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Investigation of Digestive Tract of *Sphex flavipennis* Fabrius, 1793 (Hymenoptera: Sphecidae): Morphology and Ultrastructure

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ABSTRACT

In this study, the ultrastructure and histology of digestive tract of *Sphex flavipennis* was examined by light and electron microscopy. The digestive tract divided into foregut, midgut and hindgut. There are longitudinal and circular muscle in the haemocoel side and a cuticle in the luminal side of epithelium. The epithelium made of three kinds of cells; endocrine cells, regenerative cells and enterocytes. The cytoplasm of endocrine cells filled with secretory granules and lacked infoldings of basal plasma membranes. The regenerative cells are small, undifferentiating cells. The enterocytes have well developed infoldings of basal plasma membrane that are adjacent with mitochondria.

Key words: *Sphex flavipennis, Digestive tract, Ultrastructure, Morphology*

1.INTRODUCTION

The anatomical organization of the insect digestive system in general shows the same elements, albeit with eventual differences in the proportional development of particular parts, which can be related with the insect's dietary characteristics [1]. The gut is a simple tube connecting mouth and anus [2]. As in other animals, the alimentary canal in arthropods consists of vital organs necessary for life function such as digestion and absorption of nutrients, regulation of hemolymph ionic composition and pH, and detoxification, and production of semiochemical compounds such as pheromones [3]. In addition to, the digestive tract of insects is considered an effective physical and chemical barrier against the potentially invasive pathogens that are ingested with the feeding. The digestive tract of insects consists of three parts: foregut, midgut and hindgut [2,4-6]. While the appear to be some endocrine cells, a sparse innervation and an extensive trachaeal supply, the alimentary canal can be consider as a simple epithelium one layer thick, restring on a basement membrane of connective tissue, and with a thin, discontinuous layer longitudinal and transverse muscle outside [2].

In Hymenoptera, the foregut consists of the pharynx, esophagus, crop or honey sac and proventriculus [7,8]. The proventriculus is the most specialized part of the foregut, and lies between the honey sac and midgut. As the proventriculus is ectodermic in origin, it is lined by a sclerotized cuticular intima [7,8]. The one-layered foregut epithelium is capped on the lumen side by a cuticular intima. The epithelium is surrounded externally by the muscle layers. Usually there is a single layer of longitudinal muscle, but at places there may be two layers. Outside these are a few scattered circular muscle fibers [9].

It is well known that the insect midgut is responsible for food digestion and nutrient absorption [5,10]. The midgut is endodermally derived and so does not secrete cuticle [2]. The insect midgut is generally characterized by two cell types; columnar cells and regenerative cells [11,12]. Each columnar cell contains a large, centrally located, sometimes oval nucleus. The apical cell border is clearly

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striated [11]. Regenerative cells can be form groups of cells, the regenerative nests or crypts; they may also be found as single cells scattered among epithelial cells [12-14]. These cells multiply rapidly, due to symmetric or asymmetric divisions [12,15,16]. Whereas, some of the regenerative cells enter the division and differentiate into epithelial cells, the rest of them preserve their primary structure [12]. Midgut endocrine cells in insects are characterized by presence of clear cytoplasm, secretory granules mainly in the basal area, cytoplasmic processes reaching the intestinal lumen, and absence of basal plasma membrane infoldings since they are scattered in the base of the digestive cells [17-19].

The undigested material goes straight to the hindgut where the water absorption and feces formation and elimination occur [5]. Typically, the hindgut is composed of an anterior intestine and posterior rectum. In some insects, e.g. *Locusta migratoria*, the anterior intestine is further divided into ileum and colon [20]. The Malpighian tubules act as an insect kidney, filtering the blood, and producing isosmotic primary urine, which may pass forward into the midgut, as well as posteriorly into the hindgut [2].

In this study, we investigated morphology, histology and ultrastructural features of the digestive tract of *Sphex flavipennis* by scanning electron microscopy, light microscopy and transmission electron microscop

2. MATERIAL AND METHOD

2.1. Material

Adults of *Sphex flavipennis* Fabricus, 1793 (Hymenoptera: Sphecidae) were collected from Tokat, Pınarlı village, Turkey. The samples were killed by freezing and their digestive tracts were rapidly dissected from the abdomen under a binocular microscope.

