laboratory, 25 insects were anesthetized with ethyl acetate. The head and alimentary canal of each insect were dissected in a 0.1 M sodium phosphate buffer (pH 7.2) under an Olympus SZX7 stereoscopic dissecting binocular microscope (SM) and photographed.

**Light Microscopy (LM)**

For histological investigations, the gross anatomical structures of dissected alimentary canals were removed and their gross anatomical structures were measured. Then the guts were fixed in 10% neutral formalin for 24 hours and then dehydrated for 1 hour in a progressive series of ethanol (50, 70, 80, 90 and 99.5%). The guts were subsequently transferred into xylol, and thereafter graded into solutions of decreasing series of ethanol (50, 70, 80, 90 and 99.5%). The guts were subsequently fixed in 10% neutral formalin for 24 hours and then dehydrated for 1 hour in a progressive series of ethanol (50, 70, 80, 90 and 99.5%). The samples were then transferred to hexamethyldisilazane and allowed to air dry; then the tissues were mounted on SEM stubs with double-side sticky tape. They were subsequently covered with gold by using a Polaron SC 502 sputter coater. The stubs were examined with a SEM (JEOL JSM 6060 LV) and digital photos were taken.

**Scanning Electron Microscopy (SEM)**

For scanning electron microscopy examination, 10 adult females and males were randomly selected and anesthetized with ethyl acetate. The head with mouthparts and alimentary canal attached were first removed from the body under a dissecting stereomicroscope. Then samples were fixed in 2.5% glutaraldehyde (pH 7.2, sodium phosphate buffered), rinsed three times in sodium phosphate buffer and dehydrated by using an ethanol progressive series (50, 70, 80, 90 and 99.5%). The samples were then transferred to hexamethyldisilazane and allowed to air dry; then the tissues were mounted on SEM stubs with double-side sticky tape. They were subsequently covered with gold by using a Polaron SC 502 sputter coater. The stubs were examined with a SEM (JEOL JSM 6060 LV) and digital photos were taken.

**Results**

SEM of the external head morphology of *E. ovulum* shows a pair of compound eyes, a pair of antennae, and portions of the mouthparts (Figs. 1A and B). The mouthparts consist of a labrum, a pair of mandibles, a pair of maxillae and the labium. The labrum is the upper-most of the mouthparts and located on the midline. The labrum is a single, fused plate and on its surface, there are a number of sensory setae. Beneath the labrum, a pair of jaw-like mandibles which serve as pincers can be seen. They are heavily sclerotized structures having cutting edges that move transversely to grasp or cut food (Fig. 1A). The anterior end is sharp and concave; the lower part is slightly protrudes the dorsal surface is granular and has long sensilla, and the mandibular scar is arched along the dorso-lateral axis of the mandible. The mandibular scar is prominent, wide, circular and pitted. Sensilla occur on the dorsal surface of the mandible (Figs. 1B and C). Mandibles are posteriorly attached to the arch-shaped rostrum (Figs. 1C and D). Together with the maxillae and the labial palpi they constitute the movable mouthparts (Figs. 1B to F).

There are a pair of blade-like appendages, the maxillae, behind the mandibles. The paired maxillae are bilaterally located in longitudinal clefts on each side of the labium. Each maxilla is composed of cardo, stipes, palpiifer and 3-segmented palpi. The cardo is short and flattened, the stipes and the palpiifer are longitudinal (Fig. 1F). The first and second segments of the palpi are short, the terminal segment is long and bears three types of sensilla at their apices. There are five long and cylindrical sensilla (tip 1), located medially. The edge sensilla are short and pointed (tip 2). Additionally, there is a single sensilla (tip 3) like seta at the edge (Fig. 1H).

The labium or lower lip is located ventrally and is fused along the midline. The labium is a single structure that is equivalent to the floor of the mouth although it is formed from two fused secondary maxillae (Fig. 1F). The prementum is broad and triangular, and has setae laterally. Each labial palp is composed of three smooth-surfaced segments. The basal two segments have single setae on the surface (Figs. 1E and F). The terminal segment has minute sensilla at their apices (Fig. 1G). The peduncule is very small and resembles the prementum (Fig. 1E).

