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MORPHOLOGY OF THE DIGESTIVE TRACT OF *CLADOMORPHUS PHYLLINUS* (PHASMATODEA: PHASMIDAE)

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ABSTRACT

The digestive tracts of insects may indicate life history and phylogenetic relationships among different species. Phasmatodea are chewing herbivores with an elongated body shape, which camouflages them on the vegetation on which they feed. This work evaluated the gut structure of the walking stick insect, *Cladomorphus phyllinus* (Gray 1835) (Phasmatoidea: Phasmidae), with light and scanning electron microscopy. The digestive tract of *C. phillynus* is an elongated tube with minimal external anatomical differences along its length. There is a short gastric caeca-like structure in the posterior midgut. The crop is an extensively folded storage organ and the proventriculus is covered by a thick cuticle with spine-like projections, which play a role in grinding food. The midgut has 2 anatomical regions probably involved in digestion and absorption of nutrients. The epithelial cells of the anterior midgut have bubbles of apocrine secretion, while the posterior midgut cells have a striated border. Gastric caecae-like projections are found in the posterior midgut. They are enlarged close to the midgut wall, and follow a thin and long filament, which is free in the body cavity. The epithelial cells lining the gastric caecae-like projections are cuboidal with well development striated borders, suggesting involvement in nutrient absorption. The hindgut is divided in an ileum and a rectum. The ileum epithelial cells are covered by cuticle and have cytological traits characteristic of electrolyte and water absorption. The rectum epithelial cells have no obvious absorptive features, but the rectal pads may be involved in water and electrolyte reabsorption. This work reinforces the concept that the anatomy of gut is related to the diet and body shape, and shows that the general pattern of compartmentalization of digestion in insects was maintained in Phasmatodea, but that it differs from other Orthopteroidea in some aspects.

Key Words: insects, histology, walking stick insects, gut

RESUMO

O trato digestivo dos insetos pode indicar a história de vida e as relações filogenéticas entre espécies diferentes. Phasmatodea são herbívoros mastigadores com corpo alongado que se camuflam na vegetação. Este trabalho avaliou a estrutura do intestino do bicho-pau *Cladomorphus phyllinus* através de microscopia de luz e microscopia eletrônica de varredura. O trato digestivo de *C. phyllinus* é um tubo alongado sem diferenças anatômicas ao longo de seu comprimento, exceto pela presença de uma estrutura semelhante ao ceco gástrico na região posterior do intestino médio. O papo é um órgão de armazenamento amplamente dobrado e o proventrículo é coberto por cutícula espessa com projeções na forma de espinhos. O intestino médio tem duas regiões anatômicas, e está provavelmente envolvido na digestão e absorção de nutrientes. As células epiteliais do intestino médio anterior apresentam bolhas de secreção apócrina na sua porção apical, ao passo que as células do intestino médio posterior possuem borda estriada no ápice. Estruturas semelhantes a cecos gástricos foram encontrados no intestino médio posterior, com células cúbicas que apresentam microvilosidades longas, provavelmente envolvidas na absorção de nutrientes. O intestino posterior é dividido em íleo e reto. As células do íleo são cobertas por cutícula e têm características de absorção de eletrólitos e água. As células epiteliais do reto não apresentam características de células absortivas, diferentemente das células das papilas retais, provavelmente envolvidas na reabsorção de água e eletrólitos. Este trabalho reforça que a anatomia do intestino está relacionada com a forma do corpo do inseto, e mostra que o padrão geral de compartimentalização da digestão nos insetos é mantido em Phasmatodea, mas com algumas diferenças em comparação com outros Orthopteroidea.

Palavras Chave: insetos, histologia, bicho-pau, intestino

Phasmatodea insects (walking stick or leaf insects) are herbivores who use the similarity of their body to twigs, branches, leaves, or lichens as an advantage for camouflaging themselves with vegetation (Bedford 1978). They belong to a monophyletic group included within the Orthopteroidea along with Orthoptera, Blattaria, Dermaptera, Dictyoptera, Grylloblattodea, and Mantophasmatodea (Flook & Rowel 1998). Phasmatodea comprise about 3,000 species distributed among 3 families and 500 genera (Whiting et al. 2003), including the largest known insects such as *Phobaeticus chani* Bragg, whose females reach 567 mm in length (Hennemann & Cole 2008). Females of *Cladomorphus phyllinus* Gray (= *Phibalosoma phyllinum*) are apterous and reach 220 mm in length on full growth, and thus are one of the largest insects in Brazil. Males, unlike females, are winged and reach 150 mm in length (Lima 1938; Kumagai & Fonseca 2009).

