Notes on Midgut Ultrastructure of *Cimex hemipterus* (Hemiptera: Cimicidae)

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ABSTRACT This work studied the ultrastructure of the midgut cells of *Cimex hemipterus* Fabricius (Hemiptera: Cimicidae). The midgut of adult insects was analyzed on different days after a bloodmeal, and three anatomical regions with different digestive functions were apparent. In the anterior midgut, the digestive cells had many spherocrystals, lipid inclusions, and glycogen deposits, suggesting a role in water absorption, ion regulation, digestion, and storage of lipids and sugars. The digestive cells in the middle midgut contained secretory granules in the apical cytoplasm, lysosomes, and large amounts of rough endoplasmic reticulum, suggesting that this midgut region was active in digestive processes. The posterior midgut contained digestive cells with secretory vesicles, lysosomes, rough endoplasmic reticulum, and spherocrystals, suggesting digestion and ion/water absorption. Also, there was strong evidence that the posterior midgut may be the major site of nutrient absorption. The hematophagous heteropteran groups share many of these blood digestion mechanisms.

KEY WORDS blood digestion, Triatominae, spherocrystals, perimicrovillar membranes

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The Cimicidae (Hemiptera: Heteroptera) family is composed of small obligatory blood-feeding insects that are temporary ectoparasites of vertebrates such as birds, bats, and humans (Forattini 1990, Reinhardt and Siva-Jothy 2007). Only three species can truly be considered ectoparasites of humans, commonly known as bed bug: *Cimex lectularius* L., which is distributed all over the world but is found mainly in the temperate and subtropical zones, *Cimex hemipterus* Fabricius found in the tropical regions, and *Leptocimex boueti* Brumpt, which is found in the west of Africa (Ryckman et al. 1981, Forattini 1990). Because of their blood-feeding habits and anthropophily, the bed bugs have been suspected of transmitting a variety of pathogenic agents such as bacteria, viruses, and protozoa of 41 human diseases (Burton 1963, Jupp and McElligott 1979, Forattini 1990). Particularly in relation to Chagas disease, the bed bugs showed capacity to transmit the etiological agent *Trypanosoma cruzi* between rats in the laboratory (Jörg and Natula 1982).

Hematophagous insects developed adaptations in the midgut that permit the ingestion of large bloodmeals followed by regular digestion intervals (Billingsley 1990). Midgut structural analyses of some hematophagous insects have contributed to the understanding of cellular modifications during feeding and digestion (Brown 1980; Billingsley and Downe 1985; Billingsley 1988, 1990; Okuda et al. 2002; Albuquerque-Cunha et al. 2004). For disease transmission, the midgut is a key locale for parasite and vector interactions (Kollien et al. 1998, Oliveira and De Souza 2001).

The insect midgut epithelium consists of three cell types: the principal cells, also termed digestive or columnar cells, which play a role in digestive enzyme secretion and absorption of water and nutrients (Billingsley 1990, Guedes et al. 2007); the regenerative cells, responsible for the replacement of degenerated principal cells (Martins et al. 2006); and the endocrine cells, which release hormonal peptides that control the digestive processes (Serrão and Cruz-Landim 1996b; Neves et al. 2002, 2003).

The morphology of principal cells varies according to species, alimentary cycle, and midgut region. They have microvilli and basal labyrinth, modifications of the apical and baso-lateral cell membranes that are responsible for increasing the cell surface contact with the midgut lumen and the hemolymph (Billingsley and Downe 1989; Serrão and Cruz-Landim 1995, 1996a; Terra et al. 2006). Moreover, the Hemiptera have a lipoproteic membrane system that surround the microvilli and extend to the midgut lumen. These lipoproteic membranes are know as perimicrovillar membranes (PMMs) (Lane and Harrison 1979), which are supposed to be important for protein digestion (Bill-

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ingsley and Downe 1989), compartmentalization of the digestive process (Silva et al. 1995), and amino acid absorption from diluted diets (Terra et al. 2006).