Beyond the mouthparts is the alimentary canal which is tubular structure surrounded by muscle and a tracheal network (Figs. 2A and B). The main structures of the alimentary canal of *E. ovulum* are easily distinguishable (Fig. 2A). It consists structurally of three distinct regions: the foregut, midgut and hindgut (Fig. 2A).

The foregut includes the pharynx, esophagus, crop and proventriculus (Figs. 2C, D). Muscles are attached dorsally and ventrally in the head and extend to the pharynx and esophagus (Fig. 2E). When the muscle is examined in detail, transverse striations are observed (Fig. 2F). The esophagus is elongate and terminates at the base of the crop.

A morphological cross section of the esophagus shows that it consists of lumen, intima, and a squamous epithelium which is thin, flat with external muscle layers (Fig. 3A). The intima is thick and sclerotized with folds (Fig. 3B). The intima has several well developed spine-like structures (Figs. 3C and D). Muscles are clearly visible along its length (Fig. 3B). Following the esophagus, we find the crop. In a morphological examination of the crop, bifurcated spines can be seen extending from the intima (Fig. 3F). The proventriculus follows after the crop but the transition limits are not clear (Fig. 3E). The proventriculus has a strong sclerotized intima and is the widest part of the foregut (Figs. 3G and H).
Figure 1 Scanning electron micrographs of the head region of adult *Eusomus ovulum*. (A-B) Frontal and ventral view. The mouthpart consists of a labrum (Lbr), a pair of mandibles (Md), a pair of maxillae and the labium. The labrum is a single, fused plate which bears a number of sensory setae. (C-D) A pair of mandibles which have a mandibular scar and long sensory setae. (E-F) the maxillae and the labial palpi with spines, Lp: labial palpi, Mp: maxillary palpi, Ms: mandibular scar, Pd: peduncle, Pf: palpifer, Pm: prementum, St: stripes. (G) Scanning electron micrographs of sensilla (Se) of distal segments of labial palpi. (H) The sensilla (tip 1, 2, 3) of distal segments of the maxillary palpi.
Histologically the layers of proventriculus in sequence from inside to hemocoel are lumen, intima, monolayer epithelium and circular and longitudinal muscles (Figs. 3 I and J). The proventriculus is a spherical organ formed by eight sclerotized plates (Figs. 3I and K) each bearing 2 longitudinal brushes (Figs. 3I, J, L). Additionally, its outer surface is covered with numerous tracheae and tracheoles (Fig. 3H).

The midgut, the second part of alimentary canal has two distinguishable areas, anterior and posterior sections (Figs. 4A and 5A). The anterior

![Figure 2](image-url)
region is a straight cylindrical structure (Figs. 4A and B) while the posterior region is coiled and occurs in the abdomen (Fig. 5A). It was observed that Malpighian tubules extend along the anterior midgut surface and there are tracheae on the surface (Fig. 4B). A cross section of the midgut shows that the midgut has a lumen, a single layer of columnar cells and numerous microvilli (Figs. 4C-F, 5B-D). The cells of epithelium are tall, columnar with distinct cell membranes, with centrally placed nuclei (Figs. 4D and 5B). Secretory granules can be seen in the midgut epithelial cells (Figs. 4F and 5D). Regenerative cells are found between the cylindrical epithelial cells (Fig. 4C). The posterior midgut wall produces numerous small pouch like gastric caeca (Figs. 5E and F). Tracheae can be seen among the gastric caeca (Fig. 5E). The gastric caeca, towards the end of the posterior midgut, are short, tubular, cylindrical in shape, and their surface is smooth (Figs. 5E and F). In the histological examinations, each gastric caecum is surrounded by a monolayer of cuboidal epithelium. Oval nuclei of epithelial cells

![Figure 3](image-url)
are located in the central portion of the cells. There are microvilli in the apical ends of the epithelial cells. Their lumens are wide (Fig. 5B).