The insect gut is divided into a foregut, a midgut, and a hindgut, according to their embryological origins. The foregut and hindgut arise from the ectoderm, while the midgut arises from the endoderm (Cruz-Landim 1985; Chapman 1998). The functions of the different parts of the insect gut vary among species. However, the foregut is usually involved in food storage and fragmentation, the midgut in digestive enzymes synthesis, digestion, and absorption, and the hindgut in excretion and electrolyte balance (Cruz-Landim 1985; Terra 1988; Chapman 1998).

The digestive tracts of insects may indicate the mode of life and phylogenetic relationships among different species (Terra 1990; Serrão 2001). Similar to other Orthopteroidea, Phasmatodea are chewing herbivores that feed on one or more plant species (Bedford 1976). However, data from the internal morphology of Phasmatodea are scarce. Thus, the aim of this study was to describe the morphology of the digestive tract of the adult female *C. phyllinus*.

MATERIALS AND METHODS

Insects

Adult females of *C. phyllinus* were obtained from mass rearing in the Fundação Zoobotânica, Belo Horizonte, Minas Gerais state, Brazil, where they were reared in natural environmental conditions.

Light Microscopy

Females of *C. phyllinus* were anesthetized using ethyl ether and dissected in a 125 mM NaCl solution. The digestive tract was removed and transferred to Zamboni's fixative solution (Stefanini 1967) for 24 h at room temperature. The digestive tracts were divided into the foregut, midgut, ileum, rectum, and Malpighian tubules. Next, the samples were dehydrated in an ethanol-graded series and embedded in the Leica Historesin®. Slices of 4-µm thickness were stained with hematoxylin and eosin and examined by a light microscope.

Scanning Electron Microscopy

The midguts were obtained as described, dehydrated in a graded series of ethanol dilutions, transferred to hexamethyldisilazane (HMDS) and air dried at room temperature for 10 min (Nation 1983). The organs were gold coated and examined under a LEO VP1430 scanning electron microscope in the Nucleus for Microscopy and Microanalysis at the Federal University of Viçosa.

RESULTS

The digestive tract of *C. phyllinus* is an elongated tube surrounded by muscles and tracheae, almost without external anatomical differences along its length, except for some short gastric caecae-like projections in the posterior midgut region (Fig. 1).

