Asian Journal of Biological Sciences 6 (1): 54-66, 2013 ISSN 1996-3351 / DOI: 10.3923/ajbs.2013.54.66 © 2013 Knowledgia Review, Malaysia

# Ultrastructural Study on the Midgut Regions of the Milkweed Bug, Spilostethus pandurus Scop., (Hemiptera: Lygaeidae)

Afaf A. Meguid, Hanan H. Awad, Aziza H. Omar and Heba A.S. Elelimy Department of Entomology, Faculty of Science, Cairo University, Egypt

Corresponding Author: Hanan H. Awad, Department of Entomology, Faculty of Science, Cairo University, Egypt

#### ABSTRACT

The present study aims to investigate the morphological, histological and cytological changes among the four midgut regions of *Spilostethus pandurus* adult. The adults were dissected to examine the four midgut regions. The cellular structure of the midgut was clarified by means of light and electron microscopy. The midgut consists of a single layered epithelium of two cell types. Most cells were binucleated and this may be due to active digestive properties. The midgut region has no peritrophic membrane but has internally a perimicrovillar membrane. The electron dense glycocalyx layer separates the plasma membrane from an outer or perimicrovillar membrane which is produced continuously into the midgut lumen, possibly also from secretory lysosome-like vesicles. The lysosomes and large amounts of rough endoplasmic reticulum indicate that the second, third and fourth midgut regions play a role in food digestion. The lipid in the second midgut region suggests that this region plays only a minor role in lipid absorption and energy storage. The distribution of different cell types in *S. pandurus* midgut could be correlated with the physiology of digestion and absorption.

Key words: Spilostethus pandurus, midgut, morphology, histology, ultrastructure

## INTRODUCTION

The milkweed bug, *Spilostethus pandurus*, has long been established in Egypt as one of the serious pests infesting the seeds of a great number of plants. Their economic importance has been steadily increased in recent years and the annual losses due to their ravages against vegetables and other crops are sometimes great and immeasurable. *S. pandurus* is highly polyphagous and is reported from 15 or 16 families of crop plants. Nevertheless, there are many economic records on *S. pandurus* damaging crops. In Egypt, it is found all over the country and can be encountered in fields all the year round and was recorded as a pest of sunflower seed (El-Shazly, 1995).

The alimentary canal of S. pandurus is morphologically relatively simple. There are no specialized structures such as stomodeal crop or proventriculus. Also gastric caeca are absent (Habibi et al., 2008). Woodring et al. (2007) found that the midgut regions consisted almost entirely of a three-part midgut (ventriculus), designated ventriculus 1 ( $V_1$ ), ventriculus 2 ( $V_2$ ) and ventriculus 3 ( $V_3$ ). Several investigators illustrated that the gut in hemipterous insects has been co-evolved with their food sources resulting in different structures and tissues development as the protective peritrophic membrane in the midgut of some insects has been replaced by a perimicrovillar membrane (PMM) (Terra et al., 2006; Azevedo et al., 2009; Fialho et al., 2009). In spite of the importance of S. pandurus feeding, relatively little is known about the cellular differentiation of its midgut regions. The aim of the present study was to clarify the midgut cellular structure by means of light and electron microscopy.

## MATERIALS AND METHODS

**Insect:** Milkweed bug adults, 2-days old, were used in the present study. Stock culture of *S. pandurus* was maintained in the laboratory of Entomology Department, Faculty of Science, Cairo University at 25±3°C and 65±5% RH and a photoperiod of 14:10 (L:D) h. The insects were fed on dried row sunflower seeds (*Helianthus annuus*).

Electron microscopy (EM): The each milkweed bug adults was dissected to remove the midgut which was placed in a drop of prefixation solution (2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3), for at least two hours at 4°C. Then it was rinsed overnight at 4°C in 0.1 M phosphate buffer and post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer pH 7.3 for 1 hour at 4°C. The post-fixed tissue was washed in distilled water for 2 min, followed by dehydration in a graded series of acetone. Infiltration was carried out in 1:1 acetone: Epon mixture for several hours in a slow revolving drum (3 rpm) to ensure proper Epon penetration, then embedded in Epon followed by polymerization for two days at 60°C. Ultra-thin sections were cut using MT2-B ultra-microtome, stained with lead citrate (Reynolds, 1963) for 15 min and examined using a Joel JEM-1200 EX II transmission electron microscope. Semi-thin sections were stained with toluidine blue (1%) and observed under a Cambridge light microscope.

