

Morphology and Histology of the Alimentary Canal of *Epiphaneus malachiticus* Boheman, 1842 (Coleoptera, Curculionidae)

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Abstract—This study is aimed at describing the morphological and histological structures of the alimentary canal in the adults of *Epiphaneus malachiticus* Boheman, 1842 (Coleoptera: Curculionidae), a poorly investigated species of *Curculionidae*. The alimentary canal of adult *E. malachiticus* was isolated, processed, and analyzed using stereo microscope, light microscope, and scanning electron microscope. The alimentary canal of *E. malachiticus* is morphologically and histologically divided into foregut, midgut, hindgut, and tubular structure. The foregut has pharynx, esophagus, crop which has histological layers like lumen, intimal layer, epithelium, and outer muscles. The midgut is divided into the anterior midgut and posterior midgut. The former is wide; the diameter of the latter is two-thirds of that of the former. Their wall is surrounded with muscularis externa and inner columnar epithelium. The hindgut has pylorus, ileum, colon, and rectum with such histological layers as lumen, intima, epithelium, and muscles. The Malpighian tubules are long and connected to the pylorus, they have a single layer of cuboidal epithelium. The salivary glands are absent in this species.

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In the Coleoptera, about 140000 species are phytophagous and most of these are in two superfamilies, the Chrysomeloidea and the Curculionoidea (Chapman, 2007). One of the largest groups of beetles is the Curculionoidea, an immense assemblage of more than 60000 described species arranged in 10 families (Lawrence, 1982). Weevils (Curculionidae) are a very diverse phytophagous beetle family (Steiner et al., 2016) including many serious agricultural pests damaging crops, stored grain and seeds (Casem, 2016).

Similar to other insects, the coleopteran alimentary canal is a large tube which occupies a large area in the insect body (Wigglesworth, 1973). It is generally divided into three regions: the foregut which is of ectodermal origin, the midgut which is of endodermal origin and the hindgut which is also of ectodermal origin (Sinha, 1958; Snodgrass, 1993; Rubio et al., 2008; Aldiagil et al., 2013; De Sousa et al., 2013). All these regions are involved in ingestion, storage, digestion, and absorption of food and maintenance of water balance (Borror et al., 1976; Calder, 1989; Romoser and Stoffolano, 1998).

In coleopterans, the generally short straight and tubular foregut which is the first part of the digestive system, consists of a short pharynx, a narrow tubular esophagus, crop, and proventriculus (Ekis and Gupta, 1971; De Sousa et al., 2013; Sarwade and Bhawane, 2013). In many insects, the crop is positioned between the esophagus and proventriculus and is called ‘the storage organ’ (Crowson, 1981; Snodgrass, 1993; Sarwade and Bhawane, 2013). The intima covering the proventriculus is highly developed, densely covered with usually coarse spicules and responsible for ingesting, transporting, storing, grinding, and filtering particles of food (Sinha, 1958; De Sousa et al., 2013). The noncellular intima is homologous to the external body cuticle. This layer is secreted by the epidermal cells (Sinha, 1958). The midgut is the widest part of the alimentary canal (Sinha, 1958). It is well established that the midgut of insects secretes most of the digestive enzymes and is the principal site of digestion (Gilmour, 1960; Dadd, 1970; Wigglesworth, 1972; Sarwade and Bhawane, 2013). The insect midgut includes three types of epithelial

cells: columnar, goblet, and regenerative cells showing variation in functions in various insects (Lewis, 1926; Waterhouse, 1952; Wigglesworth, 1965; Sarwade and Bhawane, 2013). The hindgut is subdivided into the pylorus, ileum followed by colon and rectum, and ending exteriorly with the anus. The hindgut is lined with a thin layer of permeable cuticle which avoids loss of useful substances (Sinha, 1958; Maddrell and Gandiner, 1980; Sarwade and Bhawane, 2013). The Malpighian tubules are excretory organs in insects arising at the junction of midgut and hindgut (Sarwade and Bhawane, 2013). The Malpighian tubules become closely associated with the rectal pads, so that the two water absorbing systems are placed in series. This system is also found in coleopterans (Sarwade and Bhawane, 2013).

Recently, the morphology and histology of the alimentary canal of different coleopterous family groups have been investigated (Sinha, 1958; Díaz et al., 1998; Díaz et al., 2000, 2003; Rubio et al., 2008; Aldiagil, 2013; De Sousa et al., 2013; Sarwade and Bhawane, 2013; Singh and Prasad, 2013). However, very few studies on the alimentary canal of Curculionidae are present in the literature. Thus, in this study we described in detail morphological and cellular structures of the alimentary canal in *E. malachiticus* adults. Knowledge of alimentary canal morphology and histology of *E. malachiticus*, which is an economically important species, is essential for better understanding of the feeding habits of this insect, the alimentary system of Curculionidae, and for the development of pest control strategies.

MATERIALS AND METHODS

Stereomicroscope (SM). In this study, adult *E. malachiticus* were collected from Özbağ, Kırşehir, Turkey in May 2018. Twenty insects were anesthetized with ethyl acetate. The alimentary canal of each specimen was dissected, its morphology was investigated under a Leica SZX7 stereomicroscope and photographed.

Light microscope (LM). For histological investigations, the alimentary systems were dissected and fixed for 24 hours in formalin liquid. Next, the tissues were dehydrated in an ethanol progressive series (70, 80, 90, and 100%), cleared in xylol, embedded in histological paraffin, and cut in to 6–7 µm-thick sections by using a Microm HM 310 microtome. The sections were stained with hematoxylen and eosine (H & E), examined under an Olympus BX51 microscope, and photographed.