The hindgut forms the last region of the gut. It consists of pylorus, ileum, colon and rectum. Six Malpighian tubules join the alimentary canal at the junction of the posterior midgut and pylorus (Figs. 6A and B). Each Malpighian tubule is an unbranched tube, being uniform in diameter (Figs. 6A and B). By sectioning a Malpighian tubule one can observe the single layer of cuboidal epithelium (Figs. 6C and D). Externally,

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**Figure 4 E. ovulum, adult:** (A, B) Malpighi tubules continuing along the anterior midgut surface (SM, SEM), (C) Longitudinal section of the anterior midgut (LM) (H & E), (D) Morphological view of the anterior midgut (SEM), (E, F) Microvilli extending in the apical of cylindrical epithelial cells. (Am) anterior midgut (SEM), (Ep) epithelium, (Lu) lumen, (Mt) Malpighian tubules, (Mv) microvilli, (Nu) nucleus, (Pv) proventriculus, (Rc) Regenerative cells, (Sg) secretory granules, (Tr) tracheae. H & E-hematoxylin and eosine, LM-Light microscopy, SEM-Scanning electron microscopy, SM-stereo microscope.
there is a thin connective sheath. On its surface, numerous tracheae and tracheoles are seen (Fig. 6C). The ends of Malpighian tubules lie parallel to the outer surfaces of the colon and rectum. Malpighian tubules present in this region have a cryptonephric arrangement. The ileum is located posterior to the pylorus (Figs. 6E and F). Histologically and morphologically, the epithelium of the ileum consists of cuboidal cells. The nuclei of the epithelial cells are round. The intima is thin and sclerotized (Figs. 6G and H).
The ileum joins with colon. Malpighian tubules can be clearly distinguished from the outer surface of the colon (Figs. 7A and B). Histologically, the colon bears muscles and is lined internally with a thick layer of cuticular intima. The epithelium is made up of cuboidal cells (Figs. 7C and D). The colon surface is surrounded by muscles and covered with a large number of tracheae and tracheoles (Fig. 7B). The colon is anterior to and contiguous with the rectum (Fig. 7A). There are rectal pads on the outer surface of the rectum (Fig. 7E). Histologically

Figure 6. E. ovulum, adult: (A, B) The connection of Malpighian tubules with pylorus (Pl) (SM, SEM), (C, D) Detailed view of Malpighian tubules (Mt) showing microvilli (Mv) on the apical surface of the cuboidal epithelium with oval nucleus (Nu), lumen (Lu), tracheae (Tr) (SEM). (E) Overview of ileum (Il), colon (Cl) and rectum (Re) (SM), (F) Outer surface morphology of pylorus (Pl), Malpighian tubules (Mt) and ileum (Il) (SEM), (G) Histological section of ileum showing lumen (Lu), intima (In), epithelium (Ep), muscles (Ms) (H&E) (LM), (H) Ileum showing lumen, intima, epithelium, muscles (SEM). H & E--hematoxylin and eosine, LM--Light microscopy, SEM--Scanning electron microscopy, SM--stereo microscope.
Figure 7. *E. ovulum*, adult: (A) General view of colon (SM), (B) Malpighian tubules (Mt) encapsulating the wall of the colon (Cl), (C) The histological section of colon showing intima (In), epithelium (Ep), Malpighian tubules (Mt), muscles (Ml) and lumen (Lu) (H&E) (LM), (D) The cross section of the colon (SEM), (E) The histological section of rectum (Rc) showing intima (In), epithelium (Ep), rectal pad (Rp), Malpighian tubules (Mt), muscles (Ml) and lumen (Lu) is filled with fecal matter (H&E) (LM), (F) Lumen (Lu), epithelium (Ep) and intima (In) in the rectum wall (SEM), (G, H) The connection of rectum (Rc) with anus (An) (H&E) (LM) (SEM). H & E-hematoxylin and eosine, LM-Light microscopy, SEM-Scanning electron microscopy, SM-stereo microscope.
and morphologically, the epithelium of rectum is made up of large cuboidal cells. Internally, the epithelium is lined by intima and externally by muscles (Figs. 7E and F). The rectum opens exteriorly through an anus (Figs. 7G and H).

