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Morphology and Fine Organization of the Midgut of *Poecilimon ataturki* Ünal, 1999 (Orthoptera: Tettigoniidae)

Damla AMUTKAN MUTLU[1]*

ABSTRACT: In order to do detailed experiments about the internal organs of the insects or to improve new methods relevant to pest management, the biology of the species should be well known. For this reason, this paper deals with *Poecilimon ataturki* Ünal, 1999 (Orthoptera: Tettigoniidae) which is a bush cricket and is known to be harmful to nature. The morphology and structure of the midgut of *P. ataturki* have been investigated using light microscopy and scanning electron microscopy (SEM). The midgut of this species has two main regions called the ventriculus and the gastric caecum. The ventriculus has a tubular structure morphologically, whereas the gastric caecum has a bulbous structure. The ventriculus has two cell types in the epithelial layer as the principle and the regenerative cells. The fine structure of the ventriculus and the gastric caecum cells revealed that the secretion activity moves from cell to lumen. These structures show a high level of similarity compared with the midgut structure of the previously studied species in the Orthoptera order despite some differences. It is thought that the enlightenment of the midgut structure of this species will contribute to science for insect pest management.

Keywords: Gastric cacea, ventriculus, histology, light microscope, scanning electron microscope (SEM)

[1] Damla AMUTKAN MUTLU (**Orcid ID:** 0000-0002-4780-8520), Gazi University, Faculty of Science, Department of Biology, Ankara, Turkey

***Corresponding Author:** Damla AMUTKAN MUTLU, e-mail: damlamutkan@gazi.edu.tr

INTRODUCTION

The Orthoptera order includes many species which are called as the grasshoppers, locusts, and katydids (Comstock and Comstock, 1888). This order is divided into two main suborders as the Ensifera which includes long-antenna species and the Caelifera which includes short-antenna species (Ünal, 2000; Biagio et al., 2009; Eren et al., 2019). These suborders are divided into many families depending on features such as wing structure, mouthparts structure, body size and color. One of these families is Tettigoniidae (Ingrisch and Rentz, 2009). Insects in the Tettigoniidae family which have more than 6.400 species known, are usually called katydids or bush crickets (Ragge, 1965; Tazegül and Önder, 2012; Cigliano et al., 2018).

The species belonging to this family are usually important for economical concerns. Even species that cannot fly can increase their numbers in ways that can affect crops and cause serious losses to rice and crops in the proper environmental conditions (Ingrisch and Rentz, 2009). Thus, they directly damage both biodiversity and the economy of agricultural land. For this reason, the control of these insects has become very important. The knowledge about invasive species provides the necessary preliminary information to form and develop appropriate control strategies. In this context, the morphology, histology and ultrastructure of the internal systems of the insects must be known in order to decide which pesticides to apply that will only affect the targeted insect without harming the environment and other living things to minimize and control the economic damages (Mead et al., 1988, Karaca et al., 2006; Willis et al., 2010; Vitale et al., 2015; Pappalardo et al., 2016; Polat, 2016; Amutkan Mutlu, 2020).

The insect digestive system has a tubular structure that extends from the mouth to the anus. It is composed of three basic regions: the foregut, where break out and storage of nutrients occurs, the midgut, where the digestion happens, and the hindgut, where the reabsorption of the feces takes place (Rost-Roszkowska, 2008; Polat, 2016; Amutkan Mutlu, 2020).

The midgut is an essential organ for insects. It is not only a main part of the digestive system. It is also known that midgut epithelial cells produce and secrete most of the digestive enzymes and constitute components of the peritrophic membrane. So, it has many critical functions for the metabolism as it is the first site of digestion (Takeda, 2012; Thorsson, 2018; Polat et al., 2019). Therefore, disruption of these midgut functions in insects belonging to the Orthoptera order, capable of rapidly increasing their populations, may supply a way and strategy for the next pest management. Based on this idea, it was aimed to enlighten the biology of this species by studying the morphology and structure of the midgut in *Poecilimon ataturki* Ünal, 1999 (Orthoptera, Tettigoniidae). To know the biology of the species will provide prior information for further studies.

MATERIALS AND METHODS

Insects and preparation of midgut samples

All experiments were done on adults of *P. ataturki*. The collection of the adult individuals was done in Bolu province, Hamidiye Village in July 2019 and brought to the laboratory. About 10 females and 10 males of *P. ataturki* were dissected under stereomicroscope. The midguts were separated from other parts of the digestive system and the gastric caecum and the ventriculus were carefully pulled apart.

Light microscopy (LM)

For histological studies with the light microscope, the gastric caecum and the ventriculus were fixed in 10% Formalin solution for 24 hours. The samples were then washed with tap water. After that, they were dehydrated in ethanol and embedded in paraffin. 5-6 µm thickness serial sections were cut using a Micron HM 310 microtome. Afterwards, the sections were stained with Hematoxylin and Eosin

(H&E) and Mallory's trichrome staining, and mounted in glass slides with Entellan (Polat, 2016). They were photographed with an Olympus BX51 photomicroscope.

Scanning electron microscopy (SEM)

The midgut pieces were fixed in 2.5% glutaraldehyde (pH 7.2) for 24 hours. Then, after rinsing in phosphate buffer, they were dehydrated in ascending ethanol series at room temperature. The specimens were then dried with critical point dryer (Polaron CPD 7501). They were mounted on SEM stubs and coated with gold by using a Polaron SC 502 sputter coater (Polat, 2016). The photographs were taken with JEOL JSM 6060 LV SEM at accelerating voltage 10 kV.

RESULTS AND DISCUSSION

As a result of the literature research on the digestive tract of Orthoptera species, it has been seen that most of the studies have focused especially on the midgut (Khan, 1964; Wanderley-Teixeira et al., 2006; Woodring and Lorenz, 2007; Rost-Roszkowska, 2008; Biagio et al., 2009; Li et al., 2018; Thorsson, 2018; Polat et al., 2019). In this study, the morphology and histology of the midgut in *P. ataturki*, which is a part of the digestive system is studied and it has been observed that the histology of the midgut in *P. ataturki* very much resembles the midgut of other studied species within the Orthoptera order. Nonetheless, in this study it is concluded that there are some differences between structure of the midgut in this species and the structure of the midgut in previously studied species. Also, there was no difference between males and females of this species.