2.2. Methods

2.2.1. Light microscopy

For the light microscopic studies, some samples were fixed in 10% formaldehyde with phosphate buffer for 24 h and other samples were fixed in Bouin's for 12h. Thereafter, the tissues were washed, dehydrated in a graded series of ethanol solutions (%70, %80, %90, %100) and finally embedded in paraffin. Paraffin sections were cut into 4-7µm thick slices and stained with hematoxylin and eosin for light microscopic examination. The sections were viewed and photographed by using a Nikon OPtihot-2 light microscope.

2.2.2. Scanning electron microscopy (SEM)

For the scanning electron microscopic studies, the alimentary canal was fixed in 3% glutaraldehyde in phosphate buffer, pH 7.2, for 4 h at 4°C. Then samples were dehydrated in a graded acetone series (%70, %80, %90, %100), critical point dried, coated with gold, and analyzed under a Jeol 5400 scanning electron microscope.

2.2.3. Transmission electron microscopy (TEM)

For the transmission electron microscopic examination, gut tissues of *S. flavipennis* were fixed in 3% glutaraldehyde in sodium phosphate buffer, pH 7.2, for 4 h at 4ºC. After fixation, samples were washed with the same buffer and postfixed in 1% osmium tetroxide and in sodium phosphate buffer, pH 7.2, for 1 h at 4ºC. Tissue samples were washed with the same buffer for 3 h at 4ºC, dehydrated in a graded ethanol series and embedded in Araldite. Thin sections were stained with 2% uranyl acetate and lead citrate. The sections were viewed and photographed under a Zeiss Libra 120 transmission electron microscope.

3. RESULTS

The digestive tract of *S. flavipennis* is a single, long tubular organ system and consists of the foregut, midgut and hindgut. The foregut consists of esophagus and a large proventriculus. The midgut situated between the foregut and hindgut and it is the longest part of digestive tract. There are two regions discernible with the unaided eye in the *S. flavipennis* midgut: anterior and posterior region. The hindgut is composed of ileum, colon and rectum. The Malpighian tubules are connected to junction region of midgut and hindgut. The hindgut and a part of midgut epithelium have many folds in abdomen. Histologically, the digestive tract, from outside to inside, consists of single-layer epithelium, lamina propria, circular muscle layer, longitudinal muscle layer and external connective tissue. There are the numerous tracheoles, which are rest on from connective tissue to inner parts. The epithelium is single layer epithelia and has various cell shapes from cubic to cylindrical. There is an intima layer in the lumen part of esophagus, proventriculus and hindgut. The thickness of intima layer of foregut is higher than hindgut.

In the SEM observation, the esophagus is a simple, straight tube. There are longitudinally folds with "V" shaped muscular fibers at the external surface of esophagus (Figure 1). In the light microscope observation, it is occurred that esophagus has a single-layer cubic epithelium and it was made folds like villi towards to lumen with intima in cross section. In this region, intima layer is almost same thickness with epithelial layer (Figure 2).

