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Ultrastructure of Digestive Canal of *Graphosoma lineatum* (Linnaeus, 1758) (Heteroptera: Pentatomidae)

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ABSTRACT

In this study, ultrastructure of digestive canal of *Graphosoma lineatum* (Linnaeus, 1758) (Heteroptera, Pentatomidae) was examined with the use of light, scanning (SEM) and transmission electron microscope (TEM). The digestive canal of *G. lineatum* consists of three distinct regions: Foregut, midgut and hindgut. The foregut and hindgut are very short. The midgut is longer and differentiated into three distinct portions: anterior, median, posterior. The anterior region is similar to a wide elongated sac, the median region is narrower than the other regions of midgut and tubular in shape, the posterior region is short and dilated. There are two pairs of Malpighian tubules at the junction of midgut and hindgut. The alimentary canal of *G. lineatum* is generally similar to that of the digestive system of other Heteroptera. The epithelial cells of the foregut are cubically arranged as a single layer. In the SEM and TEM images, the foregut is seen to be surrounded by a layer of longitudinal muscle and inner surface is formed by cell groups located over a folded basal lamina. The surface of midgut is composed of a single layer of cylindrical cells surrounded by a layer of inner circular and outer longitudinal muscles. The hindgut is composed of an extremely thin epithelial cell layer. It appears wrinkled, but does not contain microvilli.

Key words: Digestive system**,** *Graphosoma lineatum,* Heteroptera, SEM, TEM.

INTRODUCTION

Insects are among the most successful organisms in Nature. Some of them are crop pests causing considerable economic losses (Mehrabadi *et al.*, 2012), such as the Hemiptera. Members of this order both transmit disease and damage agricultural crops either indirectly or by direct feeding (Habibi *et al.*, 2008). One of the most serious pests is *Graphosoma lineatum* (Heteroptera, Pentatomidae), which is distributed throughout Europe including Turkey and preferentially feeds on umbels of Apiaceae. By its feeding, it causes injury to plant tissues, resulting in plant wilt and decreasing the quality of fruits and seeds (Schaefer and Panizzi, 2000; Yüce-Örs and Karsavuran, 2004). In spite of its importance of as a pest, comparatively little is known about the cellular anatomy of its alimentary canal (Yazdanian *et al.*, 2006). Recent studies have documented the morphology, ultrastructure, and different cell types of the digestive system of species in other orders (Çakıcı, 2008; Correia *et al.*, 2009; Rost-Roszkowska *et al.*, 2010; De Sousa *et al.*, 2013). In contrast, there are very few of examined species belonging to Hemiptera (Ghanim *et al.*, 2001; Zhong *et al.,* 2013).

The alimentary canal of insect is a tube of epithelium running a straight or convoluted course from mouth to anus. It is considered an effective physical and chemical barrier against the pathogens that are ingested with the feeding (Chapman, 1998; Levy *et al.*, 2004). Thus, the digestive tract of insects is thought to show physical alterations depending on feeding habits and food types. The digestive tract consists of three primary divisions. The foregut derived from the stomodeum, lined with cuticle continuous with that covering the surface of the body. The midgut is of endodermal origins, and the hindgut is derived from the proctodeum, again with a cuticular lining. All segments of the gut exhibit peristaltic and churning movements which serve to mix the contents and carry them along (Wigglesworth, 1965). There are regional differentiations and various evaginations throughout the gut. Anatomically, the buccal cavity is followed by the pharynx with an elaborate musculature concerned with the ingestion and deglutition of the food. Then follows the oesophagus which is usually a simple narrow tube leading to the midgut, as in Collembola and Hemiptera. The crop is located at the end of the foregut. The foregut is separated from the midgut by the cardiac valve, and at this point may be modified to form a muscular proventriculus. The midgut is in the form of a tube, and it is separated from the hindgut by the pyloric valve; when this is closed the hindgut receives only the contents of the Malpighian tubules (Wigglesworth, 1965; Chapman, 1998). When the contents enter, they are quite fluid, and the most obvious function of the hindgut in many insects is absorption of water as such in Thysanura, Dermaptera, Orthoptera and Neuroptera. The hindgut is divided into three sections; the anterior is the ileum, the middle portion, the colon, and the wider, posterior section is the rectum (Wigglesworth, 1965).

Structurally, the alimentary canal appears as a simple layer of epithelium, resting on a basement membrane, with a discontinuous layer of longitudinal and transverse muscles outside (Dow, 1987). The Orthoptera and Mecoptera which feed on solid food usually possess thick alimentary canals lined with the cuticular intima in foregut and hindgut (Liu and Hua, 2009; Wang *et al.*, 2012; Zhong *et al.*, 2015). In contrast, Hemiptera which have piercing-sucking mouth parts, and take in fluids, lack the cuticular layer (Chapman, 1998; Kerkut and Gilbert, 2013; Zhong *et al.*, 2015). Functionally, the foregut is of considerable importance in storage and digestion, but it does not play a significant role in absorption. The midgut is involved in the production and the secretion of digestive enzymes and the absorption of the nutrients. This process is different in each parts of the midgut. The anterior midgut has a great capacity of distention for storage of ingested fluids. The median and posterior midgut regions are involved in the synthesis and the secretion of digestive enzymes and nutrient absorption. The hindgut serves to reclaim useful substances before they are lost to the insect in faeces. Here absorption of water, salts and other beneficial substances takes place before excretion (Dow, 1987; Billingsley, 1990; Chapman, 1998).

Due to economic losses resulting from insect damage to crops, it is important to examine the digestive system of *G. lineatum*. The purpose of this study was to conduct a light, scanning, and electron microscopic analysis of the alimentary canal and salivary gland of *G. lineatum* to elucidate their structure so that their differences and

similarities can be recognized and identified at the ultrastructural level for comparison with those of other species causing economic damage.

MATERIALS AND METHODS

Sample preparation for light microscope (LM) investigation

Adult specimens of *G. lineatum* were field collected in Kazan, Kızılcahamam in Ankara. For light microscopic examinations, the digestive systems of ten adult males and females were dissected in a phosphate buffer (0.1 M, pH: 7.2) under a Leica EZ4D stereomicroscope. Samples were fixed in Bouin's solution for 24 h. The tissues were dehydrated in an ethanol progressive series (70, 80, 90 and 100%), embedded in histological paraffin, cut in to 6-7 µ thick sections by using a Microm HM 310 Microtome. The sections were stained with Hemotoxylin-Eosine and Mallory's Triple stain and examined under an Olympus BX51 microscope and subsequently photographed with an Olympus E330 digital camera.

Sample preparation for scanning electron microscopy (SEM)

For scanning electron microscopy, specimens were fixed in 2.5% glutaraldehyde (pH 7.2, phosphate buffered), rinsed three times with phosphate buffer, dehydrated in ascending series of ethyl alcohol (70, 80, 90 and 100%) and dried with a Polaron CPD 7501 Critical Point Dryer, then specimens were mounted by double sided tape on SEM stubs and coated with gold in a Polaron SC 502 sputter coater. The stubs were then examined with a JEOL JSM 6060 LV scanning electron microscope at accelerating voltage 5-10 kV and digital photos were taken.

Sample preparation for transmission electron microscopy (TEM)

For transmission electron microscopy, the alimentary canal of *G. lineatum* were fixed in 2.5% glutaraldehyde (pH 7.2, phosphate buffered) and postfixed in 1% OsO_4. Then OsO_4 was removed and the samples were rinsed three times with phosphate buffer. Then the samples were dehydrated in ascending series of alcohol (70, 80, 90 and 100%) and were embedded in Araldite. A Leica EM UC6 ultramicrotome was used for sectioning of the araldite block. Ultrathin sections were cut by using a Leica EM UC6 ultramicrotome and the sections were stained with uranyl acetate and lead citrate according to Reynolds (1963).The ultrathin sections were examined under the transmission electron microscope at 80-120 kV (JEOL JEM 1400) and digital photographs were taken.