The midgut of *P. ataturki* individuals comprises a ventriculus and two large anteriorly placed gastric caeca which are crescent shaped. While the ventriculus has morphologically tubular structure, gastric caeca have bulbous structure. The region where the gastric caeca connect to the digestive tract is wider, while their distal ends are narrower, directed forward and comprehend the proventriculus (Figure 1A-1B). The most prominent difference seen morphologically in the midgut is the number of gastric cecum. Li et al. (2018) reported that the gastric caecum is two bulbous in shape in *Gampsocleis gratiosa* Brunner von Wattenwyl, 1862 (Orthoptera, Tettigoniidae). *Acheta domesticus* (Linnaeus, 1758) (Orthoptera, Gryllidae) has two sac-like structure gastric caeca like the gastric caecum in *Gryllodes sigillatus* (Walker, 1869) (Orthoptera, Gryllidae) and in *P. ataturki* (Rost-Roszkowska, 2008; Biagio et al., 2009). On the contrary, *Abracris flavolineata* (De Geer, 1773) (Orthoptera, Acrididae) has six gastric caeca with anterior and posterior lobes (Marana et al., 1997). According to Xiaoming et al. (2009), *Atractomorpha sinensis* Bolívar, 1905 (Orthoptera, Acridoidea) and *Acrida cinerea* (Thunberg, 1815) (Orthoptera, Acridoidea) have six gastric caeca. The gastric caeca are the projections of the midgut that differ relevant to the feeding habits of insects (Li et al., 2018). These projections cause an increase in the surface area of the midgut and thus the gastric caeca perform more functions.

The general organizations of the gastric caecum and the ventriculus of the midgut are histologically almost identical in this species. Both of these regions of the midgut have a muscle layer, basal lamina, epithelium, and peritrophic membrane from the outer to the inner layer (Figure 2). The muscle layer of the midgut which is longitudinal and circular is well developed. A network of the trachea is also seen on the outer surface (Figure 3). The epithelium of the ventriculus which is placed on a thin basal lamina is made up of two different cell types as the principle cells and the regenerative cells (Figure 4, 6). It is seen that the connective tissue is positioned below the epithelium (Figure 5). Principal cells show monolayer sequencing. They are cylindrical cells and have round nuclei in the middle of the cells. There are brush border microvilli on the apical surface of the principle cells (Figures 4, 7). There are secretory granules secreted into the lumen between the microvilli (Figure 4, 6). The peritrophic membrane is distinguished to encircle the food bolus in the ventriculus lumen. In addition, a space is seen between the peritrophic membrane and the epithelium (Figures 2, 4, 6).

The regenerative cells are located between the principle cells in groups and are adhere to the basal lamina. Regenerative cells occur as nidi groups and are characterized by a large nucleus (Figures 4, 6, 8).

The ventriculus which is the main part of the midgut has different cell types such as the principle cells, the regenerative cells, the endocrine cells, and the goblet cells in insects. It has been observed that *G. gratiosa* has three types of cells as the regenerative cell, the principal cell, and the endocrine cell in the midgut epithelium (Li et al., 2018) as *G. sigillatus* and *A. flavolineata* reported (Marana et al., 1997; Biagio et al., 2009). The midgut of *A. domesticus* consists of the principal cells and the regenerative cells (Rost-Roszkowska, 2008; Karpeta-Kaczmarek et al., 2016) as in *Isophya nervosa* Ramme, 1931 (Orthoptera, Tettigoniidae) (Amutkan Mutlu, 2020). In this study, only two cell types: the principle cells and the regenerative cells are observed in the ventriculus of *P. ataturki*. The alimentary canal of insects displays a functional and structural organization that changes with feeding habits. Accordingly, the presence of different cell types in the ventriculus guarantees species-typical functional properties, as Caccia et al. (2019) explained.

The peritrophic membrane, matrix (PM) or peritrophic envelope is a structure, which is found in the midgut of insects that have the chewing mouthparts and feed on with solid, and secreted by the midgut epithelial cells (Pimenta et al., 1997; Chapman, 2013; Klowden, 2013; Li et al., 2018; Thorsson, 2018). The main roles of the PM include not only preventing the damage of epithelial cells by the luminal contents, but also protecting them from pathogenic microorganisms and toxins. It is also known that while it is permeable to inorganic ions and small organic molecules, it is a barrier to the passage of large molecules (Pimenta et al., 1997; Dias et al., 2019; Teixeira et al., 2019; Nayak, 2020). In this study, the peritrophic membrane is observed through the whole midgut in a similar manner to that of *G. gratiosa* (Li et al., 2018), *A. domesticus* (Thorsson, 2018), *Acrida anatolica* Dirsh, 1949 (Orthoptera, Acrididae), *Parapholidoptera spinulosa* Karabag, 1956 (Orthoptera, Tettigoniidae) (Eren et al., 2019), and *I. nervosa* (Amutkan Mutlu, 2020). Choosing a large toxic substance enough to pass through the peritrophic membrane will cause midgut epithelial cells to start to lysis with the toxic substance or insecticides. This is an important point in pest management.

Although the gastric caeca are almost identical to ventriculus, they differently display a great number of epithelial folds towards the lumen (Figure 9). Similar to the ventriculus, gastric caeca consist of a single layer of epithelial cells. They have a round nucleus in the columnar cells and are surrounded by connective tissue. The epithelial cells are placed on a thin basal lamina. There are also brush border microvilli on the apical side of the cells (Figure 10). It is observed that the secretory granules are found in different sizes between microvilli and also in the gastric caeca lumen (Figure 11). It can be said that secretions in the ventriculus and in the gastric cecum are apocrine type secretions.

When the gastric caecum is histologically investigated, it is observed that the microvillus is found on the apical region of the cells. The epithelial organization, presence of microvilli and the secretory granules in the gastric caeca show an absorption and secretion area. These structures are not only responsible for the secretion of the digestive enzymes, but also have a role in water and nutrient absorption as Marana et al. (1997), Gallo et al. (2002) and Wanderley-Teixeira et al. (2006) reported.