Scanning electron microscopy (SEM). For scanning electron microscopy, specimens were fixed in 2.5% glutaraldehyde (pH 7.2, phosphate buffered), rinsed three times in phosphate buffer, dehydrated by using an ethanol progressive series (70, 80, 90 and 100%). The tissues were dried with Hexamethyldisilazane (HMDS), then mounted by double sided tape on SEM stubs and coated with gold in a Polaron SC 502 sputter coater. The stubs were examined with a JEOL JSM 6060 LV SEM at accelerating voltage 5–10 kV, and digital photos were taken.

RESULTS AND DISCUSSION

The three parts of the *E. malachiticus* gut are easily distinguishable (Figs. 1A, 1B) and similar to those in other coleopterans (Sinha, 1958; Díaz et al., 1998, 2000, 2003; Rubio et al., 2008; Bu and Chen, 2009; Aldiagil et al., 2013; Sarwade and Bhawane, 2013; Sing and Prasad, 2013). The alimentary canal, which is a tubular, cylindrical structure, is held up properly in the body cavity with fine tracheae and tracheoles (Figs. 1A, 1B). The foregut and midgut are short, and the hindgut is longer. The midgut is differentiated into the clearly distinct anterior and posterior sections (Figs. 1A, 1B). The foregut begins at the mouth followed by the pharynx and the esophagus (Fig. 1B). This region is responsible for the transport of food particles to the crop (Figs. 1C, 1D) whose function is the temporal storage of food. The crop is thin, muscular, sac-like, and covered with simple epithelium which forms internal folds (Figs. 1E, 1F). The crop epithelial cells are covered with the intima which is non-cellular (Figs. 1E, 1F).

The proventriculus is the terminal part of the foregut. It is highly muscularized and sclerotized externally (Figs. 1C, 1D). The proventriculus usually consists of 8 heavily sclerotized chitinous basal plates each bearing 2 longitudinal brushes which extend to most of the length of the basal blade (Figs. 2A, 2B). The sclerotized plates are composed of anterior and masticatory plates. The former has transverse rows of spines. The masticatory plate is composed of stopping bristles (Fig. 2B). These sclerotized plates are located at the edge and function as gizzard and filter. The muscles are responsible for constriction of the proventriculus during the mortaring and filtering food as can be seen in Figs. 1C, 1D and 2A, 2B. Similar structures were also described by Díaz et al. (1998, 2000, 2003), Bu and Chen (2009) and De Sousa

