Fine structure of the Malpighian tubules in *Gryllus campestris* (Linnaeus, 1758) (Orthoptera, Gryllidae)

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Abstract: The Malpighian tubules are responsible for excretion and osmoregulation in insects. The Malpighian system consists of tubules that are closed at the distal end and extend from the point of junction of the midgut and hindgut. This organ helps the water and electrolyte balance of the internal metabolism by filtration with absorption of water and soluble materials as mineral salts from the hemolymph. In this study, the histology of Malpighian tubules in *Gryllus campestris* (Orthoptera, Gryllidae) has been examined in detail by light microscopy, scanning electron microscopy, and transmission electron microscopy. The distal ends of the Malpighian tubules in *G. campestris*, which are long and wiggly in shape, extend freely into the body cavity. The other end is connected to the alimentary canal. In a cross-section of the tubules, it has been observed that the outer surface of the cells is covered by muscles and trachea. The lumen is lined with a single-layer epithelium. The nuclei (which are oval or spherical in shape), rough endoplasmic reticulum, mitochondria, and spherocrystals or mineral concretions are seen in the cells. Microvilli are found in the apical membrane. When the structure of the Malpighian tubules was compared with those of other species belonging to Orthoptera and other insect orders, similarities and differences were observed.

Key words: Excretory system, Malpighian tubule, ultrastructure, electron microscope

1. Introduction
The Malpighian tubules are the excretory and osmoregulatory organs of insects, which are specialized for organic solute transport, metabolism, immune defenses, and detoxification (Wigglesworth, 1965; Chapman, 1998; Dow, 2009). They are homologous to the vertebrate nephron in the function of removing different chemical substances from the body fluids, and they are associated with water and ion transport from the hemolymph to the lumen (Wigglesworth, 1965; Chapman, 1998; Pal and Kumar, 2012). Thus, the Malpighian tubules serve the functions of both generating the primary urine and selectively reabsorbing the desirable solutes; in terrestrial insects, the filtered fluid or primary urine is passed to the hindgut and rectum, where this filtered fluid is modified as a result of reabsorption of certain substances and excretion of others to produce urine. This selective modification of primary urine occurs in the ileum, rectum, or even in the Malpighian tubules (Maddrell and Gardiner, 1974; Maddrell, 1981; Chapman, 1998).

The Malpighian tubules are present in variable numbers in different orders of insects. Although the number of the Malpighian tubules can vary from 1 to 4 in Hemiptera, 12 to 150 in Hymenoptera, 2 to 200 in Orthoptera, and 50 to 60 in Odonata, this number is 6 in Lepidoptera and 4 in Diptera. In many species, the tubules are separated into morphologically different regions and can contain different cell types (Bradley et al., 1982; Chapman, 1998; Acar, 2009; Pacheco et al., 2014; Amutkan et al., 2015). Most insects in the order Diptera have small stellate cells scattered among the principal tubule cells. In contrast with this, insects in the Heteroptera order have only one type of cells (Chapman, 1998).

The Malpighian tubules of insects consist of a long tubular structure formed from a single layer of epithelial cells and are closed at one end. However, apart from this general form of the Malpighian tubules, there is considerable variation in the form of them throughout the class of insects. Generally, the tubules are free in the hemocoel, which connect the alimentary canal at the junction of the midgut and hindgut (Roeder and College, 1953; Martini et al., 2007). Roeder and College (1953) reported that the Malpighian tubules of insects generally can be divided into four groups. They stated that the simplest of these groups is found in species of Orthoptera, Dermaptera, Neuroptera, and some Coleoptera. The
2.3. Transmission electron microscopy

For transmission electron microscopy (TEM), tissues were fixed in 2.5% glutaraldehyde with a 0.2 M phosphate buffer (pH 7.2). Postfixation was performed using 1% OsO₄ with a phosphate buffer for 1 h. The OsO₄ was then removed and the samples were rinsed three times with phosphate buffer. Dehydration was performed using a series of alcohol solutions with increasing concentrations (70%, 80%, 90%, 96%, and 100%). The tissues were embedded in Araldite. The Araldite blocks were sectioned using a Leica EM UC6 ultramicrotome, and the sections were then stained with lead citrate and uranyl acetate. Analysis and imaging were performed using a JEOL JEM 1400 transmission electron microscope at 80 kV in the Electron Microscopy Laboratory of the Faculty of Science at Gazi University, in Ankara, Turkey.