It can be said that the gastric caecum found in the alimentary canal in Orthoptera families is important for both phylogenetic (because of the morphological differences) and nutritional aspects.

Another result obtained in this study is that apocrine secretion is conducted by midgut epithelial cells. The secretory granules observed between microvilli or in the lumen in this study have been determined as in previous studies (Cristofoletti et al., 2001; Caldeira et al., 2007; Biagio et al., 2009). Some authors interpret these secretory granules as the apocrine secretion (Terra et al., 1985; Ferreira et al., 1990). On the other hand, it is also thought that it is a part of the cell renewal process (Terra and Ferreira, 1994; Aumüller et al., 1999; Caldeira et al., 2007). In addition to these views, the appearance of secretory granules is an evidence of the production and secretion of digestive enzymes in the midgut epithelial cells. Further studies will be required to identify the content of the secretory granules in the different midgut regions.

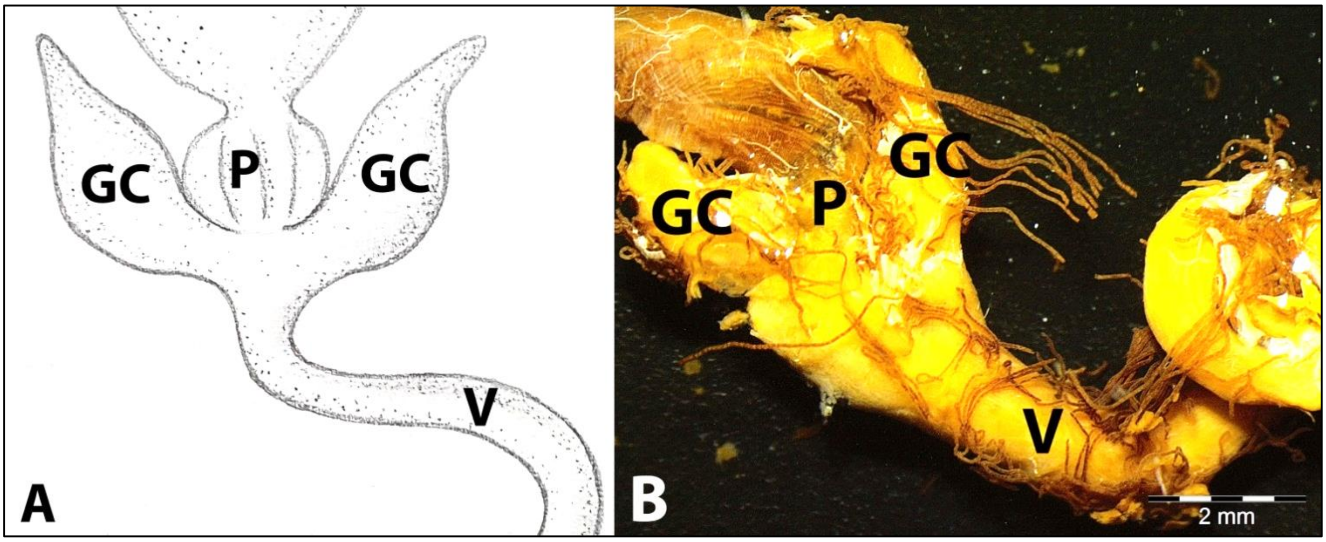

Figure 1. A. Schematic drawing of the ventral view of the midgut of *P. ataturki*. B. Stereomicroscope images of the midgut of *P. ataturki* (Scale bar: 2 mm). GC: Gastric caecum, P: Proventriculus, V: Ventriculus

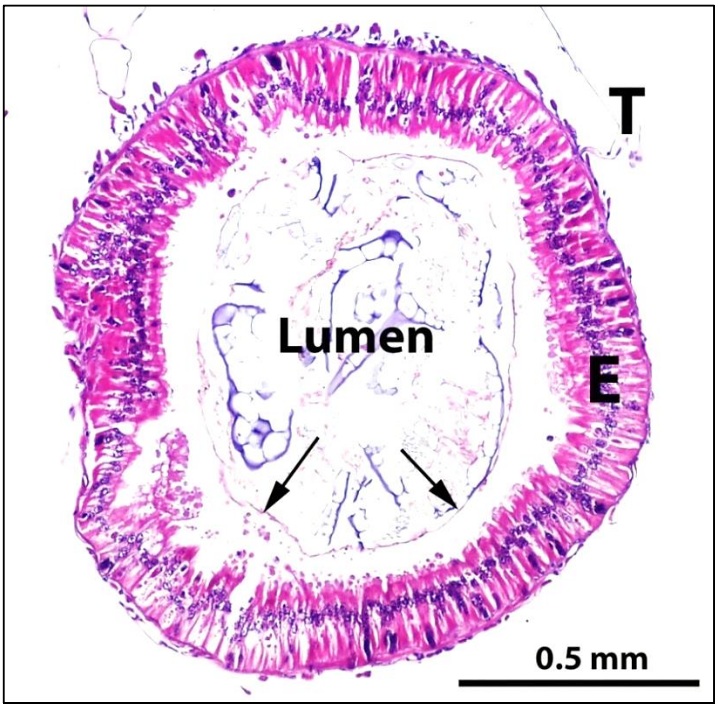

Figure 2. Light micrograph of the cross section of the ventriculus. T: Trachea, E: Epithelial cell layer, →: Peritrophic membrane (H&E staining, Scale bar: 0.5 mm)