RESULTS

Gross morphology of the digestive canal

The alimentary canal of *G. lineatum* consists of foregut, midgut and hindgut that are easily distinguishable (Fig. 1). The foregut and hindgut are very short but the midgut is longer and differentiated into three distinct sections: anterior, median and posterior. The foregut contains the pharynx, esophagus and proventriculus. The principal and accessory

salivary glands are connected to the foregut. There are two pairs of Malpighian tubules at the junction of midgut and hindgut. The hindgut consists of the pylorus and rectum.

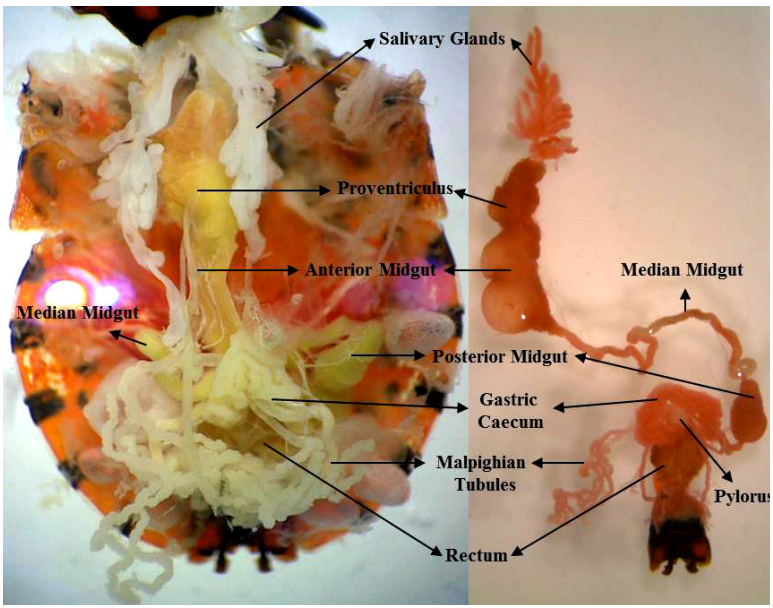

Fig. 1. Complete view of the alimentary canal in *Graphosoma lineatum*

Salivary Glands

A pair of principal and accessory salivary glands, which lie dorso-laterally and close to the end of the proventriculus, occur on both sides of the foregut of *G. lineatum* (Fig. 1). Each gland has a chitinous duct through which the gland empties into the mouth. The two principal salivary glands contain two distinct unequal sized lobes (anterior and posterior) (Figs. 2a-c). The anterior lobe, located close to foregut, is unilobar (Figs. 2b, c). The posterior lobe has many long digitate lobules that vary in size, disposition, length, and shape. It is branched in a single channel with a great number of finger-like protrusions on its margin (Figs. 2a, c). In SEM and TEM micrographs, the salivary gland is formed of a single-layer of epithelium which contains a membrane folding basally. There are microvilli on the apical region of cells. The lumen is filled with secretory vesicles (Figs. 2d-f). Nucleus with clumped heterochoromatin is present in the cytoplasm. Abundant mitochondria are visible at the apical region of the cell (Fig. 2f). Cells of the principal salivary glands are externally envelop by connective tissue cells.

The accessory salivary glands were extremely difficult to distinguish in the light microscopy section because of their small size and orientation. They are quite small and simple in structure when compared to the principal glands. They extend as a narrow tube that folded into an S shape (Figs. 3a, b). In SEM and TEM micrographs,

the accessory salivary glands are covered with a single-layer of epithelium that has basal membrane infoldings. A round space lined with cuticle is founded in the middle of accessory salivary glands (Figs. 3c, d). The cells of the accessory salivary glands contain microvilli and a great number of mitochondria between plasma membrane infolding as principal salivary glands cells (Figs. 3e-f). Heterochromatin, euchromatin and nuclear double membrane of nuclei are visible in cells of accessory salivary glands (Fig. 3e).

Proventriculus and Midgut

The proventriculus is the posterior part of foregut and is musculated. It is the most specialized part of the foregut and lies between pharynx and anterior midgut (Figs. 4a, b). The connection between the proventriculus and midgut is indistinguishable. In general, the proventriculus and all parts of the midgut have similar properties. The midgut is differentiated into three distinct sections: anterior, median, and posterior, each of which performs a different function during the digestive process. The anterior region is similar to a wide elongated sac (Figs. 4b, 5a). The median region is narrower and tubular in shape (Figs. 6a, b). The posterior region is a short dilated section (Figs. 7a, b). The proventriculus and midgut, which have roles in digestion of nutrients, are surrounded by trachea and muscles. Well-developed outer circular and inner longitudinal muscles were observed under the basement membrane (Figs. 4b, d; 5b; 6c, d; 7c). Both SEM images and cross section images illustrate that the outer and inner surfaces of the proventriculus and anterior midgut regions are indented (Figs. 4c-f). Cells of the inner surface are simple cylindrical epithelium and more uniform in length (Fig. 4c). Apical membranes of these cells are abundant in microvillus and there are many basement membrane invaginations (Fig. 5c). A continuous basal lamina lies beneath the epithelium (Fig. 5b). In the TEM images, these regions have a peritrophic membrane that surrounds the microvillus and extends to the lumen (Figs. 4g, 5d). Cells are rich in mitochondria especially under the microvilli and also abound with rough endoplasmic reticulum and lysosomes (Figs. 4g, f; 5d, g, h). In the lumen of the anterior midgut, there are a lot of symbiotic bacteria to aid digestion (Figs. 5e-f).

There are many spherocrystals that are rod-like or spherical in shape and different size lipid droplets. Numerous spherocrystals are characteristic of the whole midgut, with three membranous layers (Figs. 4g, h; 5g, h). The outer surface of the median region of the midgut is less indented than those of the proventriculus and anterior midgut regions (Figs. 6c-e). Cells of the median region of the midgut vary in height and have microvillus adjacent to the lumen (Figs. 6d-f). In addition, cells which have great numbers of vacuoles and different electron densities were observed (Fig. 6f). The posterior midgut assists absorption of water and nutrients before they can pass to the hindgut. Its outer surface is quite smooth (Figs. 7b-c). In the microscopy images, the thickness of the epithelial cells is thinner than those of previous parts (Figs. 7a, c). These cells contain plenty of lysosomes and lipid granules (Fig. 7d).