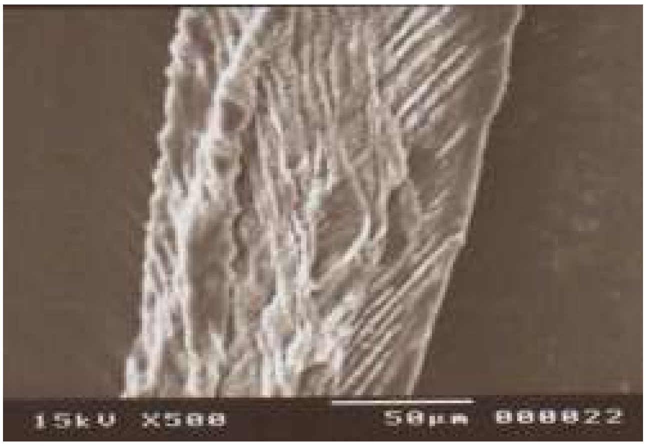

Figure 1. SEM micrograph of the esophagus in *S. flavipennis.*

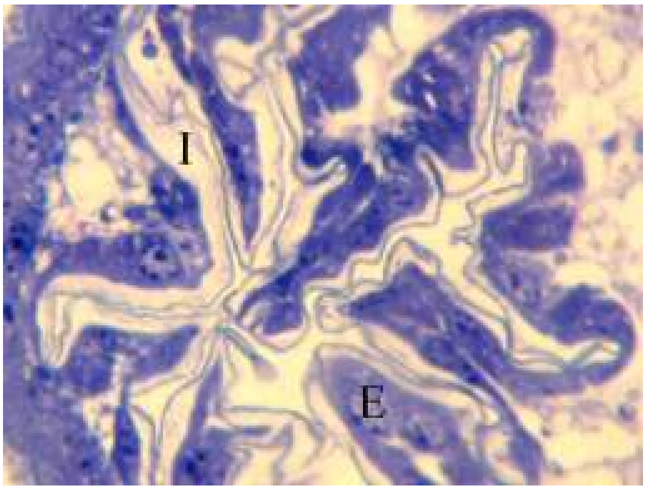

Figure 2. Light microscope micrograph of the esophagus in *S. flavipennis.* I: Intima layer, E: Epithelium, X 244.

The proventriculus is a pear shaped and beginning immediately at the end of thorax. The proventriculus is the large part of digestive tract. This region has different infoldings than the esophagus in the muscular layer, which is a parallel to digestive tract (Figure 3). In the light microscope observation, it is occurred that proventriculus has an epithelium layer, the intima layer and the hair-like structures extending to the lumen with the intima layer. The epithelium layer is a single-layer cubic epithelia and it has many folds like villi towards to lumen (Figure 4).

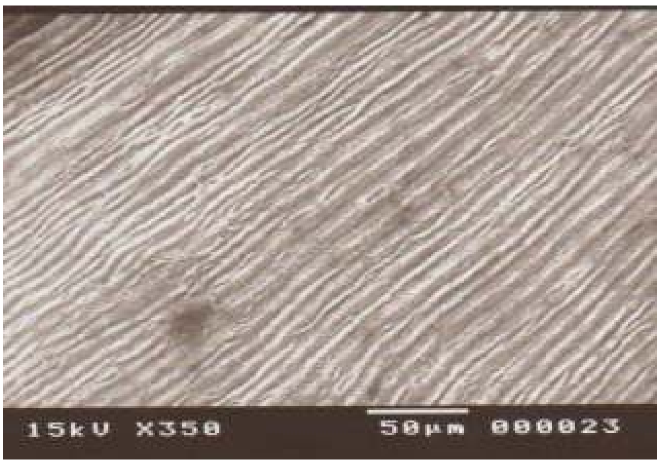

Figure 3. SEM micrograph of the proventriculus in *S. flavipennis.*

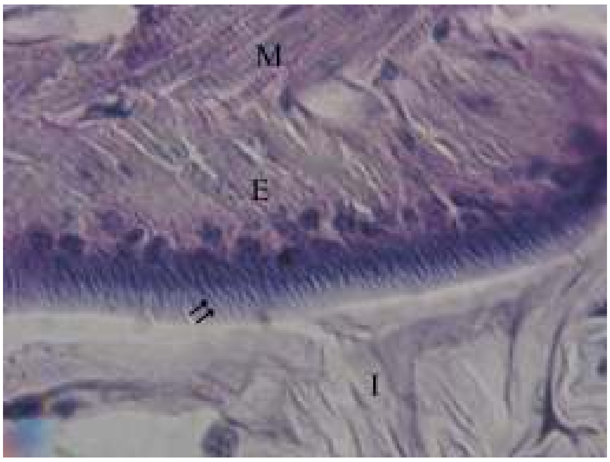

Figure 4. Light microscope micrograph of the proventriculus in *S. flavipennis.* I: Intima layer, E: Epithelium, M: Muscle layer, (⇉): Hair-like structures, X 200.