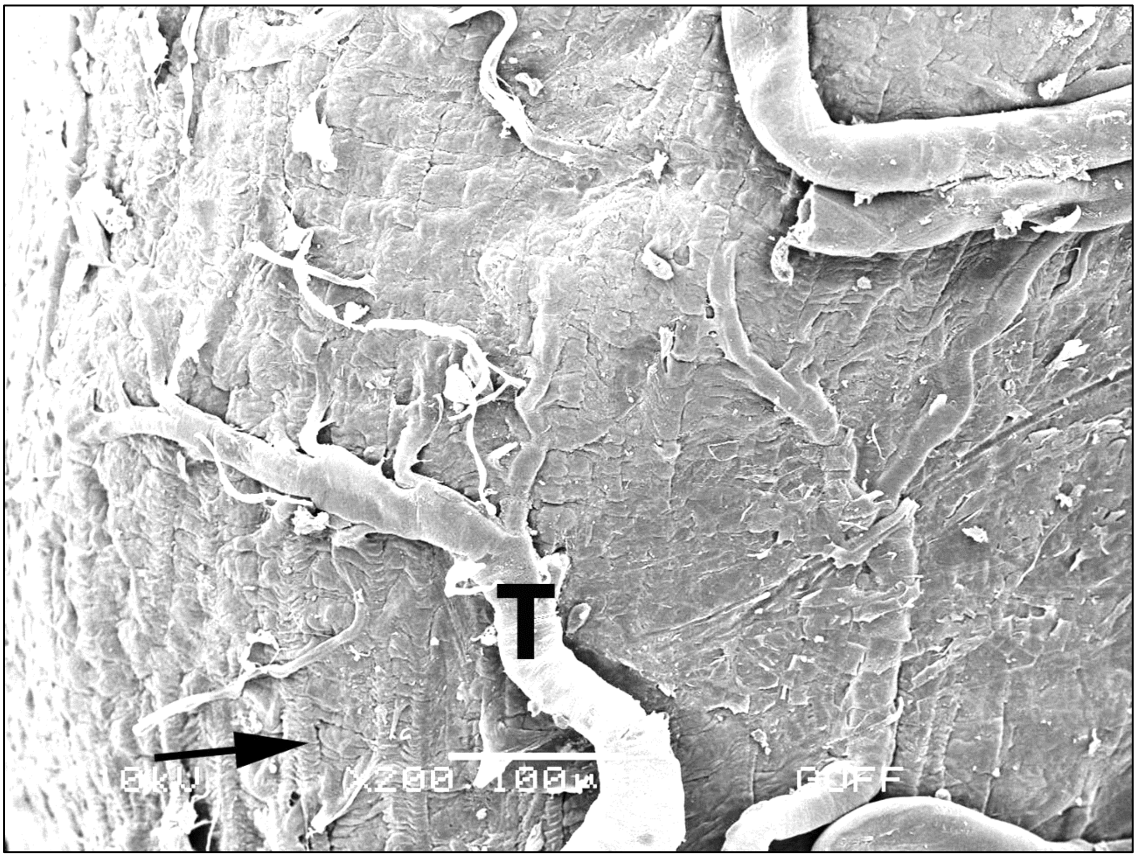

Figure 3. Scanning electron microscope images of the outer surface of the ventriculus. T: Trachea, →: Muscle (Scale bar: 100 µm)

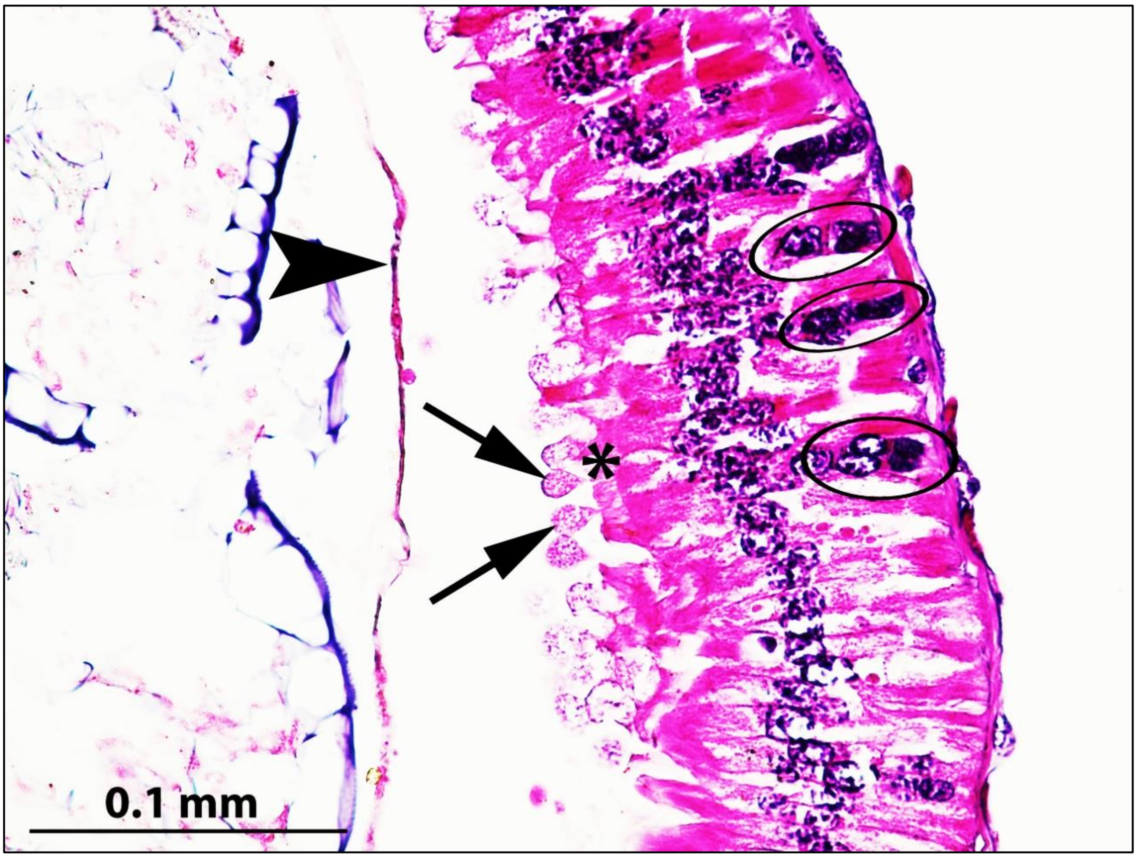

Figure 4. Light micrograph of the cross section of the ventriculus. Circled: Regenerative cells, *: Microvillus, →: Secretory granules, ►: Peritrophic membrane (H&E staining, Scale bar: 0.1 mm)