## RESULTS AND DISCUSSION

The present study revealed that the midgut (mg) of S. pandurus is composed of four different regions, 1st; 2nd; 3rd and 4th mg, respectively (Fig. 1). The midgut is found just posterior to the esophageal valve. The 1st mg region is greatly enlarged and varies greatly depending upon the quantity of food material present within it. It is almost one-third the length of the entire tract. Its wall at the anterior end usually lies in circular folds, while in the posterior; it enlarges in pear-shaped smooth walled structure. In the thoracic cavity it is completely enclosed by thick bunches of wing and leg muscles (Fig. 2a, b). The 2nd mg regions is a narrow long tubes slightly folded (Fig. 3), while the 3rd mg region, (Fig. 4a, b), is thicker and oval to spherical in shape. It narrows down into a short, narrow and smooth-walled tube forming the 4th mg (Fig. 5). The epithelial cells are fitted firmly together along the lumen cavity (Fig. 4, 5). The lumen in the 1st mg is wide and the cells are pointed or finger-shaped. The apical striated border seemed darkly stained and extended into the lumen. The circular muscles are thin consisting of almost a single strand and longitudinal muscles are few and scattered. The 2nd mg has a with wide lumen, the epithelial cells are mostly columnar and more uniform. The apical part of the cells seemed darkly stained covered with dense secretory products. The regenerative cells lie beneath the epithelial cells. The circular muscle layer consists of one strand and the longitudinal muscles are also few and scattered. The epithelial cells of the 3rd mg are mostly long columnar cells; some cells are larger than others, thus making the epithelium irregular in thickness. The lumen of this region of the gut and the apical part of the cell appears full of lipid globules that cover the apices of the cells. The nuclei are more or less centrally located and some of the cells are binucleated. The activity of the digestive epithelium is noted particularly in the third midgut region. The apical cell membrane of some cells seems to extrude into the lumen, may be due to bursting of the cells by due to the pressure of lipids. Also, regenerative cells appear with prominent nuclei underneath the columnar cells (Fig. 4a-b). A layer of two strands of circular muscles is present. The longitudinal muscle layer is very scarce in the third midgut region. The 4th mg (Fig. 5) shows that the gut seems much narrower than the previous regions. The cells are very compacting mostly columnar and irregular

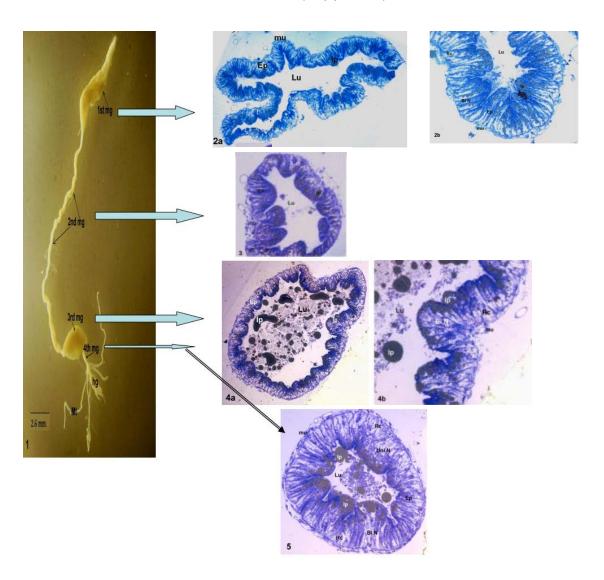


Fig. (1-5): (1) Photo micrograph of the alimentary canal of *S. pandurus*, referring to the midgut (mg) regions, 1st; 2nd; 3rd and 4th and hindgut (hg) consists of the pylorus with 4 malpighian tubule (Mt), (2-5) Photo micrograph of: (2a-b) 1st mg, (3) 2nd mg, 4(a-b) 3rd mg and (5) 4th mg regions, (semi-thin section), showing binucleated (Bi N), columnar epithelial cells protruding epically (Ep), wide lumen (Lu), lipid (lp) and muscle (mu), uninucleated cells (Uni N), regenerative cells (Rc)

in shape. Apical border shows dense lipid globules almost protruding into the lumen. The polymorphic nuclei are more or less centrally located and most of the cells are binucleated. Small regenerative cells nidi and isolated endocrine cells were scattered, in the base of the digestive cells, among several columnar cells. These endocrine cells were found in the V1-V4 midgut regions. The longitudinal and circular muscles layer was well-developed with a very thin margin compared to size of cells (Dunn and Stabb, 2005). Woodring et al. (2007) found that the midgut regions consisted almost entirely of a three-part midgut (ventriculus). The insect midgut epithelium consists

of three main cell types: the digestive cells or columnar, principal, cells which play a role in digestive enzymes secretion and absorption of water and nutrients (Guedes et al., 2007). The regenerative cells play the role of primordial cells for the newly formed epithelial cells. They are responsible for the regeneration of the entire epithelium or its individual cells (Martins et al., 2006). Endocrine cells which release hormonal peptides that control the digestive processes (Serrao and Cruz-Landim, 1996b; Neves et al., 2002, 2003). Habibi et al. (2008) reported that inspite of the fact that the morphology of the alimentary canal of Lygus hesperus showed a relatively unadorned tube compared with other hemiptera, with variably shaped compartments, the epithelial cells of the gut are relatively complex. The same author reported that the main cells in midgut of L. hesperus are endocrine and columnar cells. However, it was difficult to detect the presence of endocrine cells in the gut of S. pandurus by means of the routine microscopic techniques. The light micrographs and electron micrographs taken in the present study showed clearly that the midgut of S. pandurus is composed of a single layered epithelium resting on a continuous basal lamina or basement membrane followed by external longitudinal and internal circular muscle fibers. The epithelium is constituted composed of two basic cell types, digestive and regenerative cells. The midgut of insects has a discontinuous circular muscle layer and a longitudinal one, but the number of layers differs with species and diet (Lehane and Billingsley, 1996).