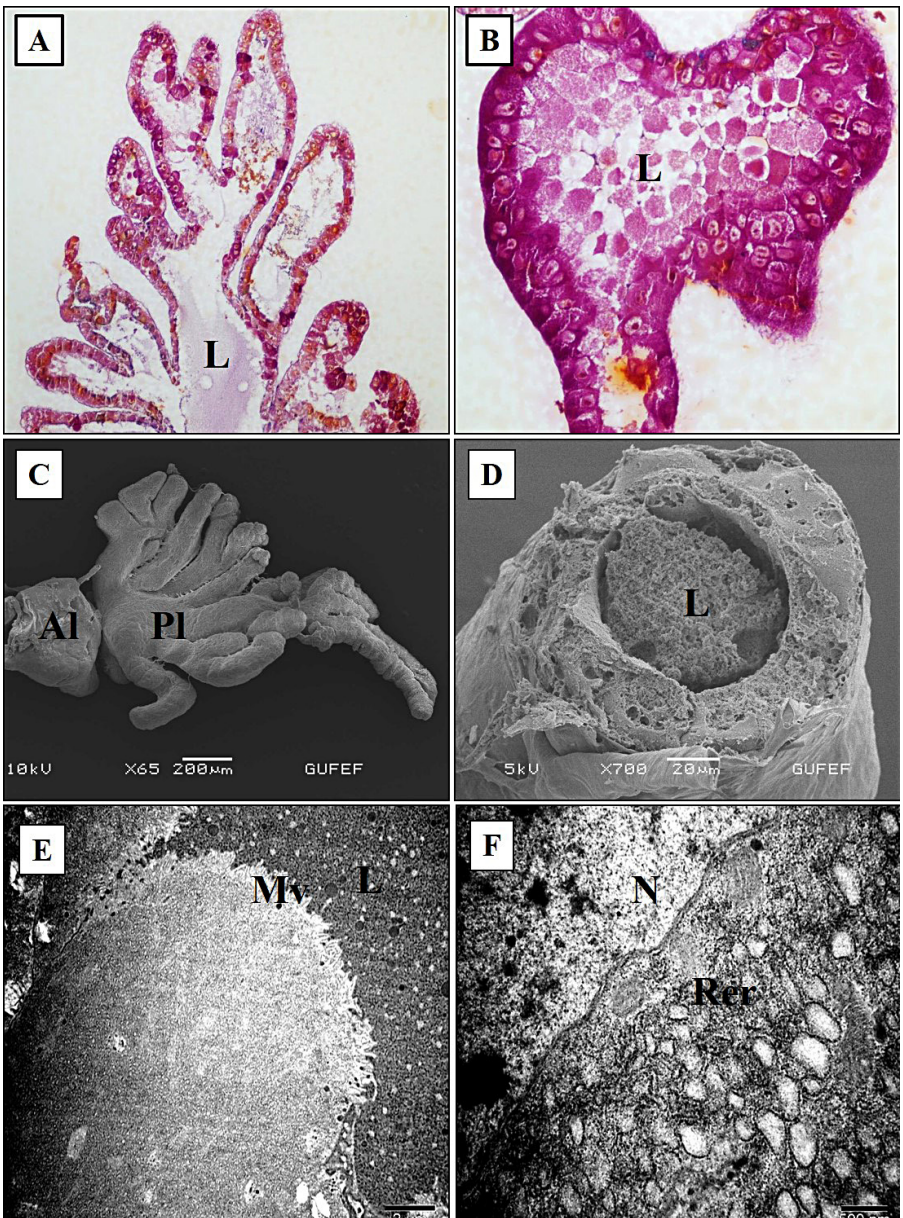

Fig. 2. Principal salivary glands of *G. lineatum*. A. Light micrograph of a longitudinal section of posterior lobe of the principal salivary glands. B. Light micrograph of anterior lobe of the principal salivary glands. C. The SEM micrograph of the principal salivary glands. D. The SEM micrograph of posterior lobes. E.-F. The TEM micrograph of cross section of principal salivary glands. Pl: Posterior Lobe, Al: Anterior Lobe, L: Lumen, Mv: Microvilli, N: Nucleus, Rer: Rough endoplasmic reticulum.

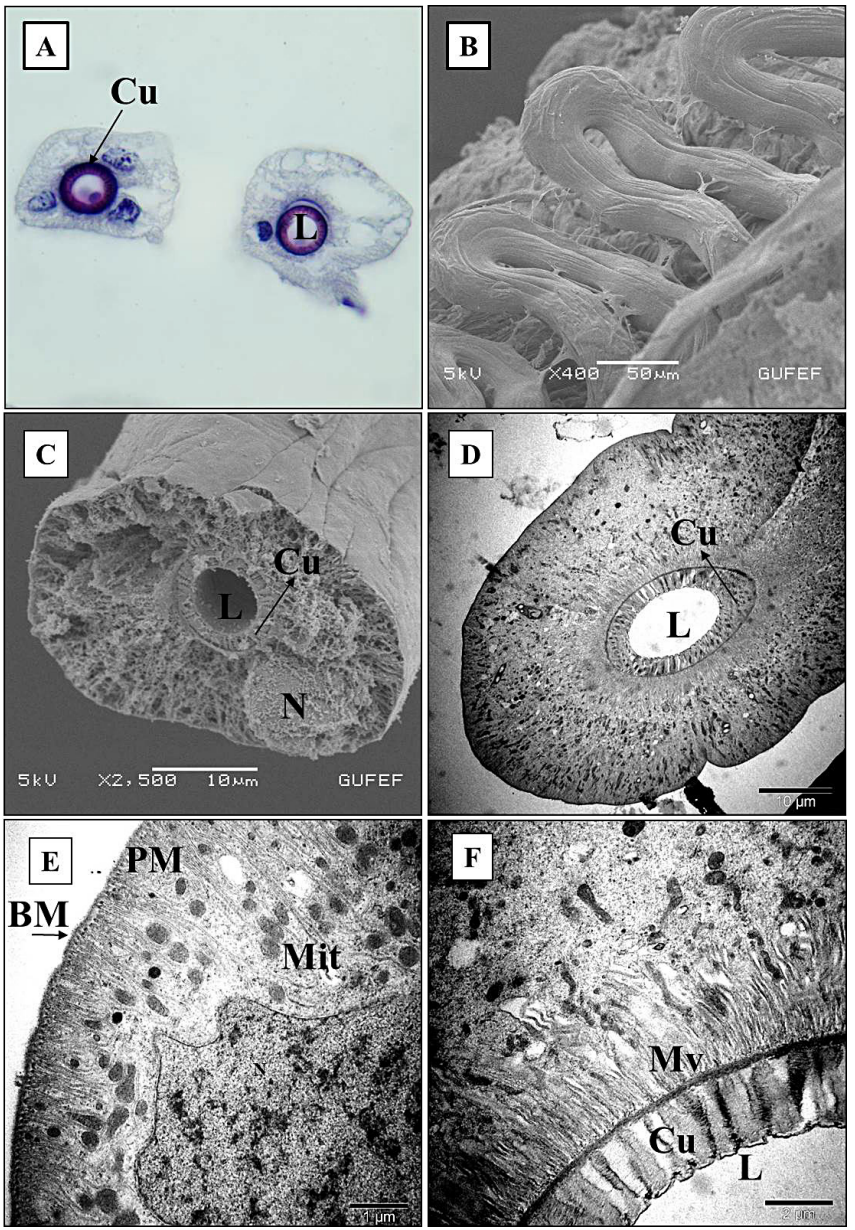

Fig. 3. Accessory salivary glands of *G. lineatum*. A. Light micrograph of a cross section of the accessory salivary glands. B. The SEM micrograph of the outer surface. C. The SEM micrograph of the inner surface. D.-F. The TEM micrograph of cross section of the accessory salivary glands showing the structure of cells. D. Over-all view. E. Plasma membrane foldings of the basal surface, lying beneath the basement membrane. F. Microvilli found under the cuticle. L: Lumen, Mv: Microvilli, N: Nucleus, Mit: Mitochondria, BM: Basement membrane, PM: Plasma membrane, Cu: Cuticle.