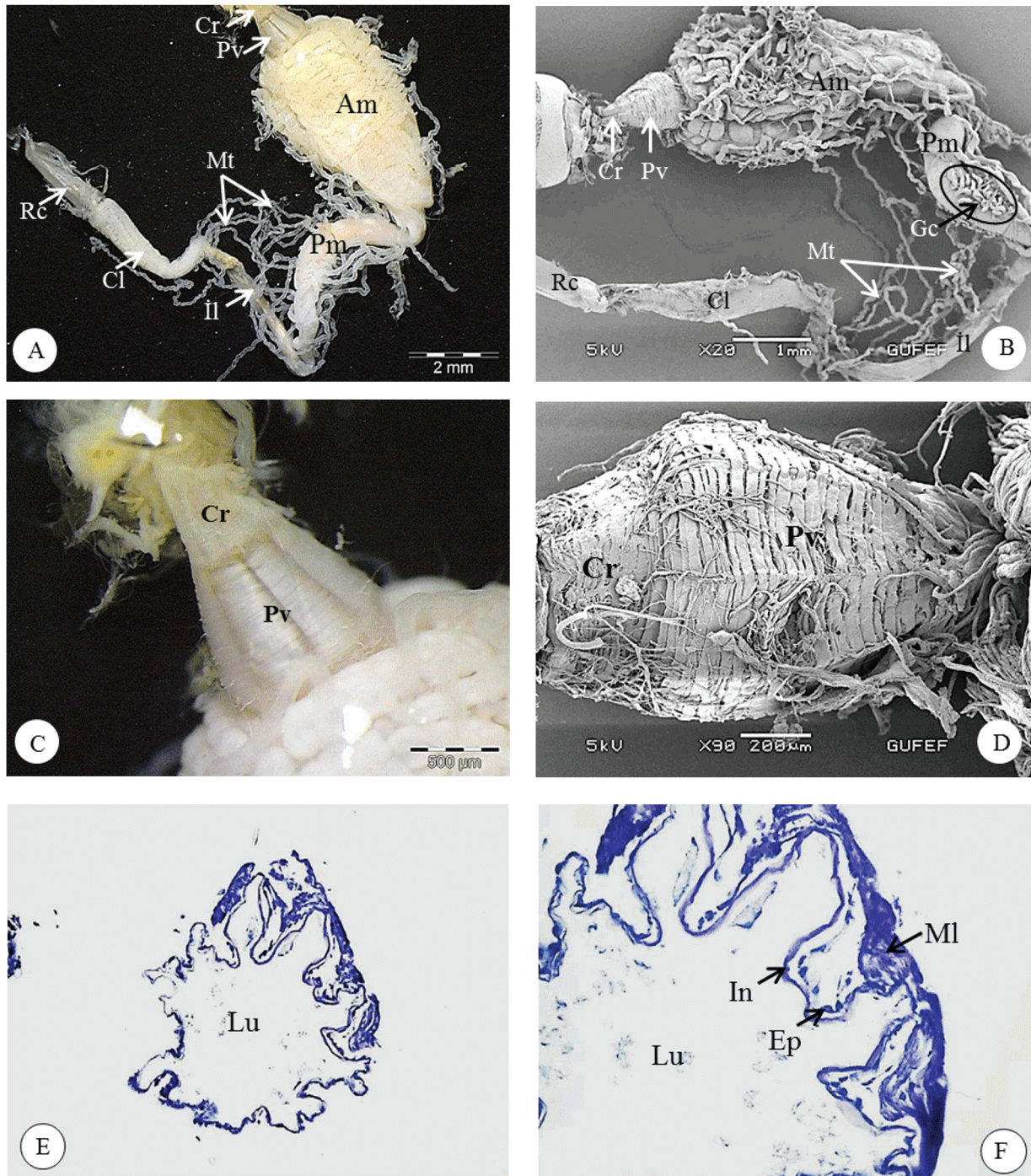


Fig. 1. *Epiphaneus malachiticus* Boh., adult: (A, B) general structure of the alimentary canal (SM, SEM), (C, D) the crop and proventriculus in foregut (SM, SEM), (E, F) the cross section of the crop (LM) (H & E) ($\times 100$, $\times 200$). (Am) anterior midgut, (Cl) colon, (Cr) crop, (Ep) epithelial cells, (Gc) gastric caeca, (Il) ileum, (In) intima, (Lu) lumen, (Ml) muscular layer, (Mt) Malpighian tubules, (Pm) posterior midgut, (Pv) proventriculus, (Rc) rectum.

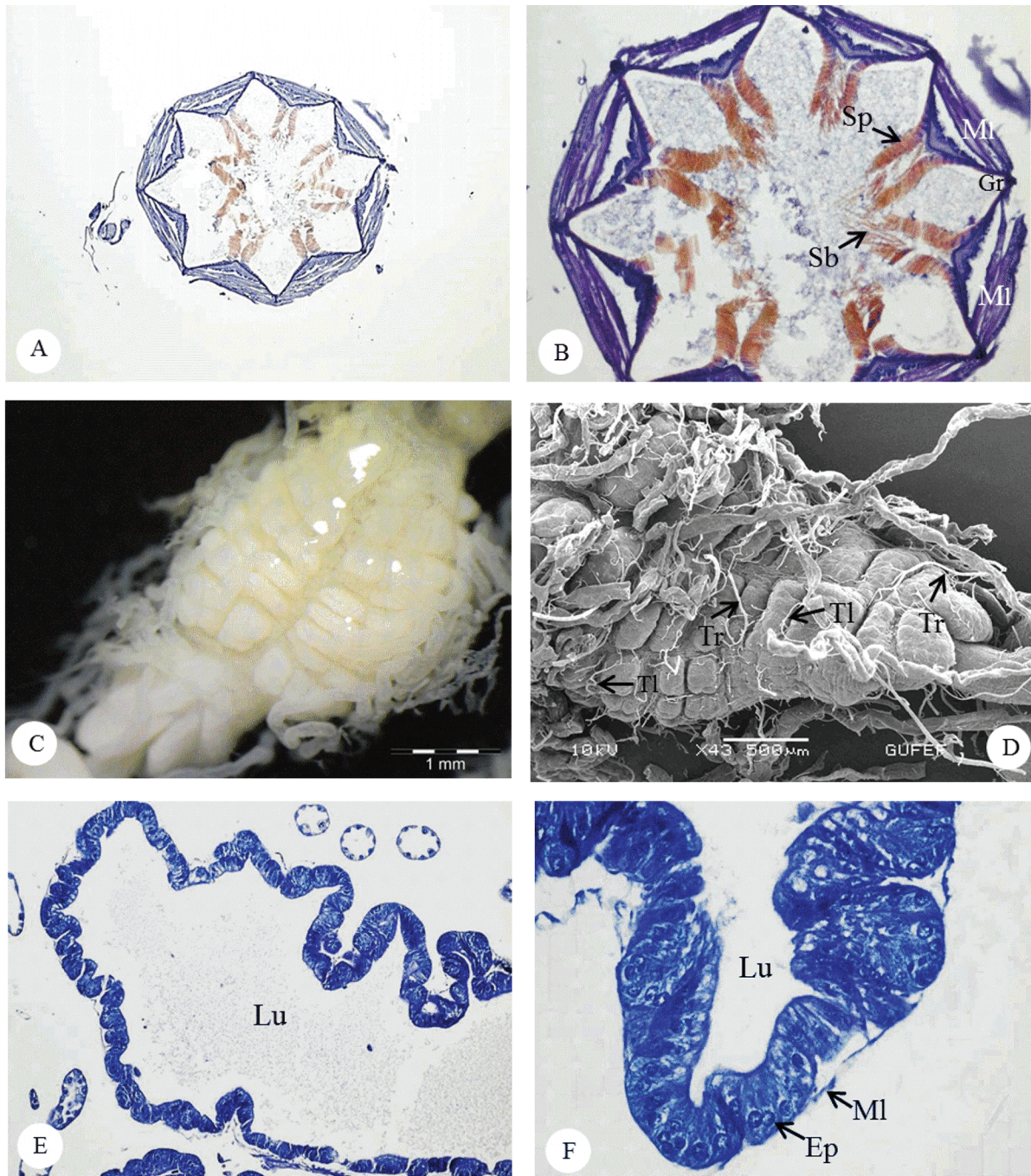


Fig. 2. *Epiphaneus malachiticus* Boh., adult: (A, B) the cross section and the sclerotized plates of the proventriculus (LM) (H & E) ($\times 40$, $\times 100$), (C, D) tracheae (Tr) and tracheoles (Tl) on anterior midgut surface (SM, SEM), (E, F) longitudinal section of the anterior midgut (LM) (H & E) ($\times 100$) ($\times 400$). (Gr) groove, (Sb) stopping bristles, (Sp) spines. Other designations as in Fig. 1.

et al. (2013). Additionally, the proventriculus outer surface has numerous tracheae and tracheoles (Fig. 1D).

The foregut of *E. malachiticus* consists of two layers. The inner layer of the foregut has a single-layered epithelium of epidermal cells with oval rounded nuclei.