**Ultrastructure of midgut regions of** *S. pandurus* adult: In the present study, it was found that the normal midgut of the milkweed bug *S. pandurus* is composed of a monolayer of epithelial cells and regenerative cells resting on a basement membrane. The luminal surface of the epithelial cells is thrown into numerous microvilli, filled with very minute fibrils. The cytoplasm of the epithelial cells is packed with organelles, mainly mitochondria, rough endoplasmic reticulum, free ribosomes, lysosomes and lipid droplets. The mitochondria are cylindrical or cup-shaped having outer and inner membranes, the inner membrane is folded into cristae which are oriented mainly transversely.

The epithelial cells of the anterior midgut (mg<sub>1</sub>) showed ultrastructural differences from mg<sub>2</sub>, mg<sub>3</sub> and mg<sub>4</sub>. A layer of perimicrovillar membrane appears adjacent to the outer border of the microvilli (Fig. 6). Compact and slender microvilli appeared covered with perimicrovillar membrane and extended toward lumen as narrow tubes forming an extended network bordered with secretory products (Fig. 7-10). Mitochondria appear small and numerous under apical region, rod-like cisternae of short RER strands, secretory vacuoles and a large number of lysosomes are also clear (Fig. 7-11). Figure 6-10 show polymorphic mitochondria, condensed under microvillar border. Figure 7 shows concentrated mitochondria and large amount of secretory vacuoles lying beneath microvillar border. Lipid globules and relatively dense secretory vacuoles were observed in Fig. 9. Adjacent cells are connected laterally with septate junctions (Fig. 10). Blebs extruding secretions from microvillar membrane and double membrane vesicles were also observed in apical cytoplasm appeared in Fig. 9 and 10. Binucleated cell appears in Fig. 8. Rough endoplasmic reticulum was observed (Fig. 10). Lamellate lysosomes were observed in midgut one (Fig. 11). The basal cell appears folded forming a basal labyrinth (Fig. 12). The basement membrane and muscle layer appear in Fig. 12 and 13.

The microvilli of mg<sub>2</sub> are not uniform as in first region mg<sub>1</sub>. Mitochondria are less condensed in the apical region. Large lipid globules appear more especially in anterior region of the cell, lysosomes are larger in size and density than in mg<sub>1</sub> and appear as large autophagic lamellate structures (Fig. 14, 16). Figure 14 shows microvilli, lipid globule, lateral cell membrane,

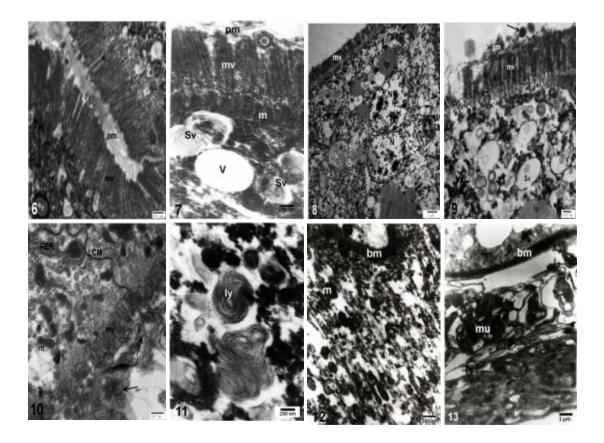


Fig. (6-13): Electron micrograph of epithelial cells from the 1st midgut region of *S. pandurus*, showing (6) Apical portion of cell, microvilli (mv) (slender and compact) and mitochondria (m) relatively dense, (7) The permicrovillar membrane (pm) covering the microvilli (mv), large vacuole (v) and large secretory vacuoles (Sv), (8) Binucleated cell (N) and lipid globules (lp), (9) Lysosomes (ly) secretory vacuoles (Sv), extruding secretion from microvillar membrane (arrow) and lipid globule (lp), (10) Blebs extruding from microvillar border (arrow), lateral cell membrane (LCM), mitochondria (m) and rough endoplasmic reticulum (RER), (11) Lamellate lysosomes (ly), (12-13) Basement membrane (bm), mitochondria (m) and muscle (mu)

lamellate lysosome and perimicrovillar membrane. Many secretions extruding into lumen appear in Fig. 15-16. Most of the epithelial cells appear uni-nucleated with one nucleus (Fig. 17-18). Figure 19 shows basal cell membrane with many infolding, basement membrane and tracheole (Fig. 20). The rough endoplasmic reticulum is relatively denser than that in mg<sub>1</sub> (Fig. 21).