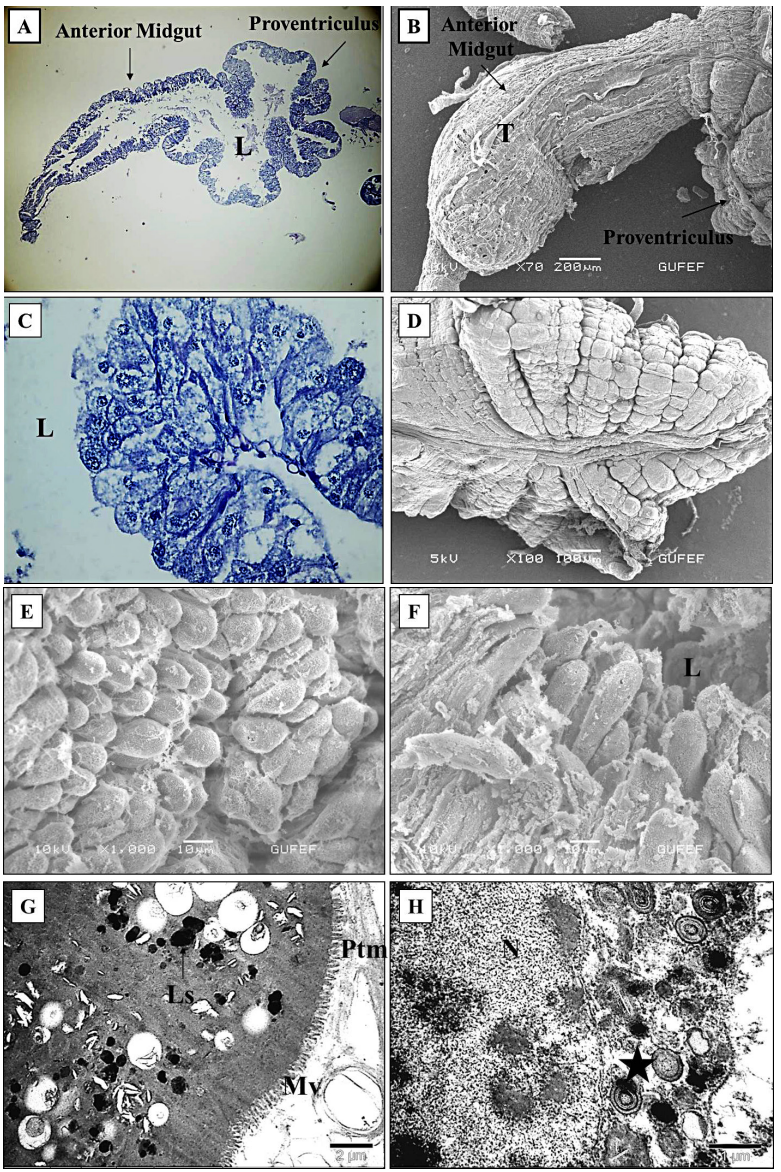

Fig. 4. Proventriculus of *G. lineatum*. A. Over-all view of the longitudinal sections of the proventriculus and the anterior midgut under a light microscope. B. The SEM micrograph of the proventriculus and the anterior midgut, surrounding by trachea. C. Light micrograph of a longitudinal section of the proventriculus. D. The SEM micrograph of the outer surface of the proventriculus. E.-F. The SEM micrograph of the inner surface of the proventriculus, shows that the cells were indented into the lumen. G.-H. The TEM micrograph of a cross section of the proventriculus. Surface of the microvilli, which are found at the apical region, are covered by a peritrophic membrane showing a great number of concentric circles (*). L: Lumen, Mv: Microvilli, N: Nucleus, Ptm: Peritrophic membrane, Ls: Lysosome, T: Trachea.

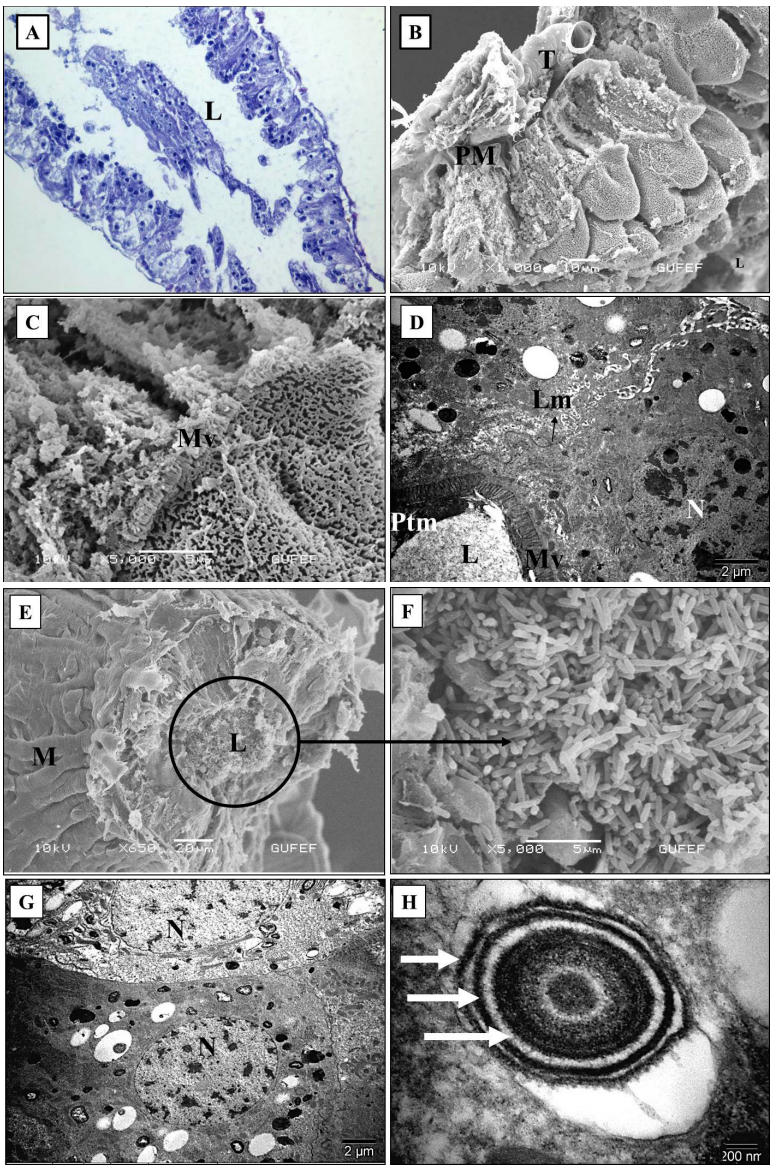

Fig. 5. Anterior midgut of *G. lineatum*. A. Light micrograph of a longitudinal section of the anterior midgut. B.-C. The SEM micrograph of the inner surface of the anterior midgut. Showing the trachea (T) surrounding outer surface, plasma membrane (PM) infoldings, microvilli (Mv), lumen (L). D. The TEM micrograph of the anterior midgut**.** Observed microvilli (Mv), nucleus (N), peritrophic membrane (Ptm), lateral membrane (Lm) and lumen (L). E.-F. The SEM micrograph of the anterior midgut which is covered with muscle (M). Lumen (L) is seen filled with bacteria. G.-H. The TEM micrograph of a cross section of the anterior midgut. Cells with different electron densities are observed. Magnification of the concentric circles (*) showing with three membranous layers.