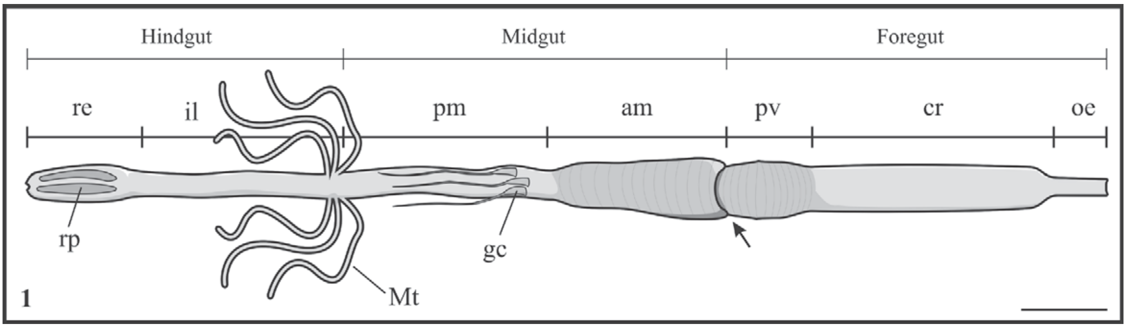


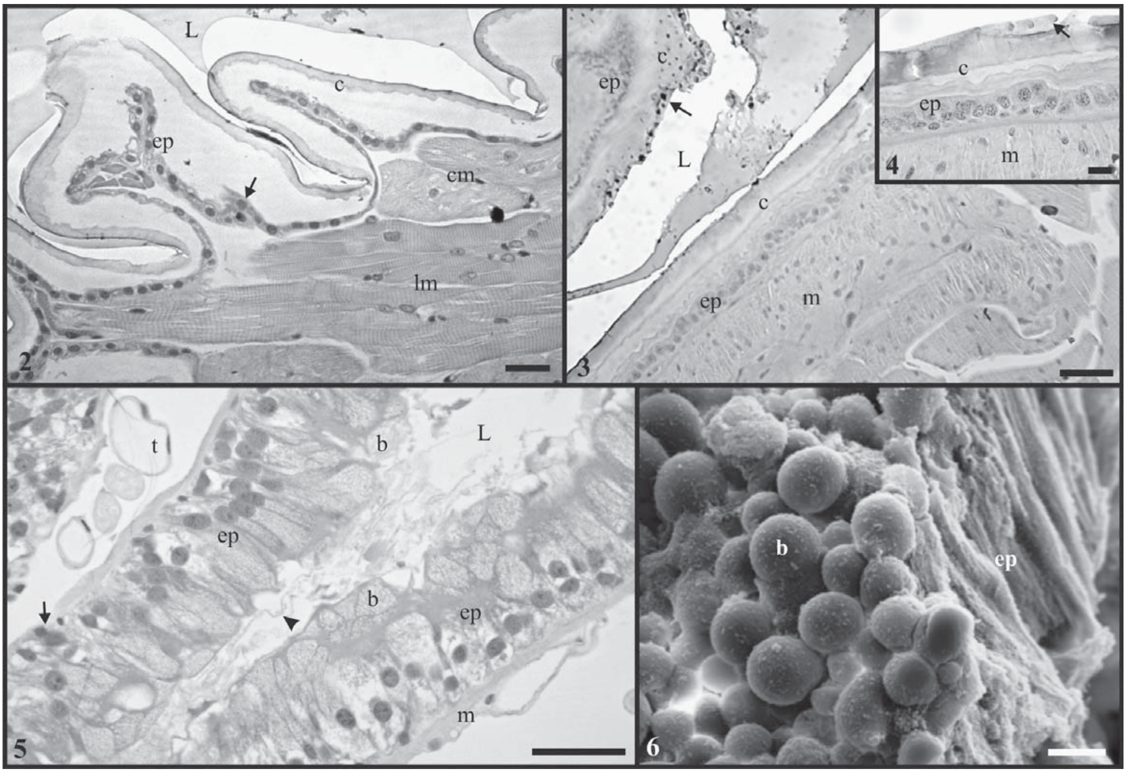
Fig. 1. Anatomy of the *Cladomorphus phyllinus* gut representing the oesophagous (oe), crop (cr), proventriculus (pr), anterior midgut (am), posterior midgut (pm), gastric-caecae like structures (gc), Malpighian tubules (Mp), ileum (il), rectum (rc) and rectal pads (rp). Arrow: transition between foregut and midgut. Bar = 1 cm.

The esophagus is a thin tube that extends across the thorax to the anterior end of the abdomen. The elongated crop takes up one-third of the foregut length with a diam slightly larger than that of the remaining gut. The proventriculus ends in a constriction as it joins the midgut. The midgut presents 2 distinct regions: the anterior midgut with a folded wall and the posterior midgut with unfolded wall, but with short tubular lateral gastric caecae. The passage of the midgut to the hindgut is characterized by the insertion of Malpighian tubules. In the hindgut, the ileum is an elongated tube with constant diam, followed by a larger tubular rectum (Fig. 1).

Histologically, the crop is lined by a single-layered epithelium with cubic epithelial cells each one containing a central and well-developed spherical nucleus with condensed chromatin. The lumen surface of the cells is coated by a thick cuticle. Externally, the epithelium is surrounded by well-developed muscle layers, consisting of inner circular and outer longitudinal layers (Fig. 2).