The microvillar borders from the microvilli of mg3 were not as compact as in the previous regions (Fig. 24-25) and the perimicrovillar membrane appears in Fig. 22. The abundance of lipid droplets and lysosomes at the apical of the epithelial cells were very high. The secretory vacuoles appear extruding their products out of the apical membrane (Fig. 25). Cells filled with large and numerous lipid globules are clear mostly in the apical region (Fig. 22-24), the basal plasma membrane was infolded forming narrow channels towards underlying haemolymph. Many

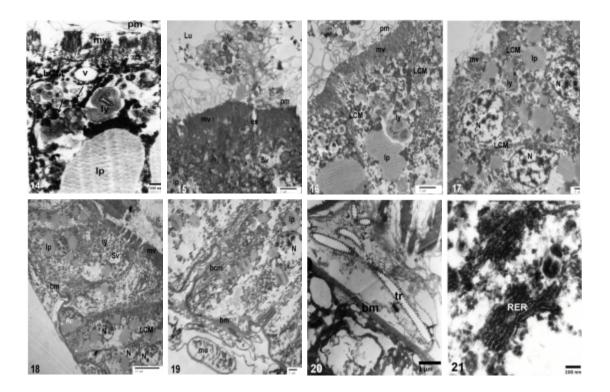


Fig. (14-21): Electron micrograph of epithelial cells from the 2nd midgut region of *S. pandurus*, showing (14) Microvilli (mv), vacuole (v), lipid (lp), lateral cell membrane (LCM), lamellate lysosome (ly), perimicrovillar membrane (pm) and electron-dense granules (arrow), (15-21) Extruding secretions (es) into lumen (Lu) from microvillar border, condensation of secretory vacuoles (Sv) under apical border, lipid globules (lp), rough endoplasmic reticulum (RER) and perimicrovillar membrane (pm) and regenerative cell (Rc) contains lipid globules, (19) Basal cell membrane (bcm), nucleus (N), lipid globule (lp) and muscle bundle (mu), (20) Basement membrane (bm) and tracheole (tr)

mitochondria appear polymorphic and relatively dense under apical cell membrane (Fig. 25). Some epithelial cells appear binucleated with two nuclei in the same cell (Fig. 24) and some cells appear uni-nucleated (Fig. 22-23). Figure 26 shows basement membrane and muscle layer. The microvilli from the mg4 were shorter than other regions, not compact and covered with perimicrovillar membrane (Fig. 28). Lipid globules are evident extruding into the lumen and epithelial cells appear binucleated (Fig. 27). Many secretions appear to be extruding from cells into the lumen (Fig. 29). The lipid globule appears surrounded by dark granules which are probably rough endoplasmic reticulum (Fig. 28). Most of the cells are binucleated (Fig. 27). Figure 28-29 show microvilli and extruding secretion from cell. Figure 30 shows muscle layer and tracheole cell appears ensheathed by connective tissue.

Very little is known about the cellular anatomy of the alimentary canal of *S. pandurus* in spite of its importance as a pest of seed plants, especially the sunflower plant which is important for the yield of its oils. In spite of the simplicity of the gross anatomy of the alimentary canal the

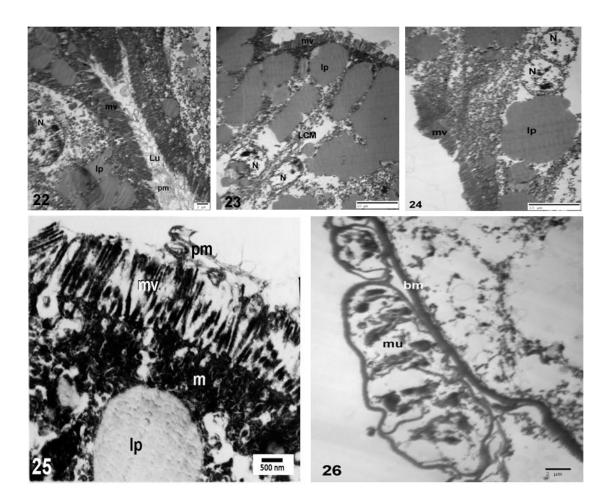


Fig. (22-26): Electron micrograph of epithelial cells from the 3rd midgut region of *S. pandurus* showing basement membrane (bm), mitochondria (m), muscle layer (mu), microvilli (mv), perimicrovillar membrane (pm), extruding secretion from microvillar border into lumen (Lu), lipid globules (lp) and nucleus (N) separated by lateral cell membrane (LCM)

investigation of its cellular anatomy was more complex. The morphology of principal cells varies according to species, alimentary cycle and midgut region. They show microvilli and basal labyrinth, modifications of the apical and basolateral cell membranes that are responsible for increasing the cell surface contact with the midgut lumen and the haemolymph (Billingsley and Downe, 1983; Serrao and Cruz-Landim, 1995, 1996a; Terra et al., 2006). The electron micrographs showed more clearly the main architecture of the different midgut regions. The four midgut regions of S. pandurus contained principal cells with distinct structural features, suggesting that there were different functions among them. The anterior region of the gut could be the main site of active transport of ions. In the first midgut region, the presence of spherocrystals suggests that the principal cells were active in water absorption and ionic regulation. The apical cell membrane or microvillar border is dense and compact and the basal cell membrane forms a basal labyrinth. The basement membrane appears dense and amorphous. It is surrounded by a layer of muscle cells and