In the Heteroptera, the blood-feeding habit is confined to members of Cimicoidea (mainly Cimicidae and Polyctenidae) and Triatominae (Reduvoidea: Reduviidae), and the hematophagy has evolved independently in these two groups (Reinhardt and Siva-Jothy 2007, Weirauch 2008). The Triatominae have been used as model organisms for midgut ultrastructural analyses in the hematophagous Hemiptera (Billingsley 1990, Albuquerque-Cunha et al. 2004) because of epidemiological importance such as Chagas disease vectors. In these bugs, the midgut is divided into anterior, median, and posterior regions, each of which performs a different function during the digestive process. The anterior midgut has a sac-like structure, with a great capacity of distention for storage of the ingested blood. The median and posterior midgut regions are tubular and involved in the synthesis and the secretion of digestive enzymes and nutrient absorption (Billingsley 1990). In the Cimicidae, however, although the midgut anatomy of *C. lectularius* was previously described (Forattini 1990), there have been no histologial or ultrastructural description of midgut for the bed bugs.

Cimex hemipterus represents the most common bed bug in Brazil, and in this work, we studied its midgut ultrastructure to study the similarities and differences in structure and blood digestion mechanisms between the bed bugs and triatomines, with new insight about the digestion in Cimicidae.

Materials and Methods

Adults of *Cimex hemipterus* were obtained from colonies held at the Entomology Department of the Oswaldo Cruz Institute (IOC), Manguinhos, RJ, Brazil. The insects were kept at room temperature on plastic tubes covered with gauze containing folded filter paper and wet cotton and were fed periodically on quail's blood (*Coturnix coturnix*).

Adult insects were starved for 10 d before feeding, followed by midgut dissections at 1, 3, 5, 7, 10, 15, and 20 d after a bloodmeal. At each time, we analyzed the midgut of three to four insects. The insects were immobilized on ice, and the midgut was dissected in 2.5% gltutaraldehyde in sodium cacodylate buffer (0.1 M, pH 7.4). After that, the midguts were divided into anterior (AMG), median (MMG), and posterior (PMG) regions. The samples were maintained in the fixative for 4 h, postfixed in 1% osmium tetroxide in sodium cacodylate buffer 0.1 M for 2 h, dehydrated in a graded acetone series, and embedded in Spurr resin. Ultra-thin sections were counterstained with 1% aqueous uranyl acetate and lead citrate (Reynolds 1963) and analyzed using a Zeiss EM109 transmission electron microscope.

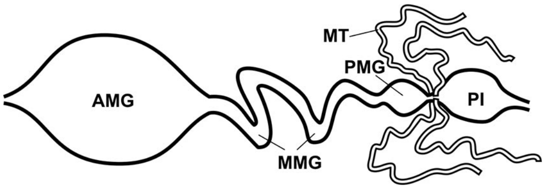

Fig. 1. Schematical drawing of *C. hemipterus* midgut. AMG, anterior midgut; MMG, median midgut; PMG, posterior midgut; MT, Malphigian tubules; PI, hindgut. Not drawn with scale.

Results

Anatomically, the midgut of *C. hemipterus* was composed of three different regions: the AMG was a sac-like structure with a great distention capacity occupying part of thorax and abdomen, the MMG was a narrow tube slightly folded, and the PMG was a dilated region before Malpighian tubules insertion (Fig. 1).

The apical surface of the principal cells of *C. hemipterus* midgut consisted of microvilli with a double plasma membrane (Fig. 2a and b). The outer one was the PMM, which began to be deposited on the first day after feeding (Fig. 2a) and remained in all observed periods. The apical cytoplasm consisted of mitochondria at the microvilli base, and the cell basal portion was characterized by well-developed plasma membrane infoldings, known as the basal labyrinth, that was also associated with mitochondria (Figs. 2c, 3e, and 4b). The cells were attached to a small continuous electron-dense basement membrane surrounded by muscle (Figs. 2c, 3e, and 4b).

Regenerative cells were found in the epithelium base, and they consisted of an electron-dense cytoplasm, an elongated mitochondria, and a rough endoplasmic reticulum near to the round nucleus (Fig. 5a). These cells were found isolated or grouped as nests in the three midgut regions.

There were morphological differences between the principal cells depending on the midgut region. However, in the same region, many of the ultrastructural characteristics of the principal cells were seen at all time points after feeding.

In the AMG, the cell cytoplasms showed great lipid inclusion amounts, glycogen deposits (Fig. 2e and f), spherocrystals with different sizes associated with rough endoplasmic reticulum (Fig. 2d), and electron-lucent vacuoles, some of them containing membrane-like material (Fig. 2e). On the 15th day after feeding, some cells showed strong electron-dense granules in the cytoplasm (Fig. 2g). On the 20th day, there was no midgut luminal content in this region, but PMM, lipid inclusions, and glycogen deposits were still found in the cell cytoplasms (Fig. 2f).