Morphologically, the midgut is almost the same thickness along its length. However, histologically, the anterior part of midgut consists of a thin intima, a single-layer of epithelium and muscle layer, from outside to inside. This intima is thin rather than in the intima layer of esophagus. There are three different cells in the epithelium of midgut; enterocytes, endocrine cells and regenerative cells (Figure 5). In the endocrine cells of anterior midgut, there are endoplasmic reticulum cisternae with dense granules in around the nucleus and in almost the every side of cytoplasm. Besides, there are secretory granules in this region (Figure 6,7). Also, it is observed that there are vacuoles in the cytoplasm of these cells (Figures 7). The nucleus is almost euchromatin structure in these cells. We were observed that there are junctional complexes in the lateral membranes of enterocytes in the midgut. The apical of these cells were surrounded by dense microvillie in the midgut (Figure 8). Regenerative cells can form groups of cells, the regenerative nests or crypts; they may also be found as single scattered among the epithelial cells (Figure 5).

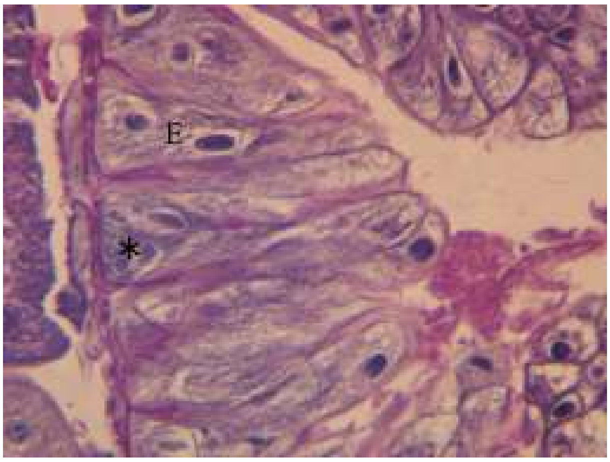

Figure 5. Light microscope micrograph of the midgut in *S. flavipennis.* E: Epithelium, (✱): Regenerative cells X 120.

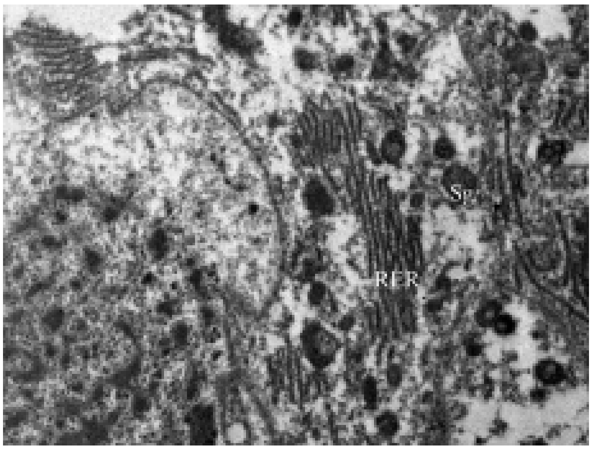

Figure 6.TEM micrograph of the endocrine cell in *S. flavipennis.* (Sg): Secretory granule, (RER): Rough Endoplazmic Reticulum X 8000

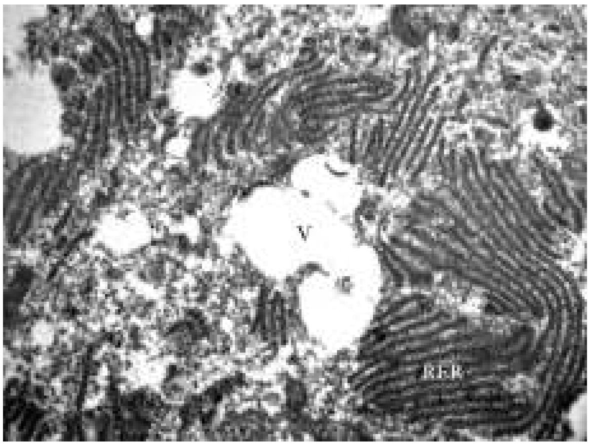

Figure 7. TEM micrograph of the endocrine cell in *S. flavipennis.* (V): Vacuole, (RER): Rough Endoplazmic Reticulum X 10000