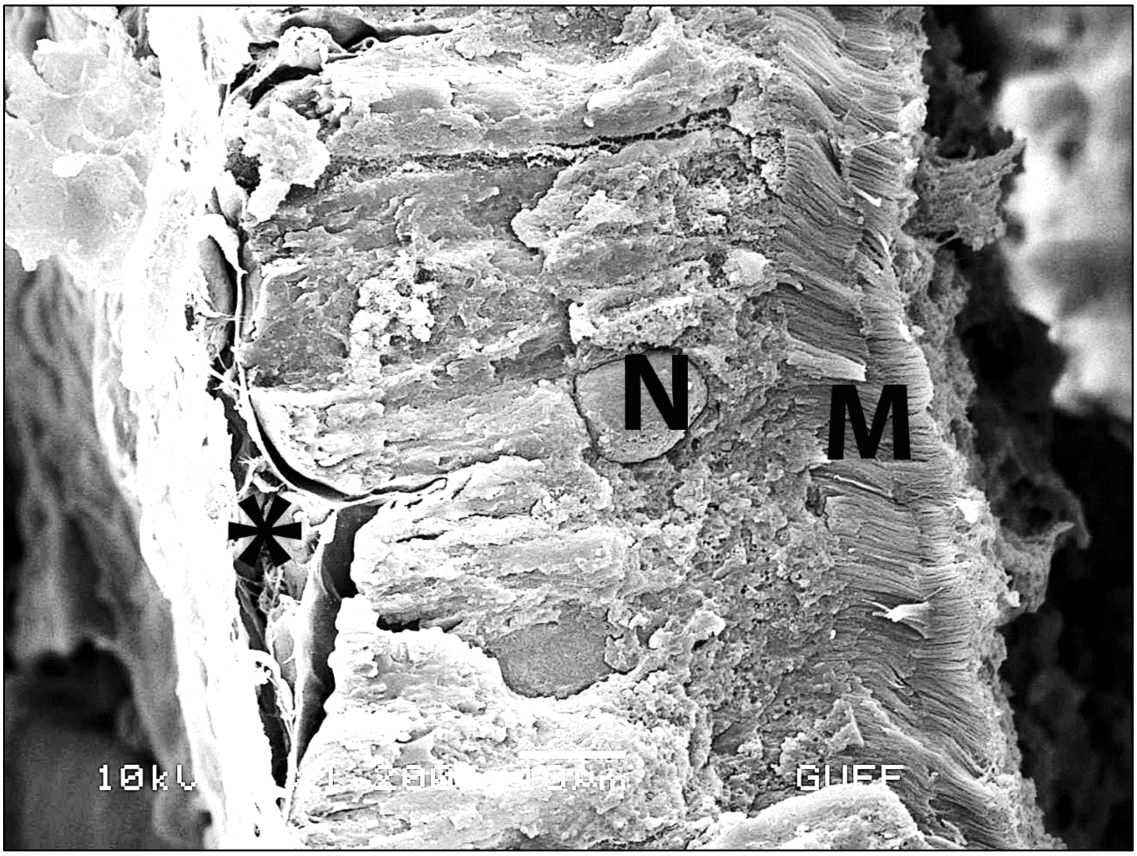

Figure 5. Scanning electron microscope images of the cross section of the ventriculus. M: Microvillus, N: Nucleus of the principle cell, *: Connective tissue (Scale bar: 10 µm)

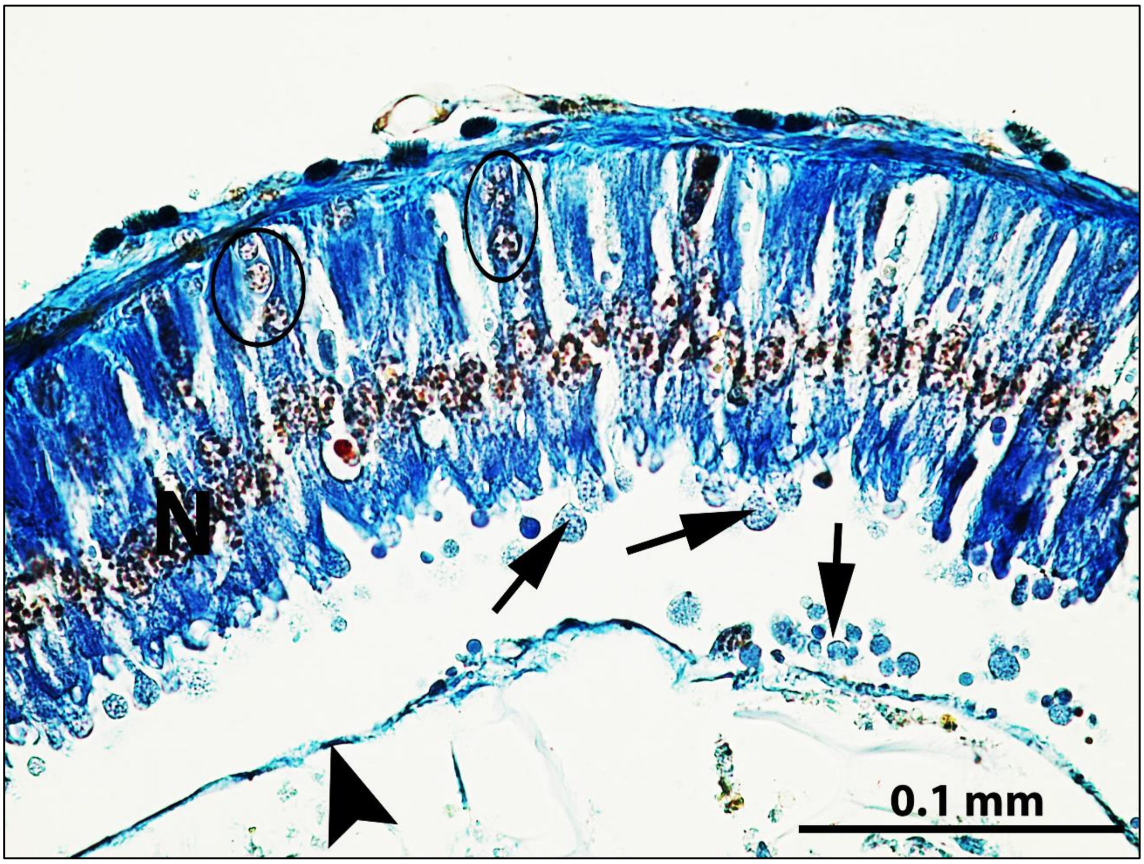

Figure 6. Light micrograph of the cross section of the ventriculus. Circled: Regenerative cells, N: Nucleus of the principle cell, →: Secretory granules, ►: Peritrophic membrane (Mallory's trichrome staining, Scale bar: 0.1 mm)