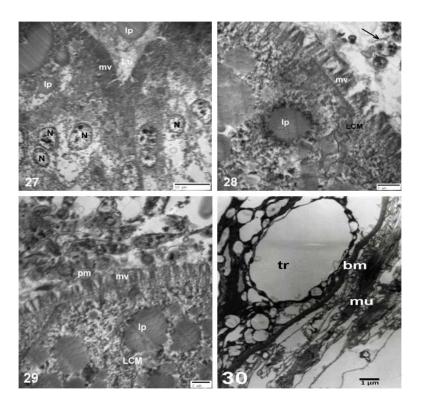


Fig. (27-30): (27-29) Electron micrograph of epithelial cells from the 4th midgut region of S. pandurus showing microvilli (mv) shorter than other region and not condensed, lateral cell membrane (LCM), lipid globules (lp), nuclei of binucleated and uninucleated cells (N) and (30) Basal cell membrane (bm), muscle (mu) and tracheole (tr)

tracheoles. The cells of the first midgut region of S. pandurus contained some lipid inclusions in the cytoplasm, suggesting that this region is not actively involved in the absorption and metabolism of lipids. The third and fourth midgut regions clearly showed that the cells contained relatively dense amounts of lipid globules. In these two regions also the secondary autophagic vacuoles were relatively dense. This may indicate that in these regions there is active digestion and absorption. Azevedo et al. (2009) suggested that the presence of smaller amounts of lipid inclusions that in the middle midgut of C. hemipterus indicates that this region plays only a minor role in lipid absorption and energy storage.

According to Habibi et al. (2008), the anterior area of the gut or ventriculus contains nearly all regenerative cells, endocrine cells and columnar cells. The middle area contains both opaque and lipoid cell types but the third or last is a lipoid zone. The mitochondria were more numerous in the apical region of the cell under the microvillar border than in the basal region. The most prominent organelles observed were the secretory vacuoles and lysosomes. Rough endoplasmic reticulum was not very prominent. The cell nucleus in midgut cells of S. pandurus appears spherical and in most cells is centrally located or just below the apical region. Most cells are binucleated, this could be due to high cell activity in which large quantities of nucleic acid move in and out of the nucleus to generate synthesis and secretion of protein enzymes. The anterior gut in hemiptera is supposed to

## Asian J. Biol. Sci., 6 (1): 54-66, 2013

be the major site of water absorption from ingested food (Silva *et al.*, 1995; Billingsley and Downe, 1989; Fialho *et al.*, 2009) and could also be the site of absorption and metabolism of carbohydrates and lipids.

Woodring et al. (2007) found that the contents of the first region ( $V_1$ ) of the gut of Oncopeltus fasciatus after freshly ingesting food from the sunflower seeds consist of 50% water, 46% lipid, 5% protein and 1% soluble carbohydrates. From the present ultrastructure micrographs, it could be concluded that the second and third midgut regions of S. pandurus could be the main sites of digestion. The perimicrovillar membrane appeared more clearly in the first region than 2nd and 3rd regions, dense deposits of lipid globules were very apparent and relatively high density of lysosomes. Rough endoplasmic reticulum was more developed. The relative density of the lipid deposits in the second and third midgut regions was relatively high. The third and fourth midgut regions of S. pandurus probably share the digestive functions with the second midgut region. However, the nutrient absorption may be more intense in the third and fourth midgut regions, because of the longer microvilli and the higher concentration of mitochondria as observed in the present micrographs. The light micrographs of the fourth midgut region in the gut of S. pandurus showed clearly that in this part of the gut the lumen was very narrow. Also the frequency number of binucleated cells was greater more in the fourth midgut region of the present investigated insect indicating high metabolic activity.

Woodring et al. (2007) reported that the junction of the midgut and hindgut in the nymphal stages of Oncopeltus fasciatus lacked a lumen. The same author reported that in the larval or nymphal stages of Oncopeltus fasciatus, 3 µL large oil drop (>85% triglycerides) accumulates in the third midgut region. The luminal plasma membrane of the midgut epithelium in insects is organized into regular folds of microvilli to increase the surface area of the cells available for absorption. The microvilli are typically covered with an electron dense fuzzy membrane termed glycocalyx. In Hemiptera the electron dense glycocalyx layer separates the plasma membrane from an outer or perimicrovillar membrane which is produced continuously into the midgut lumen (Billingsley and Downe, 1983) possibly also from secretory lysosome-like vesicles (Andries and Torpier, 1982).