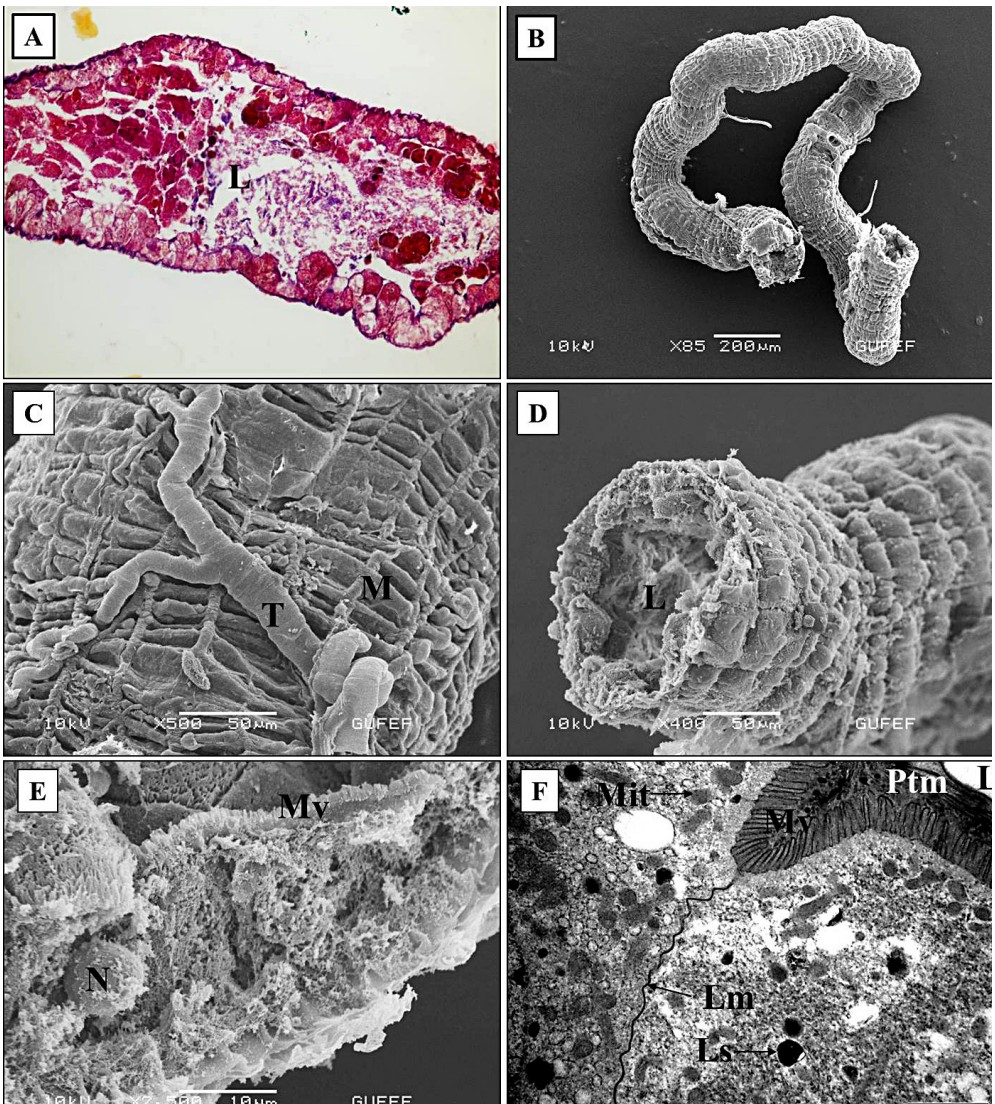

Fig. 6. Median midgut of *G. lineatum*. A. Light micrograph of a longitudinal section of the median midgut. B.-E. The SEM micrograph of the median midgut. B.-C. Shows the muscle (M) and the trachea (T) that covered the outer surface. D.-E. Illustrates the lumen (L), the microvilli (Mv) and the nucleus (N) that is found on the inner surface. F. The TEM micrograph of a cross section of the median midgut, showing the lateral membrane (Lm), well developed microvilli (Mv) extending to the lumen (L), lysosome (Ls) and mitochondria (Mit) are found in cells, peritrophic membrane (Ptm) is covered the microvilli at the apical membrane.

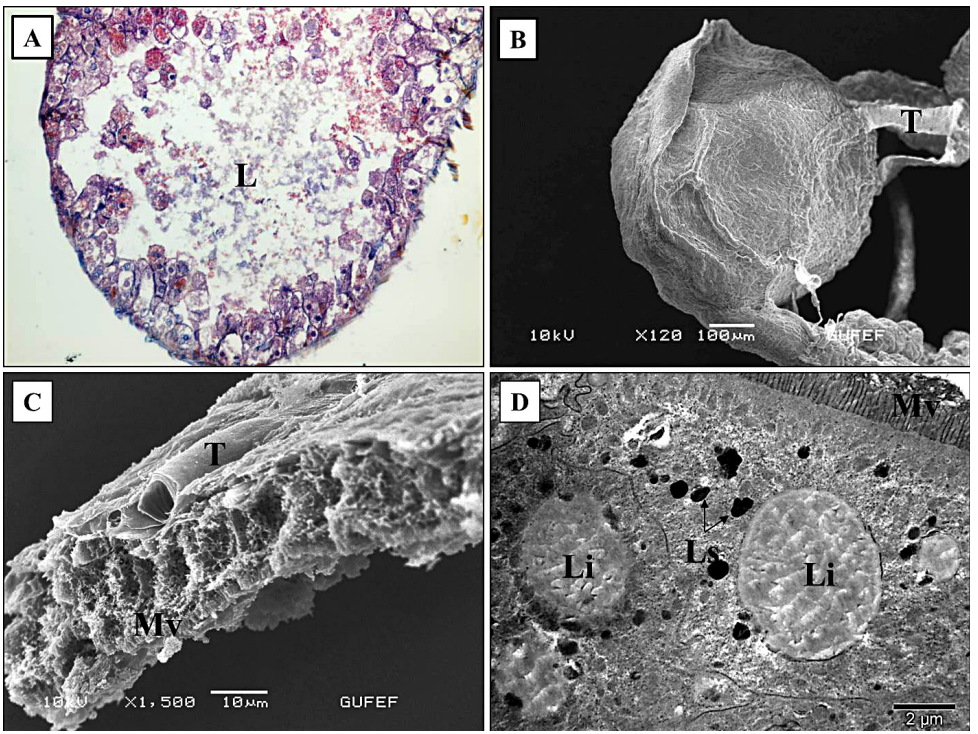

Fig. 7. Posterior midgut of *G. lineatum*. A. Light micrograph of a longitudinal section of the posterior midgut. B.-C. SEM micrograph of the posterior midgut. showing the trachea (T) surrounding the outer surface and microvilli (Mv) on the side of lumen (L). D. TEM micrograph of a cross section of the posterior midgut. illustrating the different sizes of lipid droplets (Li), lysosome (Ls) and microvilli (Mv).

Gastric Caecum

The gastric caecum is connected to the pylorus of the hindgut. It is apparently composed of four rows of plate like structures surrounding a tube (Figs. 8a, b). Each caecum is canal shaped and consists of a thin wall of epithelial cells (Figs. 8a, c). In TEM images, cells which have different electron densities show a monolayer alignment. There are a great number of infoldings in the basal membrane. Nuclei of the cells are oval-shape. Numerous mitochondria are concentrated around the nucleus (Fig. 8d). The caeca are filled with specific bacteria that help to digestive are observed in the cells (Fig. 8d).

Pylorus

The pylorus is the site of entry of the Malpighian tubules to the gut. Therefore, it is the zone in which food residue from the midgut and secretions coming from the Malpighian tubules are mixed. The pylorus, which is connected with the anterior region of hindgut is an enlarged smooth-surface region of the tract (Figs. 9a, b). The basal region of cells presented fewer plasma membrane infoldings in comparison to other parts of the

alimentary canal. Lateral membranes of cells are quite curvy. Numerous mitochondria, limited lipid droplets and variable electron density cells are observed in TEM cross sections. Microvilli are shorter compared with those in other regions (Figs. 9c-d).

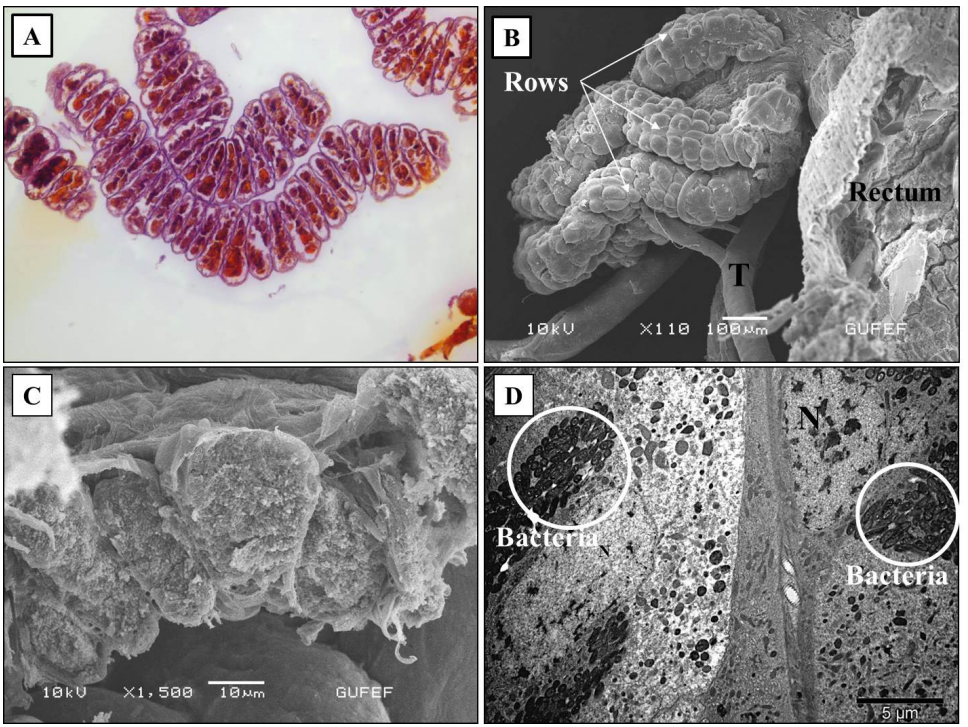

Fig. 8. Gastric caecum of *G. lineatum*. A. Light micrograph of a longitudinal section of the gastric caecum. B. The SEM micrograph of the gastric caecum which is distinguished by four rows. C. Bacteria which fill the gastric caecum. D. The TEM micrograph of a cross section of the gastric caecum. Cells with different electron densities are observed and a great number of bacteria are present.