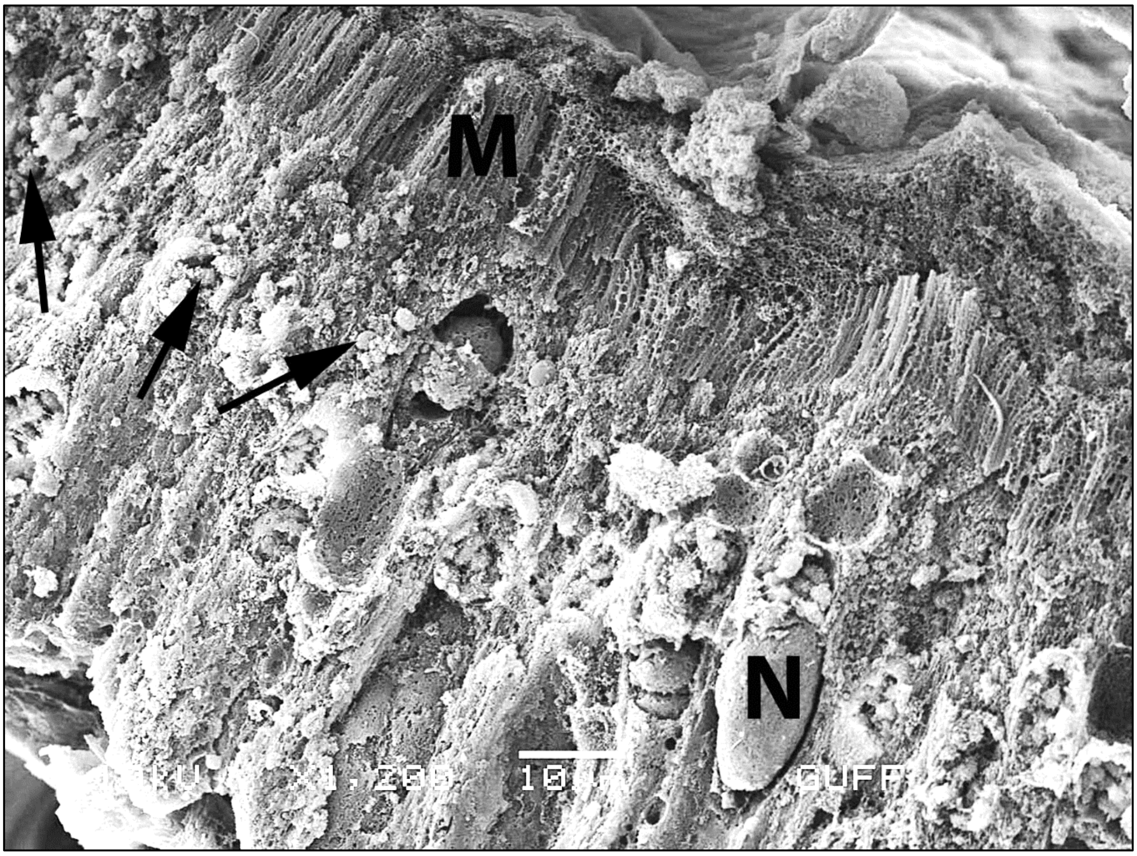

Figure 7. Scanning electron microscope images of the cross section of the ventriculus. M: Microvillus, N: Nucleus of the principle cell, →: Secretory granules in the cytoplasm of the principle cell (Scale bar: 10 µm)