The MMG principal cells showed median-apical cytoplasm with mitochondria, lisosomes, electron-lucent vacuoles carrying membrane-like material, and little granules of medium electron-density (Fig. 3a and d). Lipid inclusions were present in smaller amounts (Fig. 3b). Unlike what was observed in the AMG cells, the rough endoplasmic reticulum was abundant in the

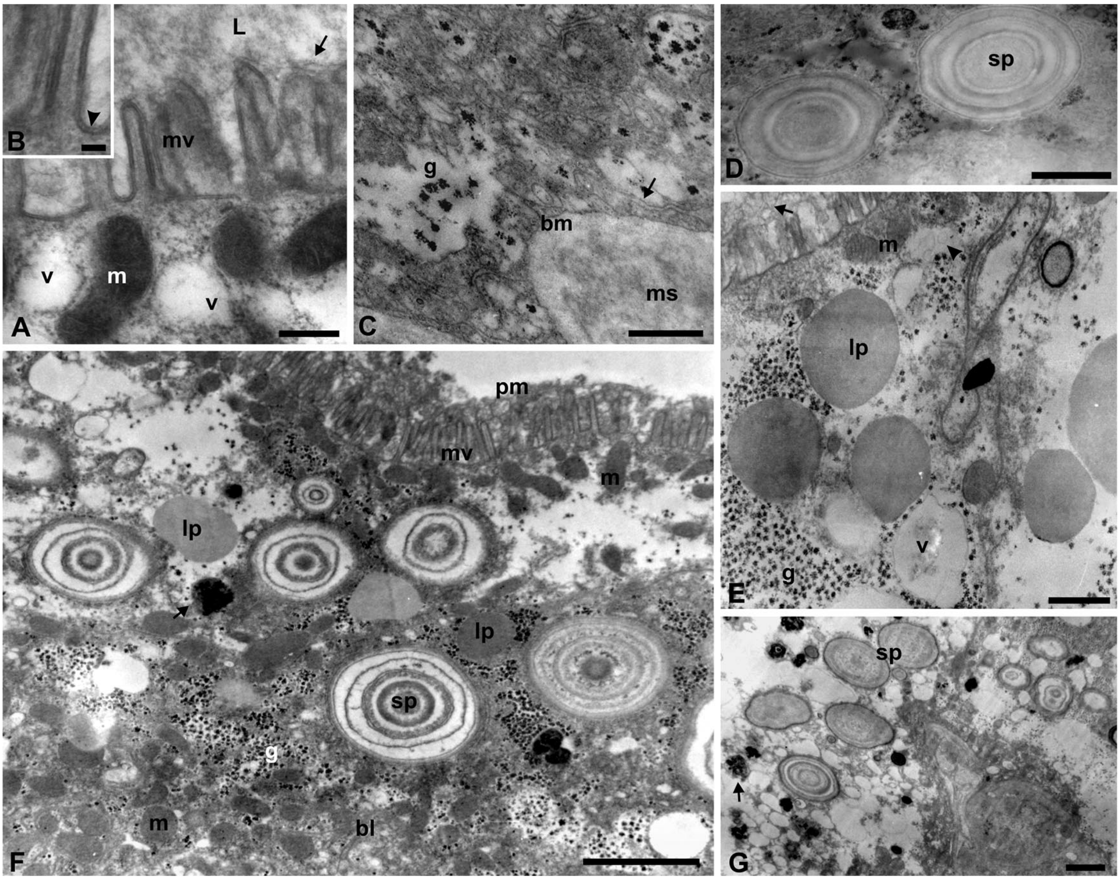

Fig. 2. Transmission electron micrographs of the principal cells of *C. hemipterus* anterior midgut at different days postfeeding. (A) Apical portion showing the microvilli (mv) with perimicrovillar membranes (arrow), mitochondria (m), and electron-lucent vacuole (v). L, lumen. Fifth day after bloodmeal. Bar = 0.2 μm. (B) Detail of a microvillus showing surface covered by the perimicrovillar membrane (arrowhead). Fifth day after bloodmeal. Bar = 50 nm. (C) Basal portion showing plasma membrane infoldings (arrow) and glycogen (g). bm, basement membrane; ms, muscle cell. Fifth day after bloodmeal. Bar = 0.5 μm. (D) Spherocrystals (sp). Third day after bloodmeal. Bar = 1 μm. (E) Median-apical portion showing lipid inclusions (lp), glycogen deposits (g), and electron-lucent vacuole (v). m, mitochondria; arrow, perimicrovillar membranes; arrowhead, membrane-like material. Fifteenth day after bloodmeal. Bar = 1.5 μm. (F) Cell cytoplasm showing spherocrystals (sp), glycogen (g), lipids (lp), strongly electron-dense granules (arrow), and microvilli (mv) with perimicrovillar membranes (arrowhead). Note the basal plasma membrane infoldings (bl) associated with mitochondria (m). Twentieth day after bloodmeal. Bar = 2 μm. (G) Median-apical cytoplasm showing spherocrystals (sp) and strongly electron-densite granules (arrow). Fifteenth day after bloodmeal. Bar = 1 μm.