Malpighian Tubules

The Malpighian tubules of *G. lineatum* are connected to the pylorus. This species has two pairs of Malpighian tubules; the end of each tubule is sealed (Fig. 9b). The closed end is located free in the body cavity and surrounded by tracheae (Fig. 10d). In this insect, the Malpighian tubules are structurally divided into two regions: proximal and distal (Figs. 10a-d). Tubule walls consist of a single layer of cuboidal epithelium (Fig. 10a). In SEM images, the outer surface of the proximal region of the Malpighian tubules is smooth and tube-shape, but the outer surface of the distal region is seen as a bead formed on the trachea (Figs. 10b, c, e). Malpighian tubule cells are also filled with large and small granules. The cells of Malpighian tubule on the side of lumen have numerous long microvilli (Fig. 10h). Malpighian tubules have well-developed basal invaginations containing numerous mitochondria. In the proximal region, bundle-shaped microvilli are observed. In a cross section of Malpighian tubules, it

is observed that the height of epithelial cells is less and the lumen is larger in the proximal region. In contrast in the distal region, the height of epithelial cells is greater and lumen is narrower (Figs. 10d, f, g).

Rectum

The rectum is a wide elongated sac located at the end of the hindgut. It becomes narrower and extends to the anus as a narrow tube (Figs. 11a, b). The wall of the rectum is covered with monolayer cubic-cylindrical epithelium in which deep infoldings are observed (Figs. 11c, e). In SEM micrographs, crystals of various sizes are seen in the lumen of the rectum (Figs. 11c-d). The inner surface of the rectum is lined by a cuticular intima that has sharply angled protrusions. Apical membranes of epithelial cells under the cuticle involve a small number of microvilli of different length and thickness (Fig. 11e). There are many basement membrane invaginations into cells and there is a basal lamina layer under them. Different shape and different sized mitochondria are observed among basal membranes (Fig. 11f).

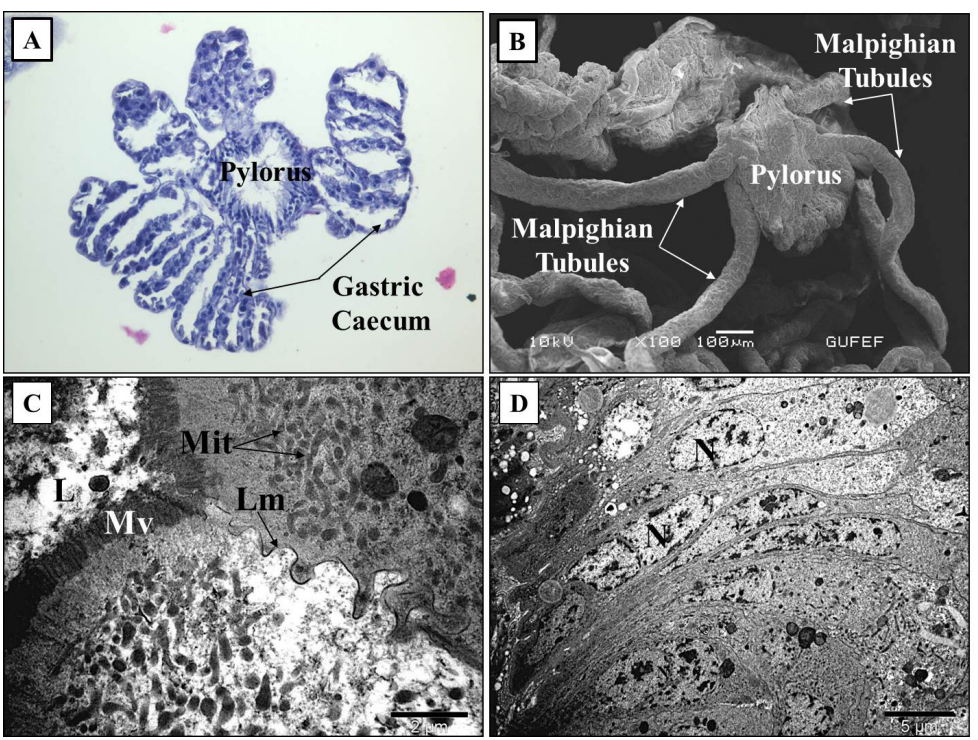

Fig. 9. Pylorus of *G. lineatum*. A. Light micrograph of a cross section of the pylorus. B. The SEM micrograph of the pylorus. Two pairs of Malpighian tubules are inserted on each side of it. C.-D. The TEM micrograph of a cross section of the pylorus. Cells with different electron densities are observed, showing the lateral membrane (Lm), nucleus (N), well developed microvilli (Mv) extending to the lumen (L), numerous mitochondria (Mit) are found in cells.

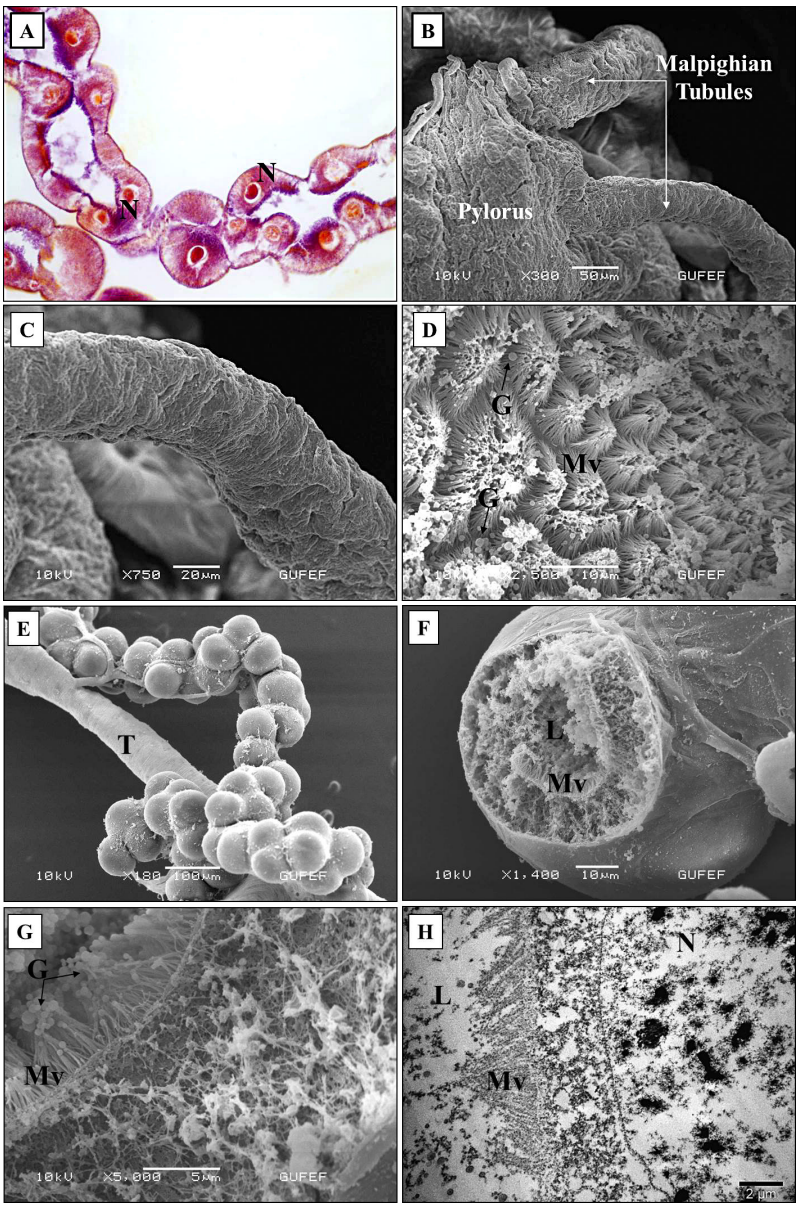

Fig. 10. Malpighian tubules of *G. lineatum*. A. Light micrograph of a longitudinal section of the Malpighian tubules. B.-C. A SEM micrograph of the proximal section. D. A SEM micrograph of the inner surface of the proximal section, showing the bundle-shaped microvilli (Mv). E. A SEM micrograph of the distal section and trachea (T). F.-G. A SEM micrograph of inner surface of the distal section illustrating the microvillus (Mv), Lumen (L), granules (G) in both lumen and epithelium. H. A TEM micrograph of a cross section of the Malpighian tubules. L: Lumen, Mv: Microvilli, N: Nucleus.