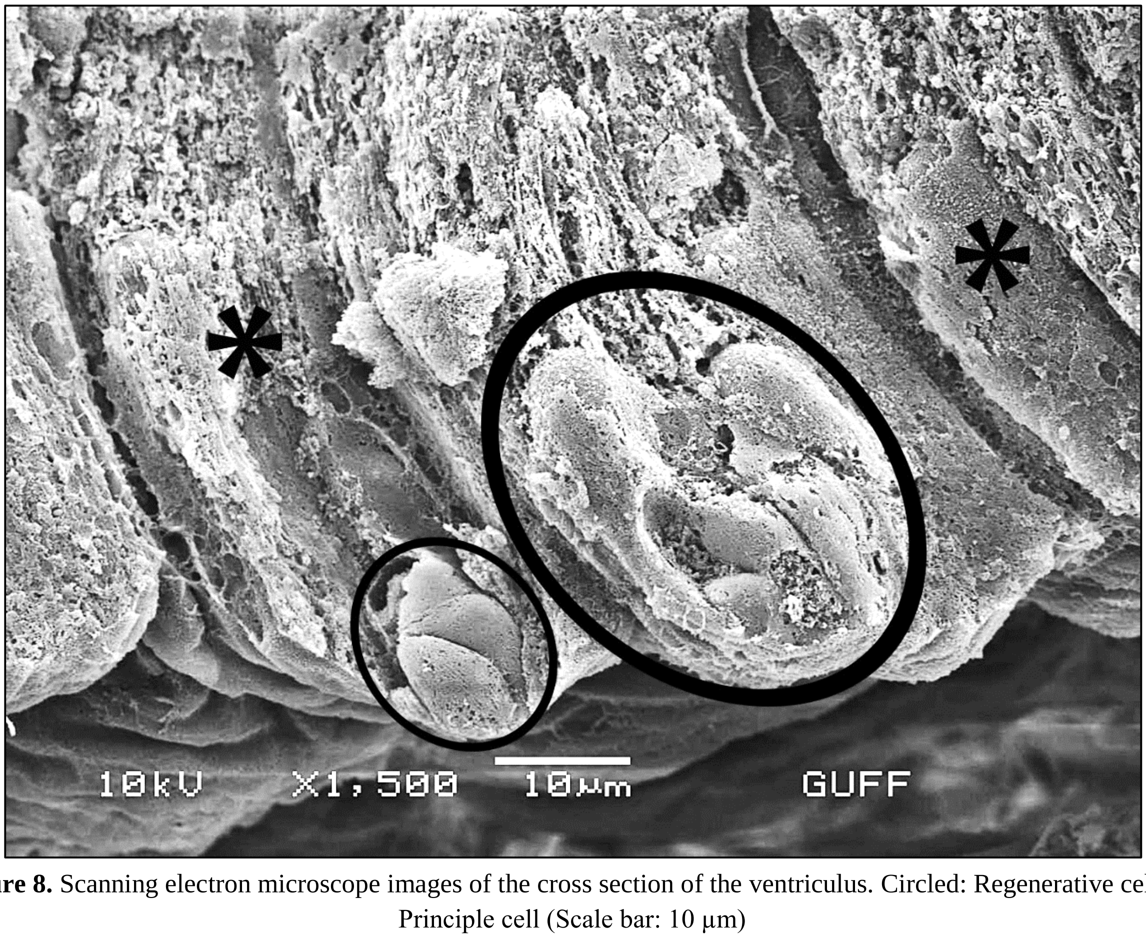

Figure 8. Scanning electron microscope images of the cross section of the ventriculus. Circled: Regenerative cell, *: Principle cell (Scale bar: 10 µm)

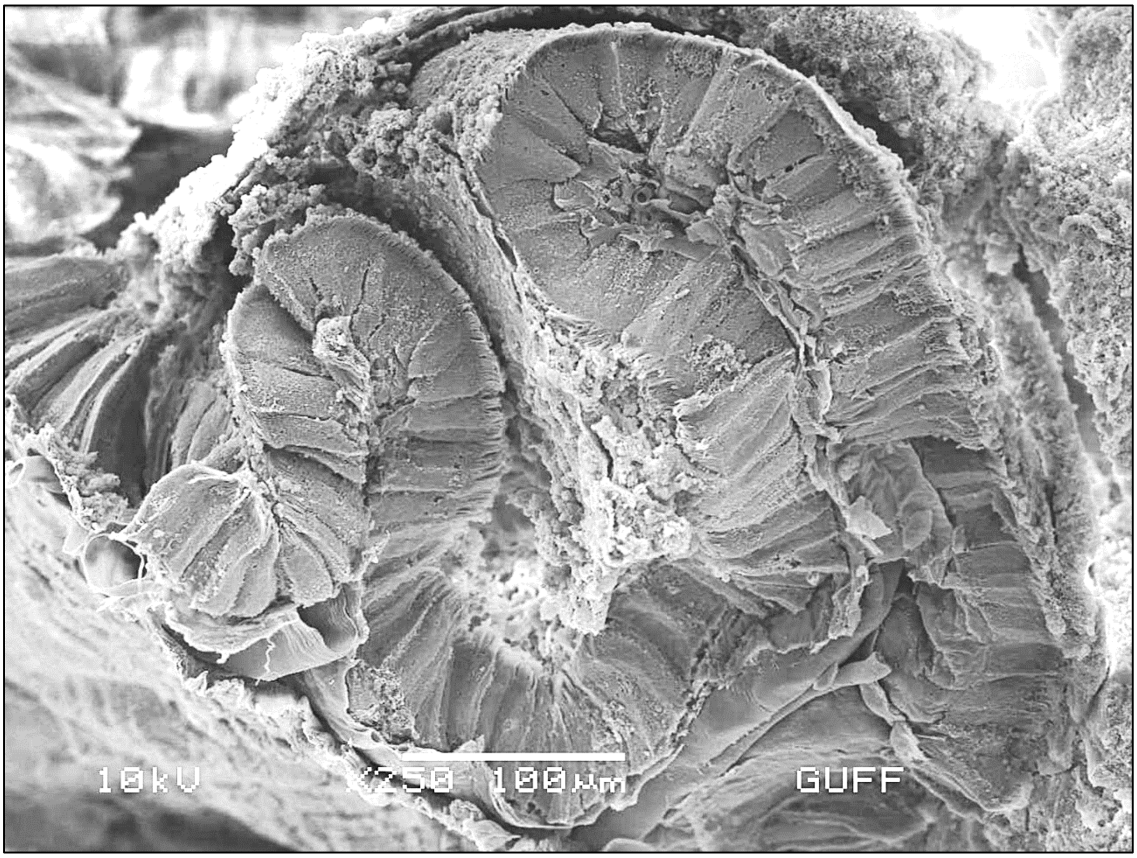

Figure 9. Scanning electron microscope images of the cross section of the gastric caecum which have the epithelial folds (Scale bar: 100 µm)