The outer layer of the foregut consists of muscles. On the outer surface of the lumen, a chitinous inner lining, the intima of which is thrown into folds (Figs. 1E, 1F and 2A, 2B) is seen, but in *Platynotus belli* Fabricius (Coleoptera: Tenebrionidae) the foregut epithelium

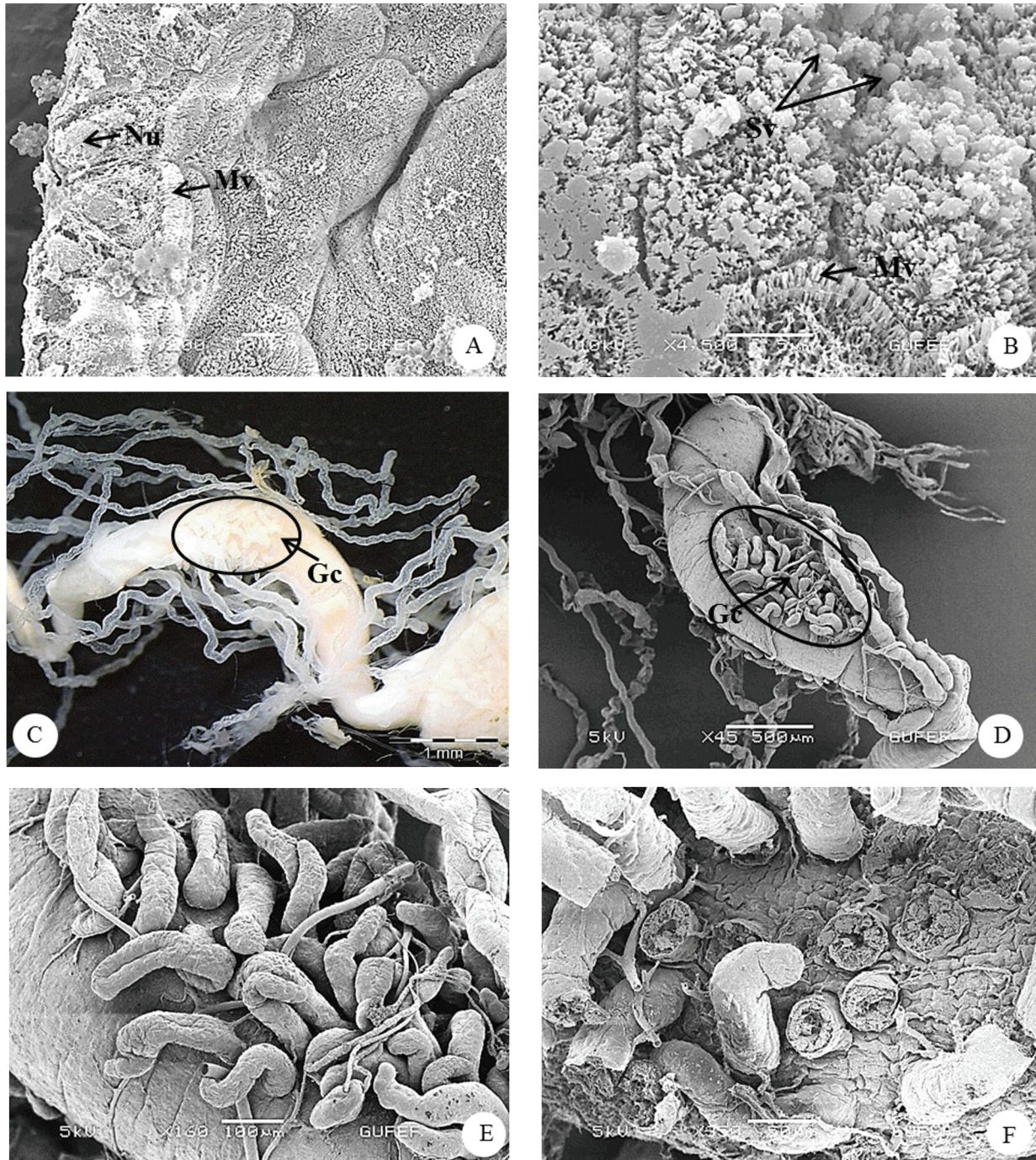


Fig. 3. *Epiphaneus malachiticus* Boh., adult: (A, B) the microvilli (Mv) of apical surfaces of the epithelial cells of the anterior midgut (SEM), (C, D) finger-like papillae of gastric caeca in the middle of the posterior midgut (SM, SEM), (E, F) the cross sections of papillae (SEM). (Nu) nucleus, (Sv) secretory vesicle. Other designations as in Fig. 1.

comprises six longitudinal folds (Sarwade and Bhawane, 2013).

The midgut of *E. malachiticus* is subdivided into the well-distinguishable anterior and posterior midguts. The anterior midgut is wide, whereas the posterior one is

long and tube-shaped (Figs. 2C, 2D and 3C, 3D). They are common in all the other described Coleoptera species (Díaz et al., 1998, 2000, 2003; Rubio et al., 2008; Sing and Prasad, 2013). The surface of the midgut has numerous tracheae and tracheoles (Figs. 2C, 2D). The