MMG (Fig. 3b and c), and there were no glycogen deposits or spherocrystals. Fifteen days after bloodfeeding, few lipid inclusions were present in the cytoplasm (Fig. 3f), whereas on the 20th day, the cells displayed large amounts of lisosomes, rough endoplasmic reticulum, and PMM.

The PMG cells had the longest microvilli, with an average length of 4.0 ± 1.1 μm in the PMG, 0.58 ± 0.14 μm in the AMG, and 0.85 ± 0.06 μm in the MMG (Fig. 4a and f). The PMG cell cytoplasms contained many lisosomes, mitochondria, rough endoplasmic reticulum, and electron-lucent vacuoles, some with membrane-like material (Fig. 4c, e, and f). Granules of medium electron-density were found among the microvilli (Fig. 4d). Five days after feeding, spherocrystals were found on the cytoplasm (Fig. 4d). After this period, some cells exhibited few granules with high electron density clustered inside vacuoles (Figs. 4e and 5b). Twenty days after feeding, the cells contained large amounts of lysosomes and PMM, and luminal content was still present. Endocrine cells of the open type (Fig. 5b), characterized by the contact with the midgut lumen, were present in this midgut region, with few secretory granules and unfolded basal plasma membrane.

Discussion

The organization of the *C. hemipterus* midgut into three major regions was similar to that found in other hematophagous and nonhematophagous Hemiptera (Jörg and Natula 1982, Billingsley 1990, Silva et al.