**Discussion**

The Curculionidae (true weevils or snout weevils) are mostly plant feeders and are economically important pests of crops (Moon, 2015). Most beetles have mouth parts designed for chewing solid food. Many beetles of the superfAMILY Curculionoidea have mouthparts at the end of distinct snouts which are responsible for penetrating, feeding, and boring holes for egg-laying (Van Zandt et al., 2003; Moon et al., 2008). Mouthparts can be separated into two basic types: those adapted for biting and chewing solid food, and those adapted for sucking up fluids (Chapman, 1982; Moon et al., 2008). The mouthparts of *E. ovulum* are similar to the weevil ancestor or plesiomorphic. The mandibles in *E. ovulum* are very strong and have been modified to cut and chew. The mandibles are a pair of hard, often tooth-like structures that move horizontally to grasp, crush, or cut food (Moon, 2015).

The gross morphological features of the mouthparts of *E. ovulum* are generally similar to those reported for other weevils (Ting, 1933, 1936; Dennell, 1942; Morimoto, 1962; Morrone et al., 1992; Lal, 1995; Bae, 2000; Bae et al., 2000; Morimoto and Kojima, 2003; Morimoto et al., 2006; Belhoucine et al., 2013; Moon, 2015). Two pairs of finger-like appendages (maxillary and labial palpi) are found around the mouth in most beetles, and they are responsible for moving food into the mouth. Each pair has lobed sensory palpi, which may detect olfactory stimuli (Moon, 2015; Ghany and Aziz, 2017).

The three parts of the *E. ovulum* gut (foregut, midgut and hindgut) are easily distinguishable and are similar to those seen in other Coleopterans (Sinha, 1958; Diaz et al., 1998, 2000, 2003; Rubio et al., 2008; Bu and Chen, 2009; Aldigail et al., 2013; Sarwade and Bhawane, 2013; Singh and Prasad, 2013; Borges et al., 2015; Candan et al., 2019).

Generally, in Curculionidae the foregut begins at the mouth followed by the pharynx, esophagus, crop and proventriculus. The foregut is responsible for the transport of food to the crop whose function is the temporary storage of food (Diaz et al., 2000; Rubio et al., 2008; Bu and Chen, 2009; Aldigail et al., 2013; Singh and Prasad, 2013; Sousa et al., 2013; Candan et al., 2019). Various authors have illustrated subtle differences among species (Sinha, 1958; Vazquez-Arista et al., 1997; Diaz et al., 1998, 2000, 2003; Rubio et al., 2008; Bu and Chen, 2009; Aldigail et al., 2013; Sarwade and Bhawane, 2013; Singh and Prasad, 2013; Borges et al., 2015; Candan et al., 2019). The spines observed within the esophageal intima of *E. ovulum* were similar to those observed in *A. diaperinus* (Coleoptera: Tenebrionidae), *Dendroctonus micans, D. ponderosae, D. pseudotsugae* pseudotsugae, *D. rufipennis* and *D. terebrans* (Coleoptera: Colytidae) (McAllister et al., 1995; Diaz et al., 2003). In contrast, the esophageal intima of *P. bellii* (Coleoptera: Tenebrionidae) lacks spines (Sarwade and Bhawane, 2013). The morphology the proventriculus of *E. ovulum* was similar to that of other weevils; the lumen contained eight sclerotized teeth extending from the intima. The intima is covered with a layer of epithelial cells and circular and longitudinal muscle layers as other species of Curculionoidea superfAMILY (Dennell, 1942; Eaton, 1942; Baker, 1984; Sanchez et al., 2000; Rubio et al., 2008; Bu and Chen, 2009; Sousa et al., 2013). The muscles are responsible for constriction of the proventriculus during the mortaring and filtering of food as can be seen in *E. ovulum*. Similar structures were also described by Diaz et al. (1998, 2000, 2003), Bu and Chen (2009) and Sousa et al. (2013).