The proventriculus consists of a single-layered epithelium of cuboidal cells each with a central nucleus containing decondensed chromatin and a prominent nucleolus. The luminal surface of the cells is coated by a thick cuticle, which has projections forming chitinous teeth, while the basal portion of the epithelium is surrounded by well-developed muscle layers (Figs. 3 and 4).

The anterior midgut presents a single epithelium of prismatic cells with a median-basal nucleus containing decondensed chromatin and a nucleolus (Fig. 5). The cytoplasm of digestive cells is filled with small weakly acidophilus granules. The apical portion of digestive cells presents bubbles that release secretions together with the part of the cytoplasm immediately below the peritrophic membrane (Figs. 5 and 6). Groups of regenerative cells are scattered at the base of



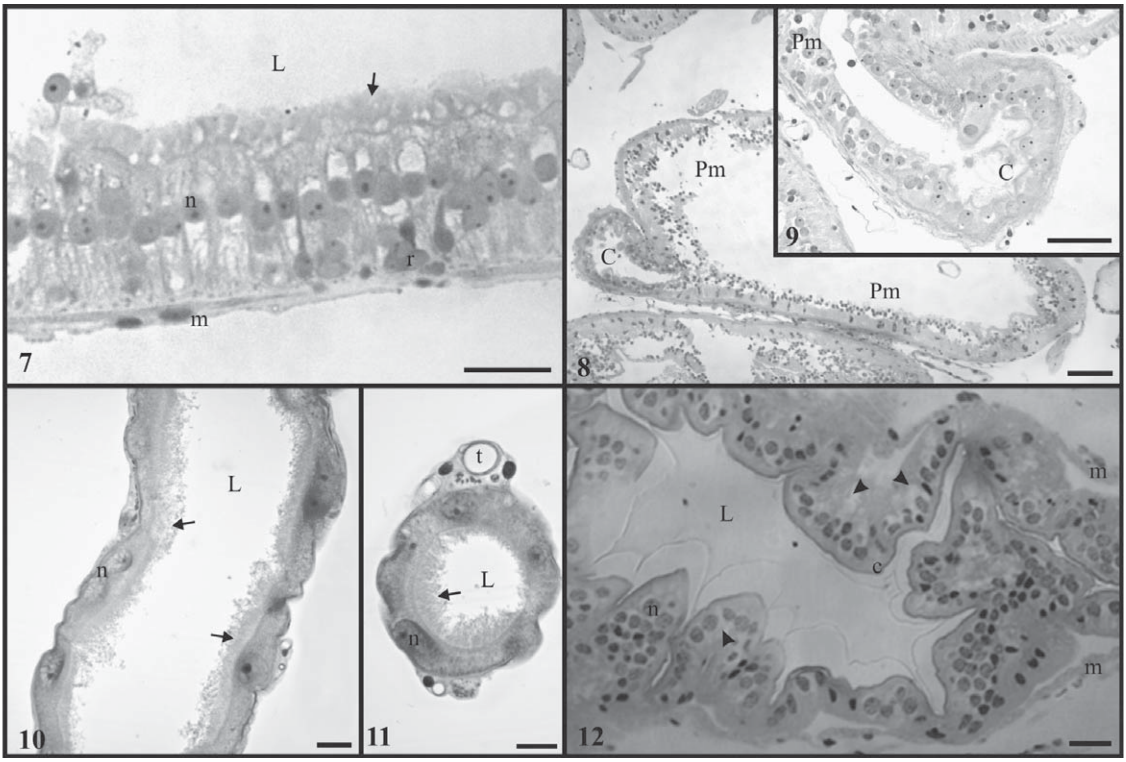
Figs. 2-6. Histological sections of gut of *Phibalosoma phyllinum.* **2.** Crop showing a folded epithelium (ep) coated by cuticle (c) in the lumen (L) and surrounded by 2 well-developed layers of circular (cm) and longitudinal (lm) muscles. Bar: 50 µm. **3.** Proventriculus with a simple epithelium (ep) coated by a thick cuticle (c) that projects teeth (arrow) into the lumen (L) and surrounded by well-developed muscle layers (m). Bar: 100 µm. **4.** Detail of the proventriculus epithelium (ep) showing the cuticle (c) with teeth projections (arrow). Bar: 20 µm. **5.** Anterior midgut epithelium (ep) showing the cell apex with vesicles and bubbles (b) of apocrine secretion. Regenerative cell nest (arrow) can be observed at the epithelium base. Bar: 100 µm. Insert: detail of the granular cytoplasm of epithelial cells. Bar = 40 µm. **6.** Scanning electron micrograph of the anterior midgut epithelium (ep) showing the bubbles (b) of apocrine secretion in the cell apex and the absence of striated border. Bar: 10 µm. Arrowhead: peritrophic membrane; L: lumen; m: muscle; t: trachea.