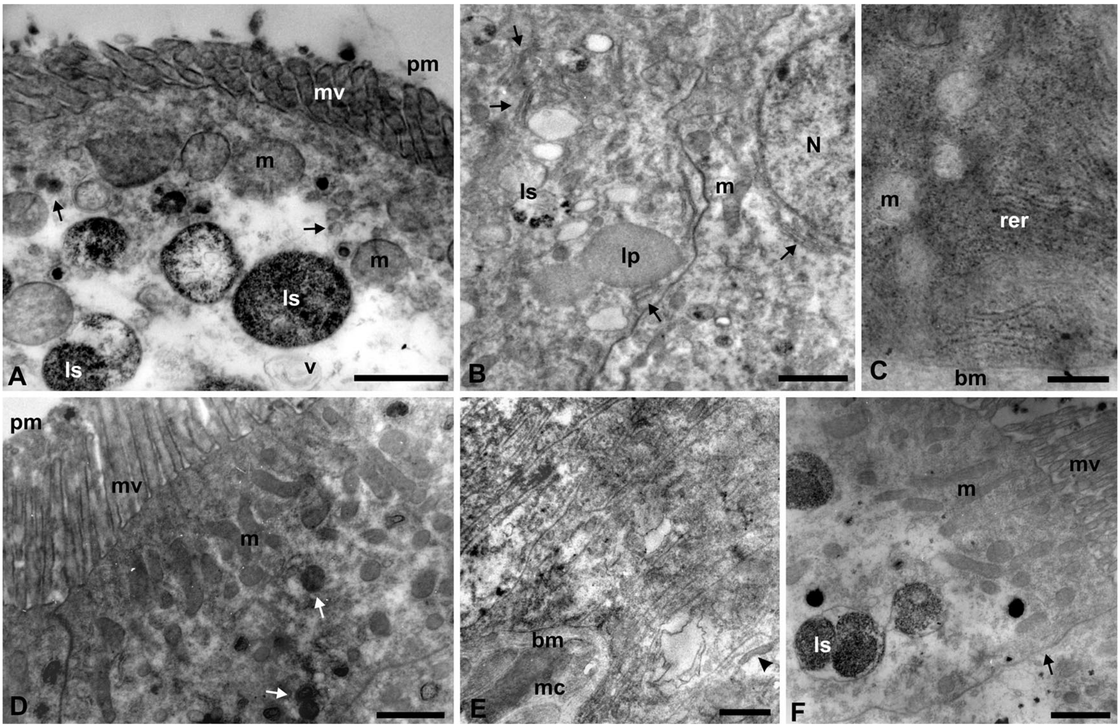

Fig. 3. Transmission electron micrographs of the principal cells of *C. hemipterus* median midgut at different days postfeeding. (A) Apical portion showing perimicrovillar membranes (pm), dilated mitochondria (m), lisosomes (ls), electron-dense granules (arrow), and vacuole with membrane-like material (v). First day after bloodmeal. Bar = 1 μm. (B) Cytoplasm of the median cell portion showing a nucleus (N) with descondensed chromatin, lipid inclusions (lp), lisosomes (ls), and rough endoplasmic reticulum (arrow). m, mitochondria. Fifth day after bloodmeal. Bar = 1 μm. (C) Basal portion showing large amount of rough endoplasmic reticulum. bm, basement membrane; m, mitochondria. Fifth day after bloodmeal. Bar = 0.25 μm. (D) Apical portion showing electron-dense granules (arrow) and mitochondria (m) associated with the microvilli (mv). pm, perimicrovillar membranes. Fifth day after bloodmeal. Bar = 1 μm. (E) Basal portion showing the well-developed membrane labyrinth. bm, basement membrane; mc, muscler cell; arrowhead, mitochondria. Tenth day after bloodmeal. Bar = 1 μm. (F) Median-apical portion showing lisosomes (ls) and mitochondria (m) associated with the microvilli (mv). Arrow, cell boundary. Fifteenth day after bloodmeal. Bar = 1 μm.

1995, Guedes et al. 2007). Despite this, a transitional segment between the PMG and the hindgut described for *Dysdercus peruvianus* Guérin-Méneville (Hemiptera: Pyrrochoridae) and *Brontocoris tabidus* Signoret (Silva et al. 1995, Guedes et al. 2007) was not found in *C. hemipterus.*

The PMM is thought to increase the digestive process efficiency (Silva et al. 1995, Terra et al. 2006). The three regions of the *C. hemipterus* midgut showed these structures on the first day after feeding, unlike *Rhodnius prolixus* Stal (Reduviidae: Triatominae), where the PMM were detected in the midgut by the second day after feeding (Billingsley and Downe 1985). This difference in the onset of PMM synthesis may be associated with the more rapid digestion and relatively shorter feeding interval seen in Cimicidae as opposed to Triatominae (Billingsley 1990, Forattini 1990). 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, Cristofoletti et al. 2003), similar to those found in principal cells of MMG and PMG of *R. prolixus* (Albuquerque-Cunha et al. 2004).

In both the phytophagous *D. peruvianus* and zoophytophagous *B. tabidus*, the PMMs are present in starved insects (Silva et al. 1995, Fialho et al. 2009), which were not found in our study. 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). This is in contrast to blood suckers that are subjected to starvation periods (Billingsley 1990, Forattini 1990). PMM production just after the bloodmeal can lead to metabolic energy economy for starved hematophagous hemipterans.

The three midgut regions of *C. hemipterus* contained principal cells with distinct structural features, suggesting that there were different functions among them. In the AMG, the presence of spherocrystals suggests that the principal cells were active in water absorption and ionic regulation. This result is in agreement with data from other Hemiptera that showed that the AMG was the major site of water absorption from the ingested food (Silva et al. 1995, Billingsley