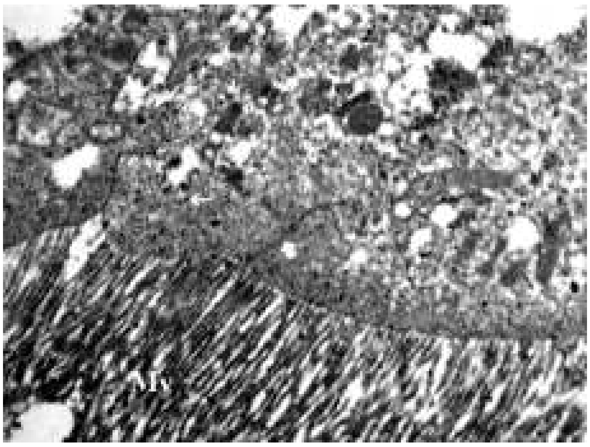

Figure 8. TEM micrograph of the enterocyt in *S. flavipennis*. (Mv): Mikrovilli, (➡): Junctional complexes, (➔): Free ribosome, X 12 500.

Posterior midgut cells also consists of three different cells; enterocytes, regenerative cells and endocrine cells. The regenerative cells were located in the basal region and nucleus has covered almost all of these cells. The apical of these cells do not extend to the intestinal lumen. The endocrine cells have a euchromatine nucleus and clearly a nucleolus within nucleus. It was observed that there are rough endoplasmic reticulum, mitochondria and secretory granules in the cytoplasm of these cells. The basal membrane infoldings of the enterocytes reached up to almost ½ length of cells. It was observed that there are abundant mitochondria between this infoldings (Figure 9).

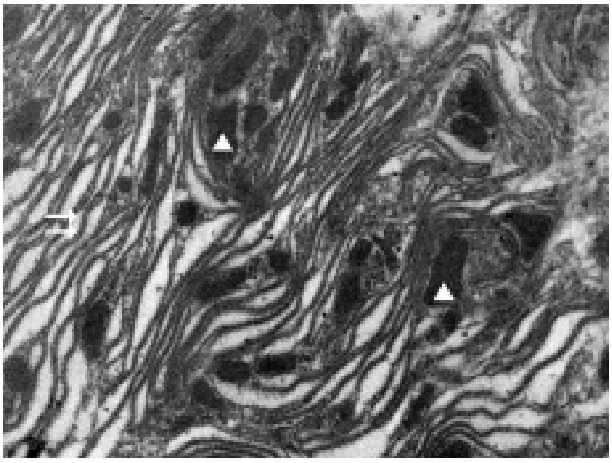

Figure 9. TEM micrograph of the endocrine cell in *S. flavipennis.* (⇉): Basal membrane infoldings, (▲): Mitochondrion, X 16000

The hindgut consists of longitudinal muscle layer, circular muscle layer, the epithelium layer that it is usually composed of cubic-shaped cells and a thin intima in part of lumen, from outside to inside. It is observed that there are subepithelial spaces between muscle layer and epithelial layer in some places of hindgut (Figure 10). The numerous of Malpighi tubules, which are attached to the combined area of the midgut and the hindgut, consists of 5-6 cells in cross section (Figure 11).

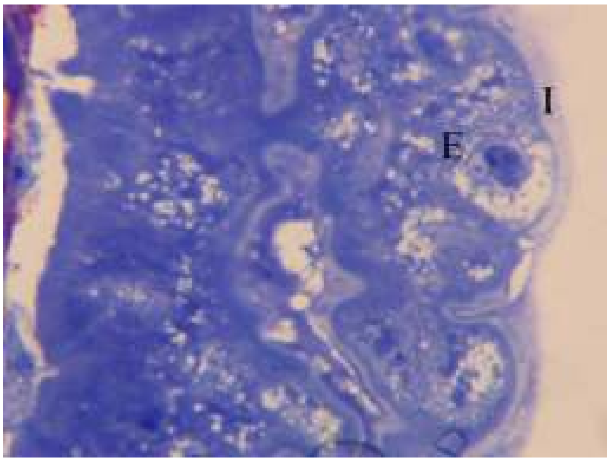

Figure 10. Light microscope micrograph of the hindgut in *S. flavipennis.* I:Intima layer, E: Epithelium, X 200.