epithelium throughout the anterior midgut. The epithelium is coated with a thin layer of muscles (Fig. 5).

The posterior midgut is lined by a single-layered epithelium of prismatic cells each with a central spherical nucleus containing a prominent nucleolus. The cytoplasm has large vacuoles weakly acidophilous, and the apical cell surface presents striated border (Fig. 7). There are also short and tubular gastric caecae-like projections in the proximal part of the posterior midgut of *C. phyllinus,* (Fig. 1, 8, 9). These gastric caecae-like projections are enlarged at their insertions in the midgut wall and project as narrowed, free tubule, within the body cavity (Figs. 1, 10, 11). In both enlarged and narrow regions of the gastric caeca-like structure, the wall presents a simple epithelium of cuboidal cells each with a well-developed central nucleus with an evident nucleolus (Figs. 9-11). The epithelial cells of the gastric caecae-like projections show a long striated border in the cell apex (Figs. 10 and 11). In the enlarged portion of the gastric caecae-like projections there is a thin layer of muscle surrounding the epithelium (Fig. 9), which is absent in the narrowed region (Fig. 11). Two thin tracheal branches run along the entire length of the narrowed region of the gastric caecae-like projections (Fig. 11).

The ileum is characterized by a folded epithelium formed by a single layer of cubic cells each with a central nucleus. The lumen surface of the cells is coated by cuticle; the basal portion of cells is less stained. Muscles cover this portion of the gut externally (Fig. 12).

The rectum of *C. phyllinus* shows a simple epithelium formed by small cuboidal cells with a central spherical nucleus with clumps of condensed chromatin and a prominent nucleolus. The lumen surface of cells is covered by a cuticular layer (Fig. 13). This gut region contains in the anterior portion, rectal pads constituted by a pseudostratified epithelium of prismatic cells (principal cells) each with a well-developed nucleus and acidophilic cytoplasm. The rectal pad cells also are



Figs. 7-12. Histological sections of gut of *Cladomorphus phyllinus.* **7.** Posterior midgut epithelial cells containing vacuoles and striated border (arrow) on the apical surface and regenerative cells (r) in the basal portion. Bar: 50 µm. **8.** Transversal section of the posterior midgut (Pm) showing the origin of the gastric caecae-like projection (C) in the midgut wall. Bar: 200 µm. **9.** Detail of the posterior midgut (Pm) and gastric caeca (C) transition. Bar: 100 µm. **10.** The gastric caeca-like projection showing striated border (arrow) at the cell apex. Bar: 20 µm. **11.** Gastric caeca-like projections in transversal section showing striated border (arrow); L: lumen; n: epitelial cell nucleus; Bar: 20 µm. **12.** Ileum showing cuboidal cells covered by a cuticle (c) and surrounded by muscle cells (m). Note the less acidophilic region in the basal portion of cells (arrowheads) that characterizes the presence of folds in the basolateral membrane. Bar: 50 µm. L: lumen, m: muscle, n: nucleus, t: trachea and tracheolar cells.

covered with cuticle. Below the principal cells, some layers of flattened cells (the basal cells) are present (Fig. 14). Both the rectum epithelium and the rectal pad are coated externally by a muscle layer (Figs. 13 and 14).

The long, thin Malpighian tubules are composed of a single layer of cuboidal cells, each cell showing a well-developed nucleus with condensed chromatin clumps. The cell apex has a striated border and the cell cytoplasm was predominantly acidophilic. Four cells formed the circumference of a Malpighian tubule (Fig. 15).