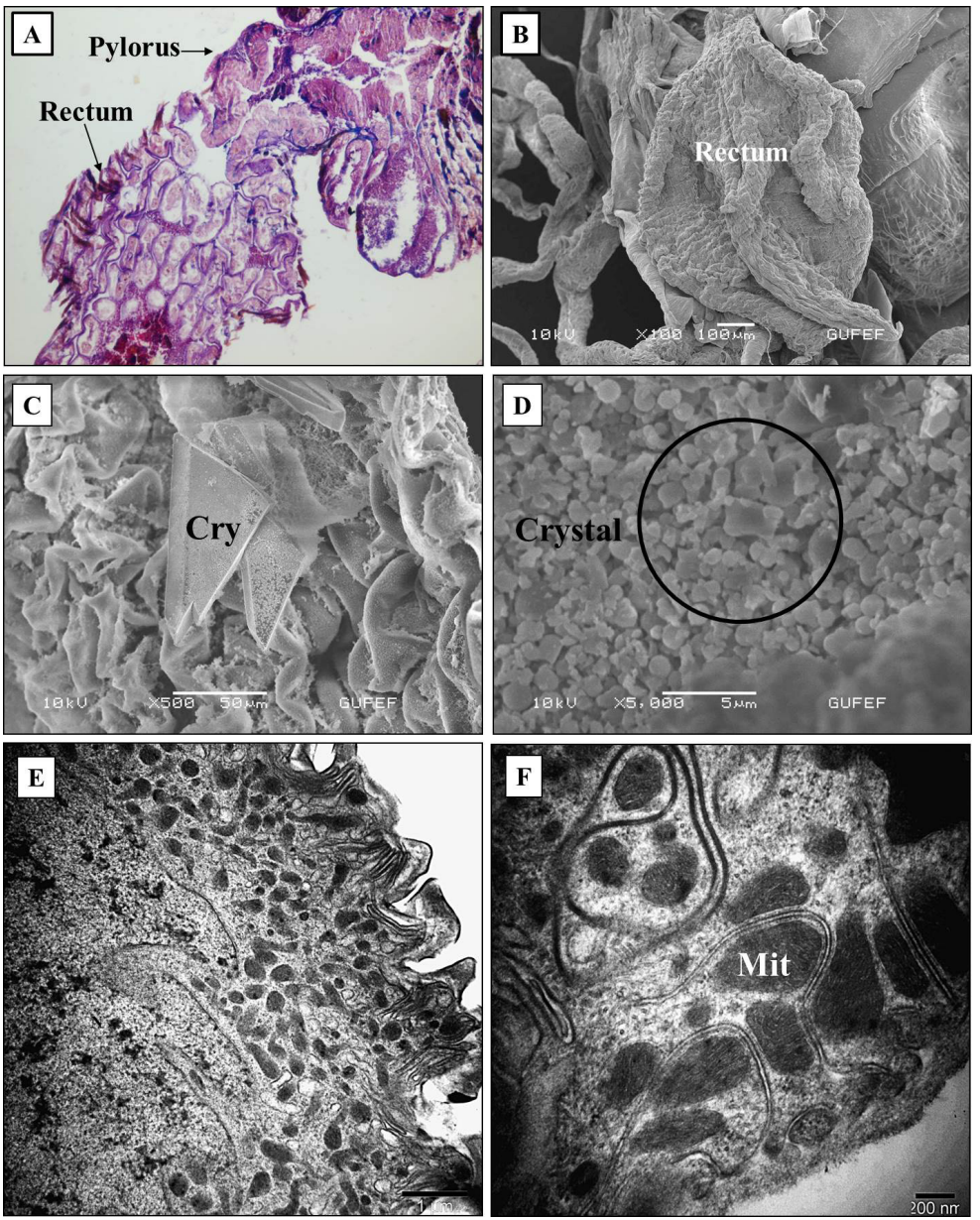

Fig. 11. Rectum of *G. lineatum*. A. Light micrograph of a longitudinal section of the rectum. B. A SEM micrograph of the outer surface of the rectum. C.-D. A SEM micrograph of the inner surface of the rectum. Crystals (Cry) of various sizes are present in the lumen.

DISCUSSION

The digestive system of insects is a long enclosed tube called the alimentary canal that extends from mouth to anus. The primary functions of the alimentary canal are digestion, absorption, production and elimination of the faeces. The digestive system is generally divided anatomically into three different regions the foregut, midgut and hindgut. The foregut is a region in which the food may be stored, filtered, and partially digested. The midgut is the primary site for digestion and absorption of food. In the hindgut occurs some absorption and faeces formation. The foregut is generally considered to consist of four sections; the pharynx, the oesophagus, the crop and the proventriculus. It is also known as the stomodaeum. The foregut of *G. lineatum* is similar to that of most insects but lacks a crop presumably lost because the organism feeds on stem and doesn't need teeth in a crop to break up food particles. The crop is lacking in most Hemiptera, but in some Heteroptera, the anterior midgut is dilated and forms a storage organ (Chapman, 2013).

The midgut consists of a ventriculus and a gastric caeca (Caecum). The ventriculus is a region where most digestion occurs. The gastric caecum provides greater area for digestion. The midgut secretes several enzymes including protease, lipase, amylase and invertase. The hindgut consists of the ileum, rectum and anus. The ileum conducts undigested food to the rectum for final processing. The rectum absorbs ions, water and small organic molecules (Chapman, 1998).

In addition, a pair of salivary gland and two pairs of Malpighian tubules are attached to the digestive tract (Yazdanian *et al.*, 2006). The salivary glands are associated with the foregut and may be important in food intake but usually not in digestion. The most important enzyme secreted by the salivary glands is amylase. In insects; the function of salivary glands is to moisten and, dissolve food as well as lubricating the mouthparts. This saliva may contain an array of compounds associated with blood intake or may be used as a fixative of the stylets of sap-sucking bugs as a *G. lineatum* (Baptist, 1941; Chapman, 1998; Ghanim *et al.*, 2001; Reis *et al.*, 2003).

The number and shape of salivary glands may vary among species as seen in studies of the salivary glands of Heteroptera. The posterior region of salivary glands of *G. lineatum* is composed of finger-like protrusions similar to those reported for *Solubea pugnax* (Hamner, 1936), but the anterior region differs from that of other species (Cecil, 1930; Barber *et al.*, 1980; Reis *et al.*, 2003). Muscles were observed covering the salivary gland epithelium. A TEM analysis of the principal salivary glands reveals a dense cytoplasm with vesiculation, a large number of electron-dense cells and rough endoplasmic reticulum. A large amount of secretion is found in the lumen. Within the accessory salivary glands, the columnar cells have a homogeneous cytoplasm. Its epithelial cells are lined with a narrow circular lumen, which is empty as in other heteropterans (Baptist, 1941). Mitochondria and length of microvilli of accessory salivary glands are more than principal salivary glands. Among species differences in salivary glands appear to be important (Baptist, 1941; Castro *et al.*, 2013). The foregut and hindgut of *G. lineatum* are short. The midgut is longer and is divided into

three parts. The anterior and posterior regions of the midgut are enlarged, while the median region is tubular. This situation is also seen in other species of Heteroptera (Hamner, 1936; Harris, 1938; Barber *et al.*, 1980; Postle and Woodward, 1988; Habibi *et al.*, 2008; Bandani *et al.*, 2010).