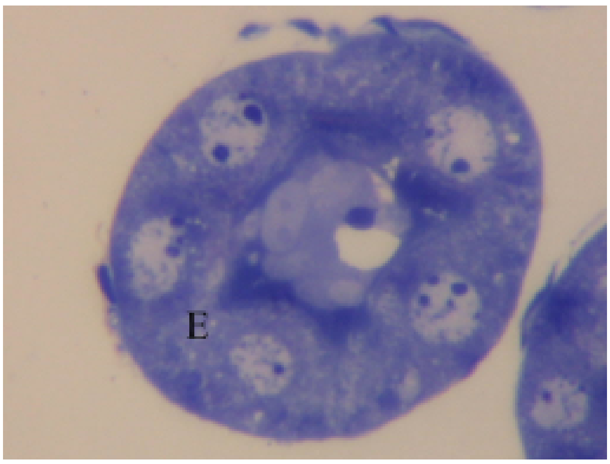

Figure 11. Light microscope micrograph of the Malpighi tubule in *S. flavipennis.* E: Epithelium, X 320.

4. DISCUSSION

As described by other many researches, the digestive tract of *S. flavipennis* consists of the foregut, midgut and hindgut [2,4-6,11,21]. It is reported that the foregut and hindgut because of ectodermic origin are lined by a cuticle layer (intima) and the midgut derives from the endoderm; therefore, it is not lined by a cuticle [21]. In our study, we observed that there are intima layer in the foregut and hindgut.

It was clearly observed that the esophagus of *S. flavipennis* has a well-developed muscle layer as described in the *Sitophilus granarius* by Baker et al. [22]. These folds may be help to progress through the channel of nutrition [23]. In previous study, it has been shown that the one-layered foregut epithelium of insects capped on the lumen side by a cuticular intima [9]. The epithelium of esophagus has a single-layer cubic cells and form folds like villi towards to lumen [3].

As described in the *Zacryptocerus rohweri* (Hymenoptera) by Roche and Wheeler [23], the proventriculus of *S. flavipennis* is shaped much like the cap of a typical mushroom. As the proventriculus is ectodermic in origin, it is lined by a sclerotized cuticular intima. In the previous study, it has been shown that this cuticle is highly sclerotized and generally many hairs arise from the upper parts of the lips to from a comb. This comb filters solid particles from the esophagus contents down to the midgut as a result of muscular action [8,22]. In our study, we observed hair-like structures (spines) extending to the lumen with the intima layer and well-developed muscular fibers in the outside of the proventriculus. A similar proventricular bulb is present in other Hymenoptera such as ants, wasps and spheciform wasps [7,8,26,25]. It is suggested that the spines in the contracted proventriculus prevent movement of food particles into the midgut and thus allow the crop to fill [22]. When the proventriculus was filled with the food, these muscles fibers may be allowed to expanded proventriculus and served as a place for store foods. In the previous study, it has been shown that proventriculus has an intima layer, a single-layer cubic epithelium and longitudinal muscles and circular muscles in the light microscopic observation [3]. The results of our observation are similar to them.

The insect midgut is the chief site both for digestion of food and absorption of nutrition, and the epithelium is responsible both for the production of many digestive enzyme and/or the uptake and transfer of nutriens to the haemolymph [26]. Apart from its role in digestion, absorbtion and excretion, the insect midgut forms a principal barrier against toxic influences from the environment [5, 27, 28]. Midgut is the longest of the gut regions, and its length varied in fed and starved conditions of the insect [9]. Generally, the insect midgut is divided into two region; anterior and posterior region [9, 22, 29]. We observed that the midgut of *S. flavipennis* consists of anterior and posterior region, which are almost the same thickness its length. However, there is a thin intima layer in beginning of anterior midgut region. This intima layer may have arrived with food from the esophagus. In the previous study, it has been reported that the midgut epithelium consists of a single layer of cells [30]. It has been shown that there are three different cells in the epithelium; enterocytes, regenerative cells and endocrine cells [27, 31]. In our study, we have observed similar results to these data. We didn't observed gastric caeca as described in the *Sitophilus granarius* by Baker et al. [22] and in the *Rhynchosciara americana* by Ferreira et al. [32].