3. Results

The Malpighian tubules of *Gryllus campestris* are relatively long, smooth tubules that end blindly in the abdominal cavity and open into the alimentary canal immediately behind the midgut. They are located at the point of junction of the midgut and hindgut. The number of Malpighian tubules of both males and females of the species displays variation between 90 and 150 tubules (Figures 1 and 2). The Malpighian tubules of *G. campestris* consist of a single-layer cuboidal epithelium with a few cells encircling the lumen (Figures 3 and 4). In SEM images, it is observed that the outer surface of the Malpighian tubules is covered with the trachea and muscles (Figure 5). In light microscopy and TEM images, the cells have a close-packed structure of microvilli on the lumen side (Figures 6 and 7). The length of the microvilli is approximately the same as the epithelium width in some regions of the Malpighian tubules (Figure 7). The most important characteristic of the microvilli is the enlargement. This bulblike enlargement is observed at the end of the microvilli. This structure and the vesicles are demonstrated to be an apocrine secretion (Figure 8). There are no mitochondria in the microvilli of the cells in the Malpighian tubules (Figure 8).

The cell nuclei, which are oval or spherical in shape, are found in the middle of the cells. The outer surface of the nuclei has a regular shape. The heterochromatin and the euchromatin regions are clearly observed in TEM (Figure 7).

The basement membrane, which is a thin structure, completely ensheaths the outer surface of the tubule. The basal plasma membrane comprises intensively well-developed infoldings. The infoldings are more or less parallel and radially aligned, and they penetrate to varying depths in the transverse section. The numerous mitochondria are organized between the basal infoldings.
There is a thin basal lamina under the basal plasma membrane (Figure 9). There are many organelles in the cytoplasm. Plenty of mitochondria are also found homogeneously in the cytoplasm, and the cytoplasm contains many bodies morphologically similar to lysosomes (Figure 11). Extensive rough endoplasmic reticulum is present in all regions of the cytoplasm. The rough endoplasmic reticulum is especially organized around the nucleus (Figure 12). There are many spherocrystals or mineral concretions that are spherical in shape and of different sizes. The early stages of the developing spherocrystals are observed in lucent vesicles.

(Figures 9 and 10).

Figure 1. Stereomicroscope image of a part of the alimentary canal and the Malpighian tubules in *Gryllus campestris*.

Figure 2. SEM micrograph of a part of the alimentary canal and the Malpighian tubules in *Gryllus campestris*. 
During development, they extend; the late stages of the spherocrystals have two or more concentric structures that have different electron densities. These are found in the central cytoplasm and close to the basal plasma membrane (Figure 13).

4. Discussion

The insect excretory system includes a number of Malpighian tubules. Its proximal region opens into the alimentary canal at the junction of the midgut and hindgut, and the distal region lies free in the abdominal...
Figure 5. SEM micrograph of the cross-section of the Malpighian tubules. Tr: Trachea; Ms: muscle; Mv: microvilli; L: lumen.

Figure 6. TEM micrograph of the cross-section of the Malpighian tubules. Muscle (arrow); N: nucleus; Mv: microvilli; L: lumen.
Figure 7. TEM micrograph of the cross-section of the Malpighian tubules. Trachea (arrow); N: nucleus; Mv: microvilli; and L: lumen.

Figure 8. The enlargements of the tips of the microvilli (★).
Figure 9. TEM micrograph of the basal side of the Malpighian tubules. BL: Basal lamina; (→): basal plasma membrane infoldings.

Figure 10. TEM micrograph of the basal side of the Malpighian tubules. Ms: Muscle; M: mitochondria; (→): basal plasma membrane infoldings.
The structure of the Malpighian tubules in Gryllus campestris displays significant similarities and differences compared with those of species investigated previously (Ramsay, 1954; Beams et al., 1955; Taylor, 1971; Acar, 2009; Pal and Kumar, 2012; 2014; Gonçalves et al., 2014; Amutkan et al., 2015; Zhong et al., 2015). While the numbers of the Malpighian tubules in Gryllus campestris range from 90 to 150, this number is 4 in Graphosoma lineatum (Heteroptera, Pentatomidae) and Sarcophaga ruficornis (Diptera, Sarcophagidae) (Pal and Kumar, 2014; Amutkan et al., 2015). Aedes aegypti (Diptera, Culicidae) has 5 Malpighian tubules (de Sousa and Bicudo, 1999). In Melanogryllus desertus (Orthoptera, Gryllidae), this number varies between 100 and 150 (Acar, 2009).