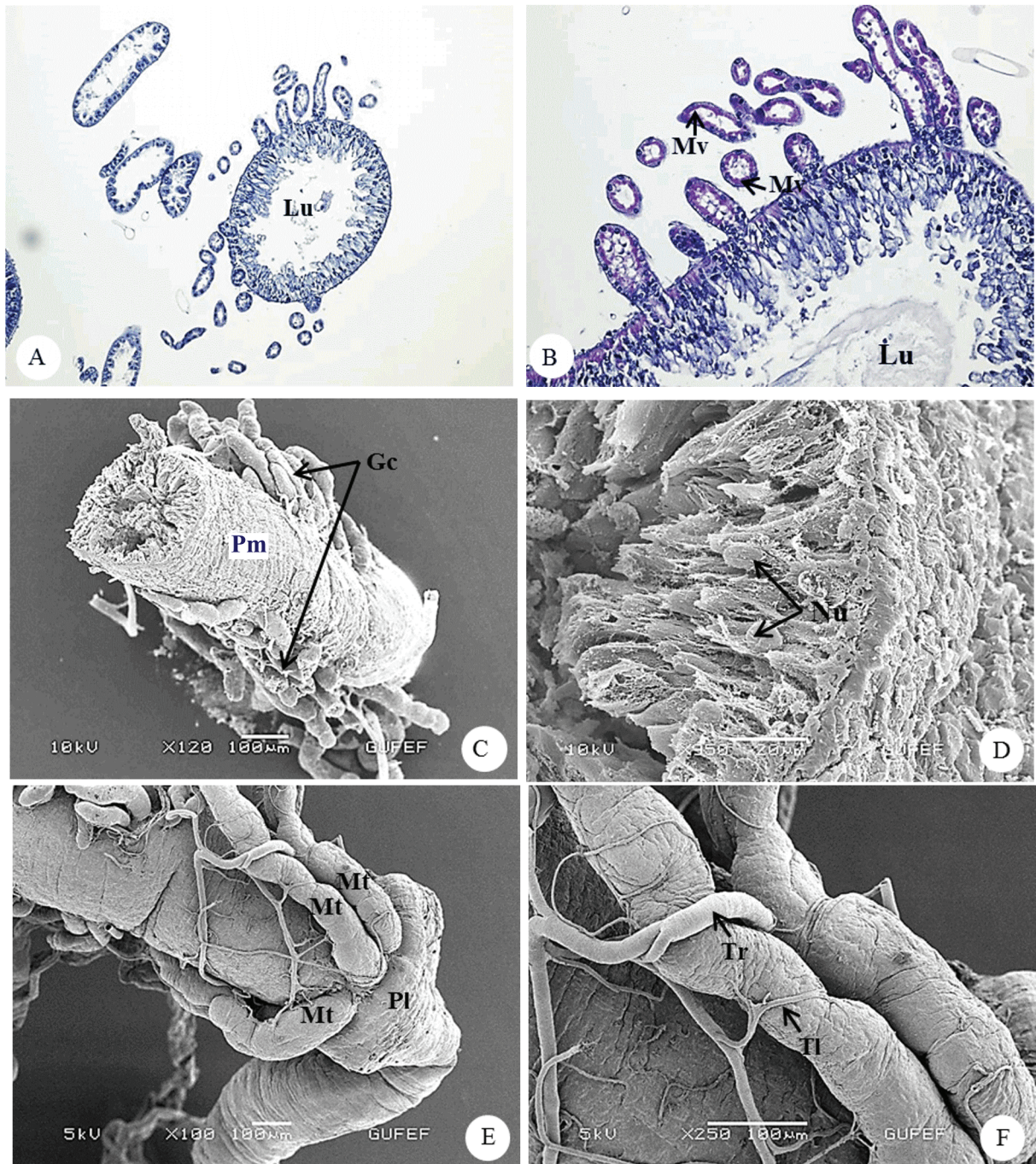


Fig. 4. *Epiphaneus malachiticus* Boh., adult: (A, B) the cross sections of cuboidal epithelium with microvilli of the gastric caeca (LM) (H & E) ($\times 40$, $\times 100$), (C, D) the gastric caeca and middle section of the posterior midgut (SEM), (E, F) the connection of Malpighian tubules with pylorus (Pl) (SEM). Designations as in Figs. 1–3.

midgut wall consists of small folds of epithelial cells with a large ovoid nucleus and of musculature. The midgut epithelium cells are elongate and columnar, arranged in a layer; the cuticle is absent (Figs. 2E, 2F and 4A, 4B). In *Tribolium castaneum* Herbst (Coleoptera:

Tenebrionidae), the midgut epithelium cells are generally larger in the anterior region of the midgut than in the posterior region (Sinha, 1958). There are histological differences also in the midgut epithelium of *Dendroctonus armandi* (Curculionidae) between the anterior