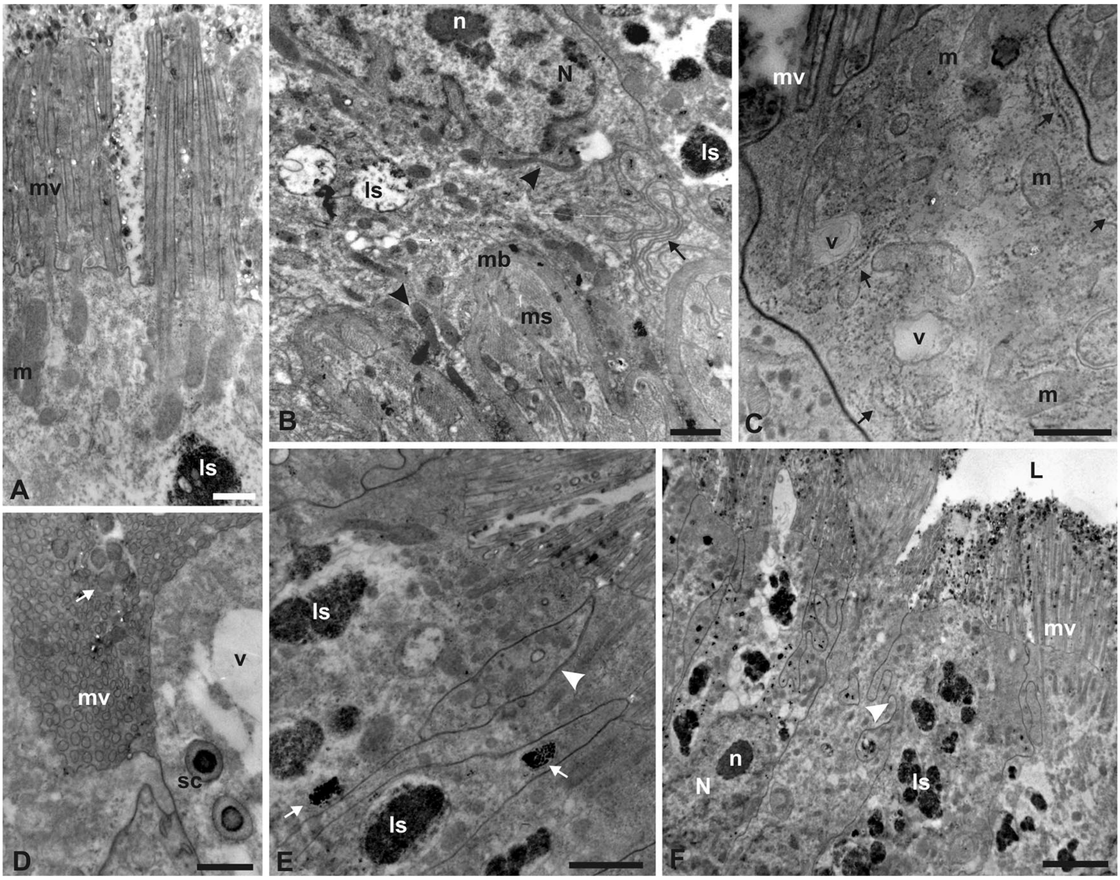

Fig. 4. Transmission electron micrographs of the principal cells of *C. hemipterus* posterior midgut at different days postfeeding. (A) Apical portion showing microvilli (mv), mitochondria (m), and lisosomes (ls). Fifteenth day after bloodmeal. Bar = 0.5 μm. (B) Median-apical portion showing nucleus (N) with descondensed chromatin and nucleolus (n), mitochondria (arrowhead), lisosomes (ls), and basal plasma membrane infoldings (arrow). Fifteenth day after bloodmeal. Bar = 1 μm. (C) General view of the cytoplasm showing mitochondria (m), electron-lucent vacuole with membrane-like material (v), and rough endoplasmic reticulum (arrow). mv, microvilli. Seventh day after bloodmeal. Bar = 0.5 μm. (D) Apical portion showing electron-dense granules (arrow) among the microvilli (mv). Note the presence of spherocrystals (sc) and electron-lucent vacuole (v). Fifth day after bloodmeal. Bar = 1 μm. (E) General view of the epithelium showing cells with lisosomes (ls) and strongly electron-dense granules (arrow). Arrowhead, cell boundary. Twentieth day after bloodmeal. Bar = 2 μm. (F) Principal cells with many lisosomes (ls). L, lumen; mv, microvilli; N, nucleus; n, nucleolus; arrowhead, cell boundary. Twentieth day after bloodmeal. Bar = 3 μm.

and Downe 1989, Fialho et al. 2009). The absorption of water and glucose by the midgut is associated with active ion transport (Barrett 1982, Terra et al. 2006, Caccia et al. 2007). Thus, the increase in spherocrystals amount in the AMG cells of *C. hemipterus* 15 d after bloodfeeding may be related to a storage of absorbed ions. Spherocrystals associated with ionic regulation and excretion were related in other insects (Gouranton 1968, Cruz-Landim and Serrão 1997).