The Malpighian tubules are surrounded by muscles in insects. They move in the hemolymph and can carry out peristaltic movements to move material from the distal end to the opening in the hindgut. Contraction of the muscles creates hydrostatic pressure across the Malpighian tubule system for the movement of water (Denholm and Skaer, 2005; Chapman, 2013). Therefore, in G. campestris, the outer surface of the Malpighian tubules is surrounded by muscle as in other species previously examined (Beams et al., 1955; Ramsay, 1955; Arab and Caetano, 2002; Martini et al., 2007).

The basement membranes of the Malpighian tubules are permeable. Uptake of colloids and proteins from the hemolymph is provided with the basement membranes. The thickness of the basement membrane varies in different orders for the class Insecta. Some species, such as A. aegypti, Calpodes ethlius (Lepidoptera, Hesperiidae), and G. campestris, have thin basement membranes (Beams et al., 1955; Ryerse, 1979; Chapman, 2013). Whether the basement membrane of the Malpighian tubules is thick or not, it serves for structural support, permeability of proteins, and ion selectivity. Additionally, it protects the tubules against pressure from muscular movement (Beams et al., 1955; Chapman, 2013).

In G. campestris, cells contain numerous secretory granules, mitochondria, ribosomes, spherocrystals or mineral concretions in different sizes, and other cytoplasmic organelles. Spherocrystals are characteristic features of the Malpighian tubule cells and have various...
roles in insects' excretory systems. They are generally known to regulate the maintenance of the appropriate mineral composition and the accumulation of nontoxic waste materials and of toxic metals. The spherocrystals also store products of mineral and organic materials (Sohal and Lamb, 1979; Wessing and Zierold, 1992; Cruz-Landim and Serrao, 1997; Delakorda et al., 2009; Lipovsek et al., 2012; Chapman, 2013; Pal and Kumar, 2014). Numerous secretory granules, which have the roles of synthesis and storage of abundant protein substances, are found in the tubule cells. Spherocrystals and secretory granules are comparably found in other species, since they have different shapes and sizes (Pal and Kumar, 2013; Zhong et al., 2015).

The Malpighian tubule cells of *G. campestris* have long and wide basal plasma membrane infoldings and extremely long microvilli. The structures of the basal plasma membrane infoldings and microvilli indicate that these are involved in the active transport mechanism that requires energy (Delakorda et al., 2009; Pal and Kumar, 2012). They also regulate an osmotic gradient and active transport of small substances by passive movement from the hemolymph into the tubule lumen (Pal and Kumar, 2013). In *Troglophilus neglectus* (Orthoptera, Rhaphidophoridae) and *Sarcophaga ruficornis* (Diptera, Sarcophagidae), a few mitochondria extend into the microvilli (Delakorda et al., 2009; Pal and Kumar, 2013). In contrast, there is a lack of mitochondria in the microvilli in *Podisus nigrispinus* (Hemiptera, Pentatomidae), *Graphosoma lineatum* (Heteroptera, Pentatomidae), *Locusta migratoria* (Orthoptera, Acrididae), and *Schistocerca gregaria* (Orthoptera, Acrididae) (Prado et al., 1992; da Cunha et al., 2012; Amutkan et al., 2015). When compared with the Malpighian tubules of other species, those of *G. campestris*, which have long microvilli (about 2–5 µm) on the apical surface, do not have mitochondria in the microvilli. The mitochondria are found in the cytoplasm, mainly in the apical region, and are associated with the basal plasma membrane infoldings. In our opinion, the well-developed basal membrane infoldings and abundant mitochondria among them in *G. campestris* are involved in active ion transportation.

Figure 12. TEM micrograph of the cross-section of the Malpighian tubules. N: Nucleus; M: mitochondria; GER: rough endoplasmic reticulum; Mv: microvilli; L: lumen.
In conclusion, comparison of the Malpighian tubules of *G. campestris* with those of other species in the Orthoptera order showed similarities. There were also morphological differences from the other insect orders. These differences can clearly be observed in the morphological structure of the Malpighian tubules and the number of tubules. We suggest that our findings provide new insights for understanding the tubule structure. Moreover, this study provides key information for future research on the Malpighian tubules. Thus, this study gives useful information on this important organ, which has different fundamental functions for insects.

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