Terra (1988) reported that although hemipteran insects lack a true peritrophic membrane, the perimicrovillar membrane (PMM) is an extracellular lipoprotein membrane ensheathing the microvilli which extend to the midgut lumen. The PMM and the microvillar membrane limit a closed space, the perimicrovillar space. This membrane is important for protein digestion (Billingsley and Downe, 1989), compartmentalization of the digestive process (Silva et al., 1995) and amino acid absorption from diluted diets (Terra et al., 2006).

Non xylem feeding Heteroptera achieve enzyme compartmentalization in the second region of the gut by means of the perimicrovillar membrane (Terra et al., 1996; Binnington et al., 1998). Woodring et al. (2007) suggested that this compartmentalization that occurs in Oncopeltus fasciatus is similar to that of the cotton stainer Dysdercus decussatus and the stink bugs. Compartmentalization of digestion was maintained by PMM as a substitute for lacking peritrophic membrane (Terra, 1990). Woodring et al. (2007) found that the serine proteases are absent in the gut of Oncopeltus fasciatus and instead cysteine proteinase is present (gut pH 6.0). The authors found high lipase activity throughout the gut but only limited amylase activities.

The perimicrovillar membrane arises from the inner membrane of the double membrane vesicles or multimembrane vesicles that likely originated from the Golgi complex or the PMM synthesis in *C. hemipterus* may be linked to vacuoles containing membrane-like structures found in the

cytoplasm of the principal cells (Silva et al., 1995). Fialho et al. (2009) suggested that the double membrane vesicles could be responsible for the formation of the perimicrovillar membrane, inspite of the fact that they could also be an indication of cell death.

The present data indicate that the midgut of *S. pandurus* is similar to that of other hemipteran insects (Cruz-Landim, 1985; Terra, 1990; Cruz-Landim *et al.*, 1996; Silva *et al.*, 1996; Serrao and Cruz-Landim, 2000; Neves *et al.*, 2002; Jarial, 2005; Martins *et al.*, 2006). *S. pandurus* as well as other phytophagous Hemiptera, such as *O. fasciatus* and hematophagous Hemiptera, such as *R. prolixus* (Billingsley and Downe, 1983) and *T. infestans* (Burgos and Gutierrez, 1976) have perimicrovillar membrane (PMM) lining the surface of microvilli in digestive cells, which is a structure commonly found in Hemiptera (Terra *et al.*, 2006; Azevedo *et al.*, 2009; Fialho *et al.*, 2009).

The PMM synthesis in S. pandurus may be linked to vacuoles containing membrane-like structures found in the cytoplasm of the principal cells. The PMM is thought to increase the digestive process efficiency (Silva et al., 1995; Terra et al., 2006). Albuquerque-Cunha et al. (2004) reported that the perimicrovillar membrane in the principal cells of the median midgut (MMG) and posterior midgut (PMG) of R. prolixus, is synthesized by the membranes of the vacuoles. This variation of PMM production could be explained by the different feeding behaviors of phytophagous, zoophytophagous and blood-sucking hemipterans. The phytophagous and zoophytophagous species can access the food source frequently in plants, without starving periods (Fialho et al., 2009).

The perimicrovillar membrane arose in Condylognatha ancestor (Paraneoptera that includes Hemiptera and Thysanoptera) that fed on phloem. The Condylognatha ancestor lost enzymes involved in initial and intermediate digestion and the peritrophic membrane (Terra, 1988; Terra and Ferreira, 1994; Terra et al., 2006). Silva et al. (2004) reported that  $\alpha$ -glucosidase is a biochemical marker for PMM in the seed sucker bug *Dysdercus peruvianus*. They also reported that adults of the major hemipteran infra orders Sternorrhyncha, Auchenorrhyncha and Heteroptera have PMM and a major membrane bound  $\alpha$ -glucosidase. Their data supported the hypothesis that PMM may have originated in the Condylognatha (Paraneopteran taxon including Hemiptera and Thysanoptera) ancestral stock and are associated with plant sap feeding.

The evolution of Heteroptera was associated with regaining the ability to digest polymers. Because the appropriate enzymes were lost, these insects instead used enzymes derived from lysosomes that are cysteine and aspartic proteinases characterized by being active in acid conditions. In the present study a high relative density of secondary lysosomes were observed in the posterior region especially in the third and fourth midgut which indicates that in this region digestion takes places.

The results of the present study are in agreement with data from other Hemiptera that showed that the anterior midgut region was the major site of water absorption from the ingested food (Fialho et al., 2009). The absorption of water and glucose by the midgut is associated with active ion transport (Caccia et al., 2007). Spherocrystals associated with ionic regulation and excretions were related in other insects (Cruz-Landim and Serrao, 1997). Furthermore, the great quantity of these cell inclusions in midgut region has important functions as an energy storage organ. The presence of secretory granules, lysosomes and large amounts of rough endoplasmic reticulum indicates that the second, third and fourth midgut regions of S. pandurus play a role in food digestion. In S. pandurus, the smaller amount of lipid inclusions in the second midgut region suggests that this region plays only a minor role in lipid absorption and energy storage.