The AMG cells of *C. hemipterus* showed a large glycogen amount and lipid inclusions in the cytoplasm, suggesting that this region is involved with the absorption and metabolism of carbohydrates and lipids. Furthermore, the great quantity of these cell inclusions at 15 d after feeding suggests that this midgut region has important functions as an energy storage organ, similar to those described for *R. prolixus* (Billingsley 1988, Billingsley and Downe 1989) and *Panstrongylus megistus* Burmeister (Reduviidae: Triatominae) (Canavoso et al. 2004). *C. hemipterus* is an insect that endures long intervals between bloodmeals because of host availability (Forattini 1990), and the storage of lipids, carbohydrates, and ions into the spherocrystals may be related to nutrient reserves for starvation periods.

The presence of secretory granules, lisosomes and large amounts of rough endoplasmic reticulum indicates that the MMG of *C. hemipterus* play a role in extra and intracellular blood digestion, such as in *R. prolixus* (Billingsley and Downe 1985, Billingsley 1988) and *Triatoma infestans* Klug (Burgos and Gutiérrez 1976). In *C. hemipterus*, the smaller amount of lipid inclusions in the MMG suggest that this region plays only a minor role in lipid absorption and energy storage.

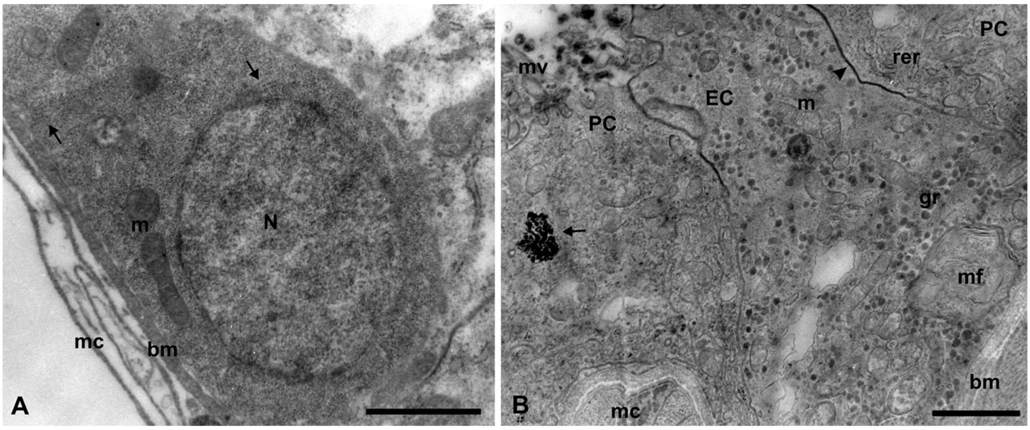

Fig. 5. Transmission electron micrographs of the *C. hemipterus* midgut epithelium at different days postfeeding. (A) Regenerative cell showing a dense and granular cytoplasm, with a rounded nucleus (N), mitochondria (m), and rough endoplasmic reticulum (arrow). bm, basement membrane; mc, muscular cell. Third day after bloodmeal. Bar = 1 μm. (B) View of an endocrine cell (EC) with electron-dense granules (gr) and mitochondria (m). Note the presence of a myelin figure (mf) and basal plasma membrane. The neighbor principal cells (PC) showing a developed basal plasma membrane infoldings (bl), rough endoplasmic reticulum (rer), mitochondria (m), and strongly electron-dense granules surrounded by a membrane (arrow). bm, basement membrane; mc, muscle cell. Arrowhead, cell boundary. Seventh day after bloodmeal. Bar = 1 μm.

The *C. hemipterus* PMG probably shares the digestion functions with the MMG. However, the nutrient absorption may be more intense in the PMG, because of the longer microvilli and the higher concentration of mitochondria near the basal plasma membrane infoldings.

Hemoglobin digestion in the insect midgut releases high amounts of heme and iron that can be toxic because of their capacity to generate reactive oxygen species. Blood-feeding insects neutralize their deleterious effects through sequestering them in insoluble high electron-dense crystals called hemozoin and hemoxisomes (Oliveira et al. 2000, Silva et al. 2006). In *C. hemipterus,* electron-dense granules similar to hemoxisomes were found mainly in the PMG cells. Although we did not provide biochemical analyses to determine their composition, their presence is suggested by the morphological similarity observed in the micrographs presented by Silva et al. (2006).

Despite the fact that the Cimicidae and the Triatominae do not share a common hematophagous ancestor, this work showed that their midguts were similar in structure and shared many adaptations for bloodfeeding.

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