DISCUSSION

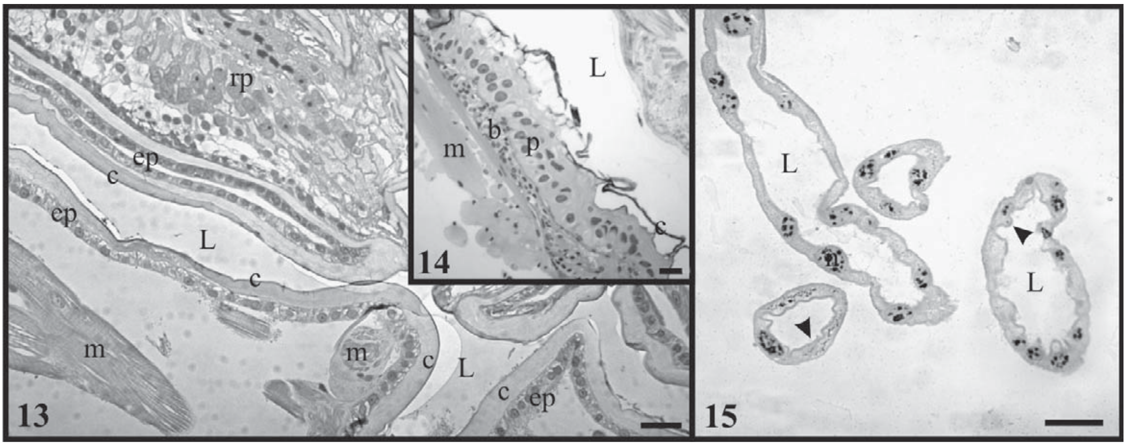
The cells of the crop of *C. phyllinus* have no morphological traits of absorptive or secretory activity. In chewing insects, the crop usually acts as a storage organ of the ingested food. This storage of food is usually associated with a crop epithelium that is highly folded when the crop is empty, but which allows distension coupled with a thick cuticle; and this occurs in the crop of *C. phyllinus*. Ingested food does not usually undergo biochemical changes in the crop because of the absence of enzyme secretion in this region (Dow 1986; Terra 1988). However, in some grasshoppers and cockroaches, initial digestion of carbohydrates, accomplished by enzymes regurgitated from the midgut, can occur in the crop (Terra 1990).

The transition between the crop and the midgut of *C. phyllinus* is marked by the presence of a proventriculus containing well-developed muscle and thick cuticle with teeth-like projections. This structure is usually engaged in grinding the food in addition to functioning as a valve that prevents backflow from the midgut (Cheesman & Pritchard 1984; Elzinga & Hopkins 1994; Szinwelski et al. 2009). Enzymatic digestion or absorption in this region is unlikely, because the proventricular cells, like those of the crop, did not exhibit characteristics of absorptive or secretory cells.

The cytological features of the anterior midgut epithelium of *C. phyllinus* suggest that this midgut region may be responsible for food digestion, since in each epithelial cell the presence of decondensed chromatin with a prominent nucleolus indicates abundant gene expression and synthesis of proteins. In addition, apocrine secretion of large cytoplasmic bubbles seems to occur in the apical portion.

In the midgut of insects holocrine secretion may occur when secretory vesicles are stored in the cytoplasm until they are released; at this time, the whole secretory cell is lost to the midgut lumen. In merocrine secretion, the limiting membrane of the secretory vesicle fuses with the apical plasma membrane, so that the cell empties its contents without any loss of cytoplasm (exocytosis). However, apocrine secretion involves the loss of the part of the apical cytoplasm in the process of releasing secretory vesicles (Terra & Ferreira 2012). Bees and *Tenebrio molitor* larvae have apocrine secretion (Cruz-Landim et al. 1996; Serrão & Cruz-Landim 2000; Cristofoletti et al. 2001), and hemipteran have microapocrine secretion (Fialho et al. 2009, 2013). The secretory mechanism in *C. phyllinus* was similar to that reported for *T. molitor* with the release of the part of apical cytoplasm of the digestive cells (Cristofoletti et al. 2001), and, thus, is characterized as the apocrine type.