The midgut of *E. ovulum* is subdivided into easily distinguished anterior and posterior parts. Both anterior midgut and posterior midgut are tube-shaped. The anterior midgut is wide whereas the posterior one is long and tube-shaped. This is similar to that described for other Coleoptera species (Diaz et al., 1998, 2000, 2003; Rubio et al., 2008; Singh and Prasad, 2013; Candan et al., 2019). The anterior midgut is responsible for allowing a sufficient mixture of digestive enzymes and food. The posterior midgut, with gastric caeca, helps to increase the efficiency of absorption. In *E. ovulum* the midgut epithelium is a layer of columnar cells, as in other species (Khan, 1964; Smith et al., 1969; Bu and Chen, 2009). The gastric caeca bear many papillae which are appearing as finger-like tubular structures. Their position, number, and arrangement vary among coleopteran species (Calder, 1989; Rubio et al., 2008). Gastric caeca are found on the posterior midgut in *E. ovulum*. A similar structure was observed in *A. diaperinus* (Coleoptera: Tenebrionidae) and *Epilachna chrysomelina* Fabr. (Coleoptera: Coccinellidae) (McAllister et al., 1995; Aldigail et al., 2013). In *Hypothenemus hampei* (Ferrari) (Coleoptera, Curculionidae), the posterior midgut is characterized by the presence of two gastric caeca (Rubio et al., 2008). However, Bu and Chen (2009) stated that approximately 160 gastric caeca are located in the middle of the midgut of *Dendroctonus armandi* (Coleoptera: Curculionidae).

In Coleopterans, both the front and posterior part of the digestive canal are characterized by presence of Malpighian tubules; they also occur between the mid- and hind-gut (Singh, 1958; McAllister et al., 1995; Diaz et al., 2000, 2003; Rubio et al., 2008; Bu and Chen, 2009; Aldigail et al., 2013; Singh and Prasad, 2013; Candan et al., 2019). In *E. ovulum*, there are six long tubular Malpighian tubules with smooth surfaces. Histologically they are composed of large cells with large, oval nuclei. Similar structures have been observed in other Coleopteran species (Sinha, 1958; McAllister et al., 1995; Diaz et al., 2000, 2003; Rubio et al., 2008; Bu and Chen, 2009; Aldigail et al., 2013; Singh and Prasad, 2013; Candan et al., 2019). The cryptonephridial system attached to the hindgut wall of *E. ovulum* is similar to that of *Dendroctonus parallelocollis, D. rhizophagus, and D. valens* (Coleoptera, Curculionidae: Scolytinae) (Diaz et al., 1998), *D. armanti* (Bu & Chen, 2009), *E. chrysomelina* (Coleoptera: Coccinellidae) (Aldigail et al., 2013), and *Odoiporus longicollis* (Coleoptera: Curculionidae) (Singh & Prasad, 2013).

As in other Coleopterans, the hindgut in *E. ovulum* consists of pylorus, ileum, colon and rectum (Sinha, 1958; Aldigail et al., 2013; Candan et al., 2019). In *A. diaperinus* (Coleoptera: Tenebrionidae), hindgut is divided into two distinct sections, the anterior small intestine and the posterior large intestine (McAllister et al., 1995). Borges et al. (2015) pointed out that the hindgut in *A. bipunctata* is composed of the ileum, rectum and rectal canal. The main functions of the hindgut are the absorption of water, salts from the feces and urine (Smith, 1968).

In the present study, the morphology of mouthparts, and the morphology and histology of the alimentary of *E. ovulum* were examined. These results were compared with those of other species of Curculionidae. It is hoped that this study will contribute to the knowledge of the digestive system of Curculionidae. Furthermore it is hoped that characters revealed may be useful in future studies; and provide information for future research into the ecology and biological control of noxious Coleoptera.
Author contribution statement

All authors contributed to this review. Conduct of experiments (NÖK), writing of the manuscript (SC, ME).

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