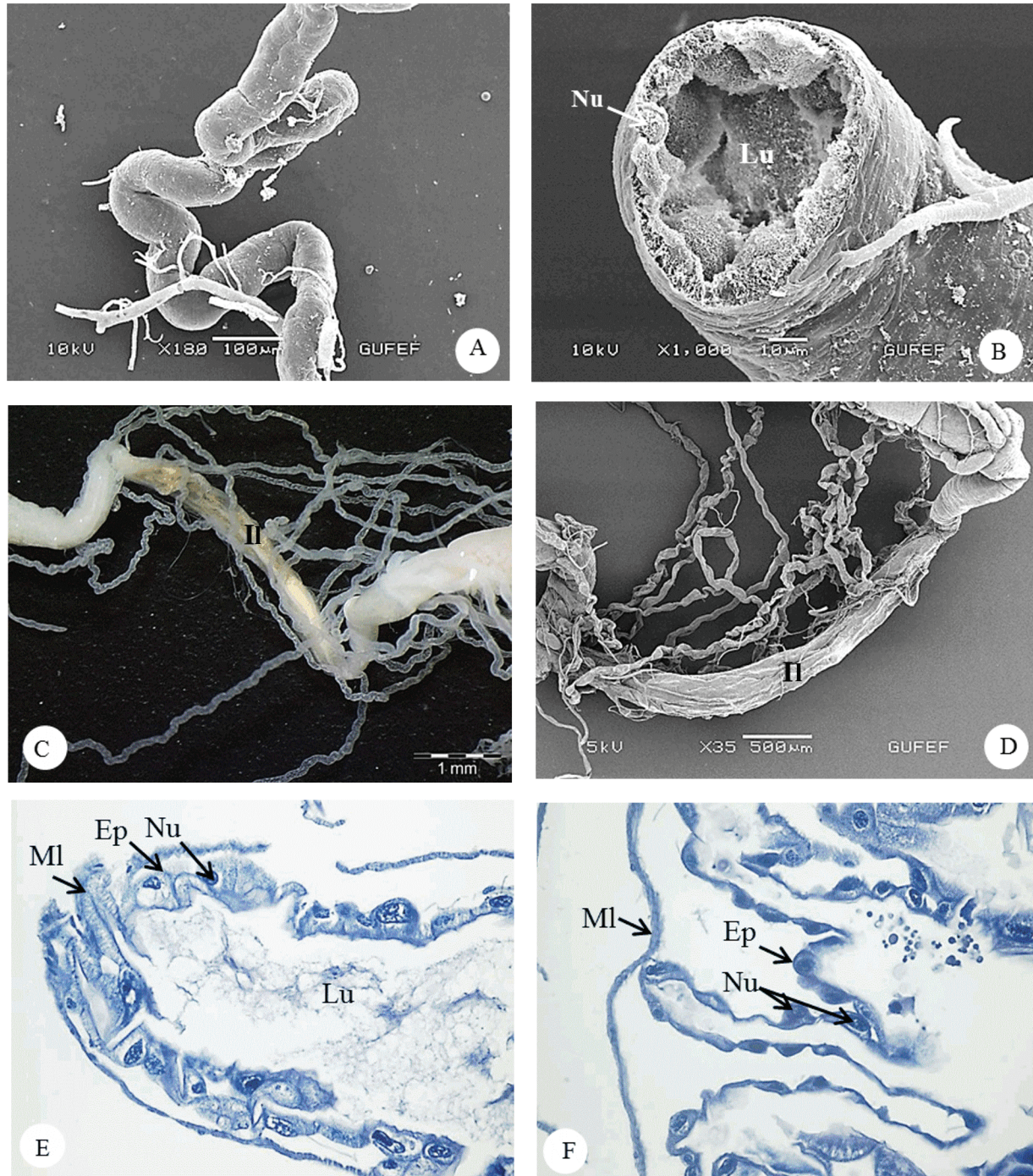


Fig. 5. *Epiphaneus malachiticus* Boh., adult: (A) tracheae and tracheoles on Malpighian tubules surface (SEM), (B) the cross section of Malpighian tubules (SEM), (C, D) the ileum between hindgut and pylorus (SM, SEM), (E, F) longitudinal section of ileum, folded cuboidal epithelium. Designations as in Fig. 1. (LM) (H & E) ($\times 200$, $\times 400$).

and the posterior midgut (Bu and Chen, 2009). In the SEM examination, the midgut lumen of *E. malachiticus* has numerous secretory vesicles (Fig. 3B). The apical surfaces of its epithelial cells have microvilli, their nuclei are ovoid and central, and the muscular tissue is

arranged in a thin layer (Figs. 2F, 3A, 3B, 4D). These structures, as in most insects, play an important role in digestion and absorption.

On each side of the middle section of the posterior midgut of *E. malachiticus* a lot of gastric caeca, which