Epithelial cells of the posterior midgut are each characterized by the presence of a well-de-



Figs. 13-15. Histological sections of gut of *Cladomorphus phyllinus*. **13.** Rectum showing the folded epithelium (ep) coated by cuticle (c) along the lumen (L) and surrounded by muscle cells (m) and the presence of rectal pads (rp). Bar: 50 µm. **14.** Detail of a rectal pad showing the principal cells (p) with thin cuticle (c) at the apex and the basal cells (b). L: lumen of the rectum, m: muscle cells. Bar: 20 µm. **15.** Malpighian tubules showing cells with striated border (arrowhead) on the apical surface. Bar: 20 µm. L: lumen of the rectum, m: muscle cells, n: nucleus.

veloped striated border relative to the digestive cells of the anterior midgut, which suggests a role of nutrient absorption; moreover the occurrence of apocrine secretion or release of cytoplasmic vesicles into the lumen was not observed. However, enzymatic digestion in the posterior midgut of *C. phyllinus* may occur by the action of enzymes linked to the epithelial plasma membrane (Billingsley & Lehane 1996).

The gastric caecae-like projections with short enlarged regions close to the midgut wall followed by a narrow and long tubule that is free in the body cavity has not been described in other Orthopteroid insects (Bracke et al. 1979; Terra 1990; Marana et al. 1997; Roszkowska 2008; Biagio et al. 2009). The gastric caecae-like projections probably play a role in nutrient absorption because the epithelial cells have extensive striated borders on their apical surfaces. In *C. phyllinus*, the gastric caecae-like projections are associated with the posterior midgut, unlike in other Orthopteroidea such as Acrididae and Gryllidae (Orthoptera) and Blattidae (Blattaria), where gastric caecae are located in the anterior midgut and act in the processes of digestion by secreting enzymes and absorbing water and nutrients (Bracke et al. 1979; Terra 1990; Marana et al. 1997; Roszkowska 2008; Biagio et al. 2009). The position of the gastric caecae-like projections in *C. phyllinus* suggests that they perform nutrient absorption from food previously digested in the anterior and posterior midgut.

The hindgut – subdivided into ileum and rectum – is responsible for excretion and water reabsorption from food and primary urine produced by Malpighian tubules; thus contributing to the osmotic control of insects (Chapman 1998). Herbivorous insects usually obtain water from leaves and much of the water present in the primary urine produced by the Malpighian tubules is reabsorbed in the ileum and rectum (Audsley & Phillips 1995; Azuma et al. 2012). Histologically, the ileum cells of *C. phyllinus* have characteristics of fluid transport cells, based on a basal less acidophilic region that is indicated by the presence of a developed system of folds in the basal membrane. The latter creates spaces for intercellular transport of water and small molecules (Cruz-Landim 1994; Santos & Serrão 2006).

In the rectum, water absorption occurs primarily through the rectal pads. The rectal pads are composed of 3 cell types: the columnar principal cells, which remain in contact with the gut lumen, and which are covered by a thin cuticle; the junctional cells, which are tall and narrow and which separate the principal cells of the epithelium of the rectum; and the basal cells, which are positioned below the principal cells, and which separate them from the hemocoel (Chapman 1998). In Hymenoptera and Lepidoptera, the rectal pads present 2 layers of principal cells, while in other orders the rectal pads consist of only one layer of cells (Noirot & Noirot-Timothée 1976; Chapman 1998). The rectal pads of *C. phyllinus* have only one layer of principal cells arranged as a pseudo-stratified epithelium over the basal cells, as is also observed in cockroaches (Noirot & Noirot-Timothée 1976). The model of water absorption by rectal pads involves the formation of an osmotic gradient in the intercellular spaces by the active transport of ions by the principal cells (Noirot & Noirot-Timothée 1976; Garayoa et al. 1999).

Comparison of the digestive tract of *C. phyllinus* with those of other Orthopteroidea showed differences in apocrine secretion and the occurrence of gastric caecae in the posterior midgut. However, the general pattern of the compartmentalization of digestion in insects was maintained, i.e., with the foregut involved in storage and breakdown of food, the midgut involved in digestion and absorption of nutrients, and the hindgut involved in water reabsorption.

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