In insects, the proventriculus is a very variable in form. It often occurs as a simple valve at the proximal end of the midgut, projecting a short distance into the midgut lumen. It is reported to separate the partly digested food in the midgut from food in the crop (Chapman, 1998; 2013). It has been found that the proventriculus belongs to foregut in different insect orders. The proventriculus of *Zacryptocerus rohweri* (Hymenoptera, Formicidae) was examined because of pollen-feeding, thorns are seen in it (Roche and Wheeler, 1997). The proventriculus of *Hypothenemus hampei* (Coleoptera, Curculionidae, Scolytinae) contains eight tooth-like structures (Rubio *et al.*, 2008). Studies by Ahmad and Afzal (1978) revealed that the proventriculus of *Catacanthus incarnatus* (Heteroptera, Pentatomidae) consisted of a small spherical sac. In contrast, tooth-like structures were not observed in *G. lineatum*'s proventriculus. However, apical regions of cells protruding into the lumen or cells containing single layer cylindrical epithelium are seen as other species (Ahmad and Afzal, 1978; Barber *et al.*, 1980).

The tubular part of the midgut is known as the ventriculus. Many insects bear a single caecum, usually at the proximal end of the midgut, such as in Gryllidae while some dipteran larvae have two caeca; Acrididae have six; cockroaches and larval Culicidae have eight and some Coleoptera and Heteroptera have multiple caeca that are variable in position. The cells of the midgut are actively involved in the production and secretion of digestive enzymes and the absorptions of the nutrients. For this reason, the lumen of the midgut is covered with a peritrophic membrane. The peritrophic envelope can be categorized as either Type 1, produced by the entire length of the midgut, or Type 2, produced by specialized tissues within the anterior midgut, but there is much variation within each type. The role of the peritrophic envelope in the mechanical protection appears self-evident; it also protects against many potential microbial pathogens (Chapman, 1998).

A four segmented midgut has been reported in some Pentatomorpha bugs such as *Dysdercus peruvianus* (Silva *et al.*, 1995; Terra and Ferreira, 2012). In contrast, some Pentatomorpha and Cimicomorpha bugs have been reported to have two or three regions in their midguts (Billingsley, 1990; Azevedo *et al.*, 2009; Fialho *et al.*, 2009). The authors found that the midgut of *G. lineatum* consist of three distinct regions. The main midgut cells of *G. lineatum* are columnar and contain cells with different electron densities. These cells are structurally differentiated according to the region of the gut, especially, in terms of the shape, size, number and type of vesicles; the length and abundance of microvilli may vary within each region of the midgut (Lehane, 1998). Santos *et al.*, 1984 and Brown *et al.*, 1985 characterized different epithelium cells along the midgut of *Erinnyis elio* (Lepidoptera, Sphingidae) and *Aedes aegypti* (Diptera, Culicidae). Mehrabadi *et al.*, 2012 reported the main midgut cells

of *Eurygaster integriceps* as being columnar and endocrine cells. In heteropteran species, there is no cuticle layer covering the midgut epithelium. Cells are simple cylindrical cubic epithelium. Lengths of microvilli may change throughout the midgut (Cecil, 1930; Hood, 1937; Cheung and Marshall, 1982; Boonsriwong *et al.*, 2007). Our observations of *G. lineatum* reveal a well-organized and extensive brush border of microvilli occurs adjacent to the lumen in the epithelial cells, and microvilli length change throughout the midgut. In addition, rough endoplasmic reticulum and secretory vesicles are found in the midgut cells. In our SEM micrograph, bacteria which have a digestive function are observed in the anterior midgut.

Caecal bacteria were first observed in crushed specimens of the chinch bug, *Blissus leucopterus* (Hemiptera, Blissidae). Researchers studying the structure of gastric caecum observed a great number of bacteria in the gastric caeca of various Heteropteran species (Glasgow, 1914; Hirose *et al.*, 2006). These researchers observed that these bacteria were not only always present in the caeca of adult bugs, but also present in the alimentary canal during all stages of development. In a similar study, Hirose *et al.*, 2006 investigated the gastric caecum of *Nezara viridula* (L.) (Heteroptera, Pentatomidae) and found and named the bacteria *Pantoea* sp. The gastric caecum which is distinguished by the four rows, is always full of bacteria in this and other Heteroptera. Similarly, the gastric caecum of *G. lineatum* has four rows and the bacteria were always present in the caeca.

The Malpighian tubules serve as the main excretory organs of insects. The tubular system consists of branching tubules closed at one end extending from sides of the alimentary canal that absorbs solutes, water, and wastes from the surrounding hemolymph (Srivastava and Bahadur, 1961; Gaino and Rebora, 2000a, 2000b). The tubules are connected to the intestines through a transition region that runs from the midgut to the hindgut; they then connect to the hemocoel and deliver fluids to the hemolymph. The basic function of the Malpighian tubule involves the transport of fluids from the hemolymph to the lumen (Rigoni and Conte, 2014). The wastes then are released from the organism in the form of solid nitrogenous compounds (Chapman, 1998). The physiology and structure of insect Malpighian tubules can be altered by the presence of parasites, which include viruses, bacteria, protozoans, nematodes, and entomophagous insects (Rigoni and Conte, 2014).

The Malpighian tubules may be present in variable numbers in different orders of the class Insecta, for example, in Odonata the number of the Malpighian tubules varies between 50 and 60 tubules in Hemiptera from 1 to 4, in Lepidoptera that number is 6, in Hymenoptera they range from 12 to 150, in Orthoptera from 2 to 200, while the Diptera have only 4 tubules. In many species, the tubules are separated into morphologically different regions and each region is homogeneous for one cell type (Wigglesworth, 1965; Pacheco *et al.*, 2014). The number and the thickness of Malpighian tubules varies among different insect orders (Gaino and Rebora, 2000a, 2000b; Arab and Caetano, 2002). Malpighian tubules of *Tibicen* (Hemiptera, Cicadidae) and *Magicicada* (Hemiptera, Cicadidae) are bead-shaped and each segment is of

different length (Rakitov, 2002). According to Xie *et al.* (2011), pores are found on the outer surface of Malpighian tubules of *Ceroplastes japonicus* (Hemiptera, Coccidae) and well-developed microvilli are seen at the side of lumen. Compared with other studies, there are only four Malpighian tubules in *G. lineatum* and these are proximally paired. These tubules are separated into two segments of unequal length. In our observation, these tubules start as a tube, than continuous bead-shaped and lying free in abdominal cavity. The ultrastructure of the Malpighian tubules of *G. lineatum* has been researched in detail by Kalender (1997).

The rectum is the final section of the gut. The rectum's function is to absorb water from the faeces before they exit the body. Absorption in the rectum is a key component of the regulation of the body water (Chapman, 1998). The rectum of *G. lineatum* is similar that of other species of Hetroptera including Pentatomidae (Cecil, 1930; Harris, 1938; Barber *et al.*, 1980; Ghanim *et al.*, 2001). The anterior end of the rectum is formed by an extension of the pylorus and the posterior end tapers to form the anus. A well-developed rectum has an epithelial cell layer and a thin cuticular intima as seen in the rectum of *Eurygaster integriceps* (Mehrabadi *et al.*, 2012). The cells of the rectum of *Philaenus leucophthalmus* (Hemiptera) are relatively small and uniform, and there is very little variation in the thickness of the chitinous lining (Cecil, 1930). By contrast, the thickness of the epithelial layer in *G. lineatum* increases in width in the region of the nucleus. Instead of multiple microvilli, intense infoldings are seen at the apical regions of cells, such as observed in *Carpocoris pudicus* (Heteroptera, Pentatomidae) (Metin, 2014).

In summary, insect digestive system is structurally complex and varied from species to species. The gut is both an important and major barrier for insect control agent. Understanding of the cellular and morphological structure and processes of insect gut will help for getting under control of the pests. Therefore, we have described for the first time the ultrastructure and morphology of the alimentary canal of *G. lineatum.* The general structure of the alimentary canal of *G. lineatum* exhibits similarity to that of previously described species in Heteroptera. However, some morphological ultrastructural differences are also found within Heteroptera. The similarities and differences of the alimentary canal among other species of Heteroptera might be informative for taxonomic and phylogenetic analysis of Hemiptera and also other hemipterans. These results show that these bugs have an alimentary canal found in Heteroptera. When differences of histological and morphological structures are paid attention, this study obtains the key information for future research into digestive system biology, ecology and biological control agents of the Heteroptera. Also, it contributes to the knowledge of the alimentary system of Heteroptera.

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