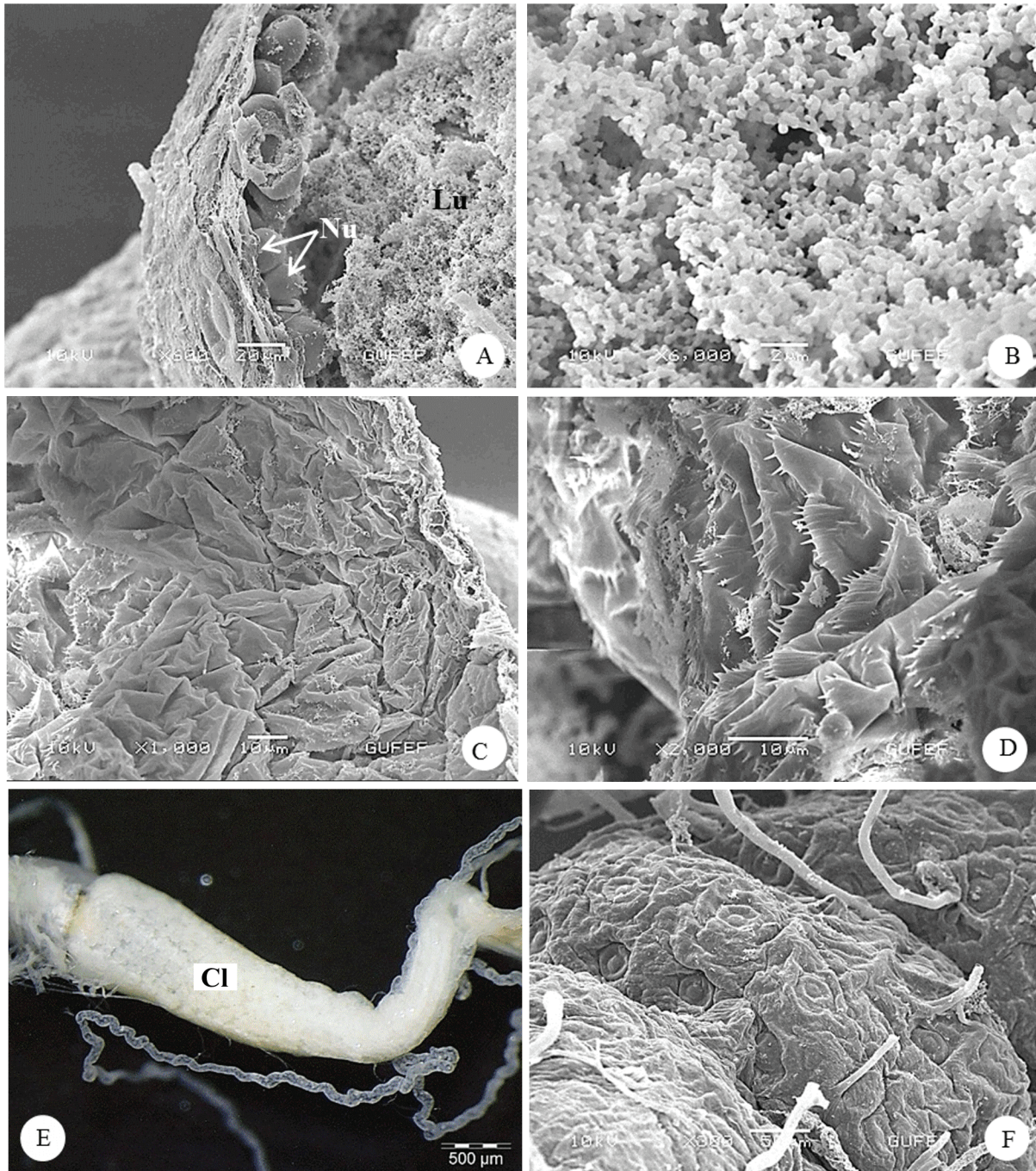


Fig. 6. *Epiphaneus malachiticus* Boh., adult: (A, B) a large number of bacteria in ileum lumen (SEM), (C, D) the spiniform structures and folds on the ileum interior surface (SEM), (E, F) the surface of the colon (SM, SEM). Designations as in Figs. 1, 3.

are short finger-like papillae, are located and they are conducive to increasing the efficiency of absorption (Figs. 3C–3F, 4C). The gastric caeca bear a lot of papillae, their position, number, and arrangement varying between weevil genera (Calder, 1989; Rubio et al., 2008). In *Dendroctonus parallelocolis* Chapuis, *D. rhizophagus* Thomas et Bright, and *D. valens*

LeConte (Coleoptera, Curculionidae: Scolytinae), at the end of the posterior midgut, there are gastric caeca which are small and numerous (Díaz et al., 1998). In *Hypothenemus hampei* (Ferrari) (Coleoptera, Curculionidae: Scolytinae), the posterior midgut is characterized by the presence of two gastric caeca (Rubio et al., 2008). In *Dendroctonus armandi*, approximately 160 gastric