## CONCLUSION

The alimentary canal is simple and similar to the "lygus type" but its cellular anatomy and function of the different regions indicate different digestive functions. The histology and ultrastructure of the midgut cells showed that the cells are very unique in their architecture. The Hemiptera lack a peritrophic membrane, but possesses a perimicrovillar membrane that compartmentalizes the gut internally. Most of the cells are binucleated and this may be due to active digestive properties.

The cellular anatomy and function and the distribution of different cell types in *S. pandurus* could be correlated with the physiology of digestion and absorption, which in turn could be related to the insect feeding mechanisms and how these cell types impact their functions.

## ACKNOWLEDGMENTS

This work was funded by the Faculty of Science, Cairo University, to whom the authors express their profound appreciation. The authors also thank Dr. Heba Abo El Ezz for her assistance in technical guidance.

## REFERENCES

- Albuquerque-Cunha, J.M., C.B. Mello, E.S. Garcia, P. Azambuja, W. De Souza, M.S. Gonzalez and N.F. Nogueira, 2004. Effect of blood components, abdominal distension and ecdysone therapy on the ultrastructural organization of posterior midgut epithelial cells and perimicrovillar membranes in *Rhodnius prolixus*. Mem. Inst. Oswaldo Cruz, 99: 815-822.
- Andries, J. and G. Torpier, 1982. An extracellular brush border coat of lipid membranes in the midgut of *Nepa cinerea* (Insecta, Heteroptera): Ultrastructure and genesis. Biol. Cell., 46: 195-202.
- Azevedo, D.O., C.A. Neves, J.R. dos Santos-Mallet, T.C.M. Goncalves, J.C. Zanuncio and J.E. Serrao, 2009. Notes on midgut ultrastructure of the tropical bed bug *Cimex hemipterus* Fabricius (Hemiptera: Cimicidae). J. Med. Ent., 46: 435-441.
- Billingsley, P.F. and A.E.R. Downe, 1983. Ultrastructural changes in posterior midgut cells associated with blood feeding in adult female *Rhodnius prolixus* Stal (Hemiptera: Reduvidae). Can. J. Zool, 241: 2574-2586.
- Billingsley, P.F. and A.E.R. Downe, 1989. Changes in the anterior midgut cells of adult female *Rhodnius prolixus* (Hemiptera: Reduviidae) after feeding. J. Med. Entomol., 26: 104-108.
- Binnington, K.C., M.J. Lehane and C.D. Beaton, 1998. The Peritrophic Membrane. In: Microscopic Anatomy of Invertebrates, Harrison, F.W. and M.L. Byand (Eds.). Vol. 11, Wiley-Liss, New York, pp. 747-758.
- Burgos, M.H. and L.S. Gutierrez, 1976. The intestine of Triatoma infestans. I. Cytology of the midgut. J. Ultrasctruct. Res., 57: 1-9.
- Caccia, S., M. Casartelli, A. Grimaldi, E. Losa, M. de Eguileor, F. Pennacchio and B. Giordana, 2007. Unexpected similarity of intestinal sugar absorption by SGLT1 and apical GLUT2 in an insect (*Aphidius ervi*, Hymenoptera) and mammals. Am. J. Physiol. Regul. Integr. Comp. Physiol., 292: 2284-2291.
- Cruz-Landim, C. and J.E. Serrao, 1997. Ultrastructure and histochemistry of the mineral concretions in the midgut of bees (Hymenoptera: Apidae). Neth. J. Zool, 47: 21-29.
- Cruz-Landim, C., 1985. Ultraestrutura e funcao do tubo digestivo dos insetos. An. Acad. Sci. Sao Paulo., 6: 28-41.