It is suggested that the midgut can be considered the biggest endocrine organ of insect [19]. It is known that the secretory activity of gastrointestinal tract is important for the process of nutrients digestion and absorption [19]. As described by Brown et al. [17], Billingsley and Downe [33], Raes and Verbeke [34] and Neves et al. [35], the endocrine cells of anterior midgut were characterized with clearer cytoplasm than the digestive cells, rough endoplasmic reticulum, secretory granules, and without basal labyrinth. It is known that the endocrine cells are connected with the surrounding cells by septate junctions close to the apex [34]. In this study, anterior midgut endocrine cells show same ultrastructural characteristics with the midgut endocrine cells described for insects.

In numerous insect groups, the regenerative cells may form characteristic regenerative cells groups, which lie between the basal parts of epithelial cells [12,36]. These cells play important role in the epithelial regeneration, due to their capability for intensive proliferation and differentiation, which leads to the formation of new epithelia after degeneration [12,36,37]. It is known that these cells never possessed a surface exposed to the midgut lumen and the nucleus occupied most of the cell volume [33]. The regenerative cells have little cytoplasm which are few ribosome, mitochondria, endoplasmic reticulum and Golgi apparatus profiles suggesting low metabolic activity in these cells [31]. We have shown that the regenerative cells in the midgut of *S. flavipennis* are similar to described by those authors.

The enterocytes were numerous in midgut regions and theirs nucleus was generally centrally located [24]. These cells are concerned with enzyme secretion and with the absorption of the products of digestion [28]. As described by other many researches, the enterocytes of midgut in insects lay on the basal lamina and apically there lined by microvilli which border the gut lumen [10, 22, 26, 28-30, 38, 39]. Furthermore; same researchers described well-developed rough endoplasmic reticulum, free ribosomes, mitochondria, secretory granules and Golgi complexes in the enterocytes. It has been shown that basal region of these cells characterized by infoldings of the plasma membrane associated with mitochondria. This localization of mitochondria is typical in the epithelia involved in the ionic active transport [10, 28, 29]. It is known that the midgut cells are laterally joined by a junctional complex composed of long continuous junctions in the apical part and gap junctions in the basal part [10, 20, 28, 30]. In this study, the anterior and posterior midgut enterocytes show same ultrastructural characteristics with the midgut enterocytes described for insects.

The hindgut of most insects may be morphologically subdivided in pylorus, ileum, colon and rectum and it is internally lined by a continuous epithelium covered by a cuticular intima [5]. It is reported that this intima of the hindgut is thinner than the foregut because this region is responsible for water and nutrient absorption [40]. In the previous study, it is shown that the epithelium of hindgut composed of cubic cells [5]. The epithelium rests on basal lamina and there are circular muscles and outer longitudinal muscles, as described by many researchers [5,30,40], suggests a greatly increased surface area for absorption and bacterial attachment as well as an ability for the hindgut to move and mix its contents [23]. We have shown that hindgut of *S. flavipennis* were similar to described by those authors.

Malpighian tubules play a role in the excretory process and in the iron water homeostasis [40]. In the excretory process in insects, the primary isoosmotic urine is produced in the Malpighian tubules and discharged into the ileum, where its volume and the composition are adjusted along the hindgut according to insect's needs. This way, substance such as water, ions and metabolic residues are reabsorbed from the material present in the lumen [40]. In our study, we observed that the numerous of Malpighi tubules, which are attached to combined area of the midgut and the hindgut, consists of 5-6 cells in cross section.

In this study, we were investigated morphological, histological and ultrastructural features of digestive tract of *S. flavipennis*. The features of foregut, midgut and hindgut were found to have differences from both morphological and histological structures which help us to clarify and better understand each particular organ in the alimentary canal of this species.

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