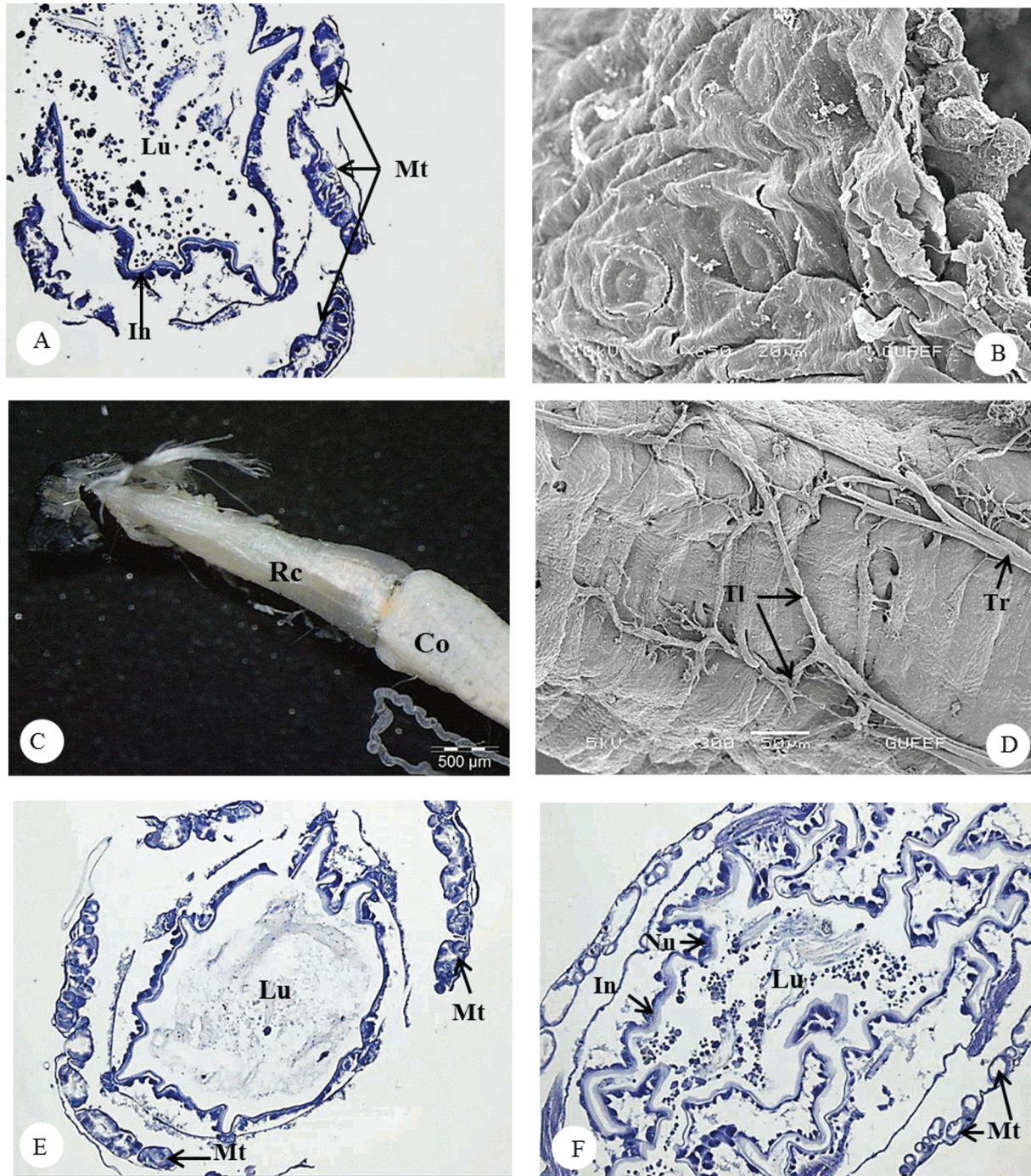


Fig. 7. *Epiphaneus malachiticus* Boh., adult: (A, B) the cross section and longitudinal section of Malpighian tubules encapsulating the wall of the colon intima (H & E) (LM, SEM) ($\times 200$), (C) rectum and colon, (D) the rectum surface (SM, SEM), (E, F) Malpighian tubules encapsulating the wall of the rectum (LM, SEM) ($\times 200$). Designations as in Figs. 1–6.

caeca are located in the middle of the midgut (Bu and Chen, 2009). Aldiagil et al. (2013) pointed out that there is no clear intestinal caecal diverticulum in the *Epilachna chrysomelina* (Coleoptera: Coccinellidae) midgut.

Histologically, the gastric caeca of *E. malachiticus* have a simple cuboidal epithelium with microvilli. They are wrapped in a thin layer of connective tissue of the midgut (Figs. 4A, 4B). The morphology and histology of the gastric caeca were reported to be similar in *Den-*

troctonus approximatus Dietz, *D. mexicanus* Hopkins, *D. frontalis* Zimmermann, and *D. brevicomis* LeConte by Díaz et al. (2000), but in the latter species the gastric caeca are situated at the end of the posterior midgut (Díaz et al., 2000).

The hindgut of *E. malachiticus* is responsible for excretion and water re-absorption from food and for primary urine produced by Malpighian tubules, thus contributing to the osmotic control of insects (Chapman, 1998). The pylorus is located at the junction of the midgut and the hindgut (Fig. 4E). The pylorus contains six Malpighian tubules which are arranged in two sets, are long and tubular structures, and their surfaces are flat; but on their surface numerous tracheae and tracheoles are seen (Fig. 4F). Similar observations on Malpighian tubules were made by Sinha (1958), Díaz et al. (2003), Rubio et al. (2008), Bu and Chen (2009), Aldiagil et al. (2013), and Sing and Prasad (2013). Additionally, according to Sing and Prasad (2013), *Odoiporus longicollis* (Oliv.) (Coleoptera: Curculionidae) has 6 Malpighian tubules arranged into two sets, of which the first is smaller than the second one, but in *E. malachiticus*, the Malpighian tubules are found to be of the same size. The Malpighian tubules are composed of cuboidal cells with oval nuclei and microvilli (Fig. 5B). These tubules are generally long and narrow (Fig. 5A).

The hindgut with the pylorus is followed by the front part, the ileum, then by the colon and the rectum. The ileum of *E. malachiticus* is yellowish, in the form of a long tube, and is located between the pylorus and the colon (Figs. 5C, 5D). This structure is similar to that in *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Sinha, 1958). Histologically, the ileum wall of *E. malachiticus* from the outside to the inside consists of muscles, cuboidal epithelial cells which are thrown into a number of irregular folds, and intima (Figs. 5E, 5F). The lumen has a lot of bacteria clumps (Figs. 6A, 6B), and the intima of the ileum has spiniform structures (Figs. 6C, 6D). The same structures were reported by Sarwade and Bhawane (2013) for *Platynotus belli* (Coleoptera: Tenebrionidae).

The colon and rectum epithelia of *E. malachiticus* consist of regularly arranged cuboidal cells (Figs. 7A, 7B and 7E, 7F). In the cross sections, the distal endings of Malpighian tubules are completely surrounded by the wall of the colon and rectum (Figs. 7A, 7E, 7F). The Malpighian tubules are located just underneath

the peritoneal sheath and extend at least to the end of the colon; they constitute the cryptonephridial system with the hindgut wall. This structure is similar to that of *Dendroctonus parallelocollis*, *D. rhizophagus*, and *D. valens* (Coleoptera, Curculionidae: Scolytinae) (Díaz et al., 1998), *D. armandi* (Bu and Chen, 2009), *Epilachna chrysomelina* (Coleoptera: Coccinellidae) (Aldiagil et al., 2013), and *Odoiporus longicollis* (Coleoptera: Curculionidae) (Sing and Prasad, 2013).

The colon of *E. malachiticus* is a straight tube leading to the rectum (Figs. 6E, 7C). The rectum is surrounded with muscles and a large number of the tracheae and tracheoles (Fig. 7D).

The hindgut epithelial cells are quite different from those of the foregut and midgut; they are cuboidal and thrown into a number of irregular folds. According to Sinha (1958), similar structures are present in the *Tribolium castaneum* hindgut. The epithelium of the hindgut regions is uniform in *E. malachiticus*, whereas in *T. castaneum*, the epithelial layer is thinner in the ileum and rectum and thicker in the colon (Sinha, 1958).

Our data show that the alimentary canal of *E. malachiticus* has the same structural pattern which is observed in other coleopterans (Sinha, 1958; Díaz et al., 1998, 2000, 2003; Rubio et al., 2008; Aldiagil, 2013; De Sousa et al., 2013; Sarwade and Bhawane, 2013; Singh and Prasad, 2013). However, *E. malachiticus* has morphological and histological differences in the shape, position, number and arrangement of the gastric caeca, hindgut epithelial cell structure, and the intima of the ileum which has spiniform structures and bacteria in its lumen.

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ADDITIONAL INFORMATION

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