- Cruz-Landim, C., J.E. Serrao and R.L.M. Silva-de-Moraes, 1996. Cytoplasmic protrusions from digestive cells of bees. Cytobios, 88: 95-104.
- Dunn, A.K. and E.V. Stabb, 2005. Culture-independent characterization of the microbiota of the ant-lion *Myrmeleon mobilis*. Appl. Environ. Microbiol., 71: 8784-8794.
- El-Shazly, M.M., 1995. Effect of temperature on development and population growth rates of *Spilostethus pandurus* (Scopoli) (Hemiptera: Lygaeidae) in Giza, Egypt. Insect Sci. Appl., 16: 17-25.
- Fialho, M.C.Q., J.C. Zanuncio, C.A. Neves, F.S. Ramalho and J.E. Serrao, 2009. Ultrastructure of the digestive cells in the midgut of the predator *Brontocoris tabidus* (Heteroptera: Pentatomidae) after different feeding periods on prey and plants. Ann. Entomol. Soc. Am., 102: 119-127.
- Guedes, B.A.M., J.C. Zanuncio, F.S. Ramalho and J.E. Serrao, 2007. Midgut morphology and enzymes of the obligate zoophytophagous stinkbug *Brontocoris tabidus* (Signoret, 1963) (Heteroptera: Pentatomidae). Pan-Pac. Entomol., 83: 66-74.
- Habibi, J., T.A. Coudron, E.A. Backus, S.L. Brandt, R.M. Wagner, M.K. Wright and J.E. Huesing, 2008. Morphology and histology of the alimentary canal of *Lygus hesperus* (Heteroptera: Cimicomoropha: Miridae). Ann. Entomol. Soc. Am., 101: 159-171.
- Jarial, M.S., 2005. Electron microscopic study of the anterior midgut in *Cenocorixa bifida* Hung. (Hemiptera: Corixidae) with reference to its secretory function. Zool Sci., 22: 783-790.
- Lehane, M.J. and P.F. Billingsley, 1996. Structural and Ultra Structure of the Insect Midgut. In: Biology of the Insect Midgut, Lehane, M.J. and P.F. Billingsley (Eds.). Chapman Hall, UK., London, pp: 3-25.
- Martins, G.F., C.A. Neves, L.A. Campos and J.E. Serrao, 2006. The regenerative cells during the metamorphosis in the midgut of bees. Micron, 37: 161-168.
- Neves, C.A., L.L. Bhering, J.E. SerrAo and L.B. Gitirana, 2002. Framed-like midgut endocrine cells during the metamorphosis in *Melipona quadrifasciata* anthidioides (Hymeonptera, Apidae). Micron, 33: 453-460.
- Neves, C.A., L.B. Gitirana and J.E. Serrao, 2003. Ultrastructure of the midgut endocrine cells in *Melipona quadrifasciata* anthidioides (Hymenoptera, Apidae). Braz. J. Biol., 63: 683-690.
- Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17: 208-212.
- Serrao, J.E. and C. da Cruz-Landim, 1995. The striated border of digestive cells in adult stingless bees (Hymenoptera, Apidae, Meliponinae). Cytobios, 83: 229-235.
- Serrao, J.E. and C. da Cruz-Landim, 1996a. A comparative study of digestive cells in different midgut regions of stingless bees (Hymenoptera: Apidae: Meliponinae). J. Adv. Zool., 17: 1-6.
- Serrao, J.E. and C. da Cruz-Landim, 1996b. Ultrastructure of midgut endocrine cells in workers of stingless bees (Hymenoptera, Apidae, Meliponinae). Iheringia Ser. Zool., 81: 151-156.
- Serrao, J.E. and C. da Cruz-Landim, 2000. Ultrastructure of the midgut epithelium of Meliponinae larvae with different developmental stages and diets. J. Apic. Res., 39: 9-17.
- Silva, C.P., A.F. Ribeiro and W.R. Terra, 1996. Enzyme markers and isolation of the microvillar and perimicrovillar membranes of *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae) midgut cells. Insect Biochem. Mol. Biol., 26: 1011-1018.
- Silva, C.P., A.F. Ribeiro, S. Gulbenkian and W.R. Terra, 1995. Organization, origin and function of the outer microvillar (perimicrovillar) membranes of *Dysdercus peruvianus* (Hemiptera) midgut cells. J. Insect Physiol., 41: 1093-1103.

## Asian J. Biol. Sci., 6 (1): 54-66, 2013

- Silva, C.P., J.R. Silva, F.F. Vasconcelos, M.D.A. Petretski, R.A. Damatta, A.F. Ribeiro and W.R. Terra, 2004. Occurrence of midgut perimicrovillar membranes in paraneopteran insect orders with comments on their function and evolutionary significance. Arthropod Struct. Dev., 33: 139-148.
- Terra, W.R., 1988. Physiology and biochemistry of insect digestion: an evolutionary perspective. Braz. J. Med. Biol. Res., 21: 675-734.
- Terra, W.R., 1990. Evolution of digestive system of insects. Annu. Rev. Entomol., 35: 181-200.
- Terra, W.R. and C. Ferreira, 1994. Insect digestive enzymes: Properties, compartmentalization and function. Comp. Biochem. Physiol., 109: 1-62.
- Terra, W.R., C. Ferreira and J.E. Baker, 1996. Compartmentalization of Digestion. In: Biology of Insect Midgut, Lehane, M.J. and P.F. Billingsley (Eds.). Chapman and Hall, London, pp: 419-486.
- Terra, W.R., R.H. Costa and C. Ferreira, 2006. Plasma membranes from insect midgut cells. An. Acad. Bras. Cienc., 78: 255-269.
- Woodring, J., K. Hoffmann and W.L. Matthias, 2007. Feeding, nutrient flow and digestive enzyme release in the giant milkweed bug, Oncopeltus fasciatus. Physiol. Entomol., 32: 328-335.