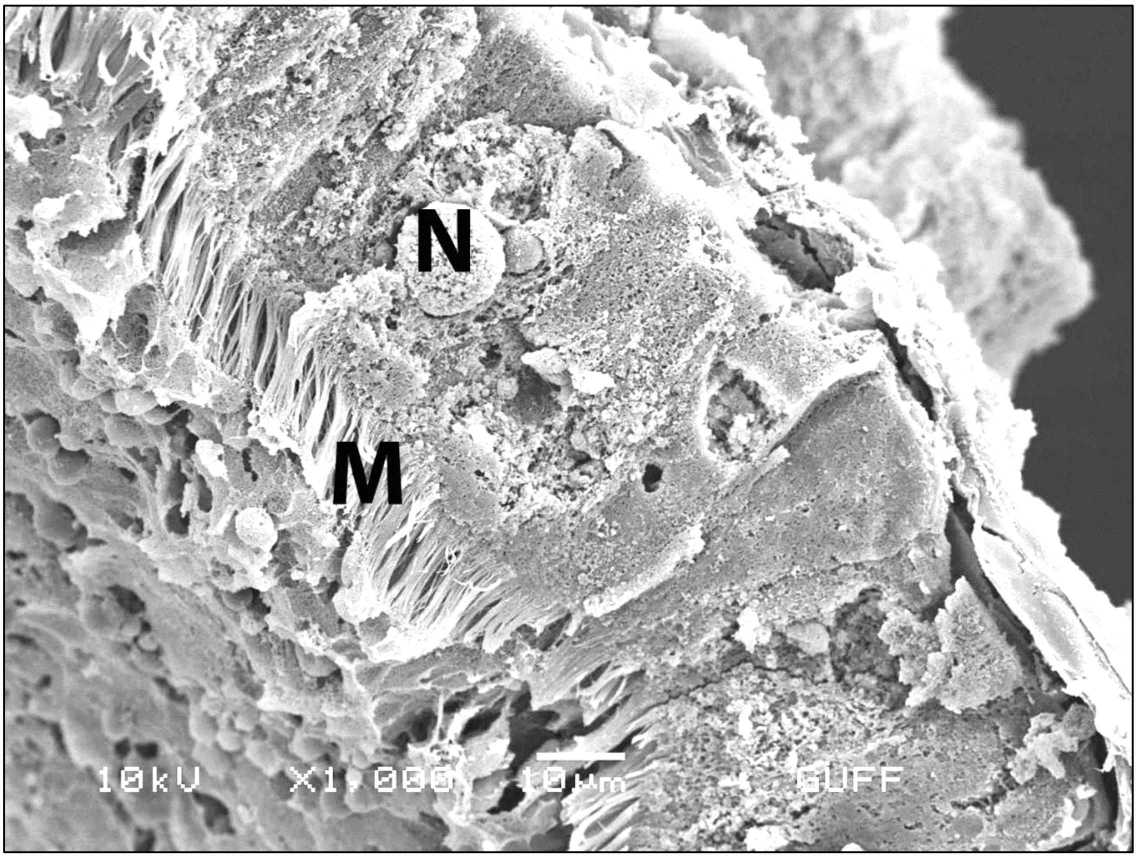

Figure 10. Scanning electron microscope images of the cross section of the gastric caecum. N: Nucleus, M: Microvillus (Scale bar: 10 µm)

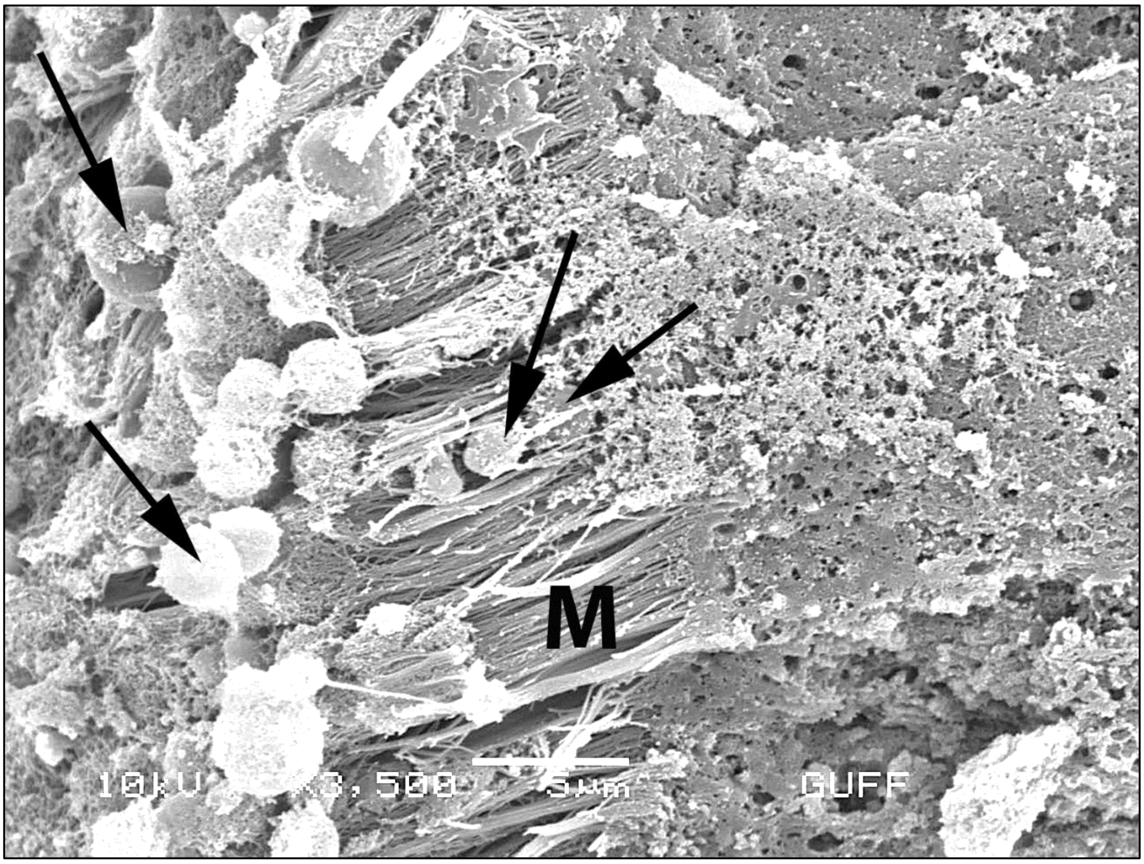

Figure 11. Scanning electron microscope images of the cross section of the gastric caecum. →: Secretory granules, M: Microvillus (Scale bar: 5 µm)

CONCLUSION

Results of this study show that the morphological and histological structures of the midgut in P. ataturki share considerable similarities with the midgut structure of other Orthoptera species, while there are also some morphological and histological differences. This study is aimed at providing preliminary information for other studies to be carried out on this insect species or to develop new methods in insect control.

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Conflict of Interest

The author declares that there is no conflict of interest.

Author's Contributions

I hereby declare that the planning, execution and writing of the article was done by me as the sole author of the article.

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