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Anatomy and histology of the alimentary canal and ovarioles of *Ceraeochrysa cubana* adults

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Abstract

Lacewings are generalist widespread predators with potential for use in biological control programs. The objective of this study was to describe the anatomy and histology of the alimentary canal and the ovarioles of *Caraeochrysa cubana* (Hagen) (Neuroptera Chrysopidae) adults. In the foregut the crop, proventriculus stomodeal valve are well developed. In the midgut the digestive cells, had a well-developed striated border, evident peritrophic matrix. Externally the midgut wall showed a layer of circular muscle followed by longitudinal ones. The mid-hindgut transition was characterized by the opening of numerous Malpighian tubules that were thin and formed by a single layer of cuboidal cells with small nucleus. The final portion of the alimentary canal of *C. cubana* was formed by a sac-like rectum that showed the wall with a layer of flattened cells and six rectal pads characterized by an inner layer of columnar cells and an external one of flattened cells, separated by a narrow space. The ovaries were of meroistic polytrophic type, like other species of the order Neuroptera.

Key words: gut, ovary, lacewings.

Introduction

Lacewings are generalist predators with worldwide distribution found in diverse habitats (Albuquerque *et al.*, 1994; Chen *et al.*, 2006). The food resources used by these insects varies with the insect developmental stage, with larvae feeding on aphids, grasshoppers, whiteflies, thrips, and eggs of various insects (Canard, 2001; McEwen *et al.*, 2001), whereas adults consume honeydew, pollen grains and nectar (New, 1975; Principi and Canard, 1984; McEwen *et al.*, 2001).

Chrysopidae larvae are predators (Stelzl and Devetak, 1999; Tavares *et al.*, 2012) and have potential as control insect pests, such as aphids, whitefly pupae and moths (Stelzl and Devetak, 1999; McEwen *et al.*, 2001; Pappas *et al.*, 2007; Barbosa *et al.*, 2008). *Caraeochrysa cubana* (Hagen) larvae are potential agents of biological control of lace bug pest (Tingidae) in palm (Serrano *et al.*, 1988) and citrus crops (Venzon and Carvalho, 1993). Adults are mostly phytophagous (except the genus *Chrysopa*) (Stelzl and Devetak, 1999), with chewing-biting mouthparts and do not interfere directly in the biological control, but they are used in mass rearing (Biagioni and Freitas, 2001; Mantoanelli and Albuquerque, 2007) and are important to be studied because contribute to the reproductive success and maintenance of the populations in the field.

The alimentary canal (Ickert, 1968; Principi and Canard, 1984; Woolfolk *et al.*, 2004; Hemalatha *et al.*, 2014; Scudeler *et al.*, 2016) and the female reproductive tract (Kubrakiewicz *et al.*, 1997; Garbiec and Kubrakiewicz, 2012) of lacewings adults have been studied in a limited number of species. In this context, *C. cubana* is a species that had been little studied with this approach. Although those studies provide important data on the morphology, association with symbiotic microorganisms and insecticides effects, detailed information on the anatomy and histology of the alimentary canal and ovarioles of *C. cubana* are scarce and, therefore, additional studies are necessary for shed light on an important potential biological control agent. Morphological studies of internal organs are important for understanding the basic organization them as well as for propose functional hypothesis and contribute to comparative studies with insects reared in different conditions.

This knowledge will be useful in comparative studies of the anatomy and histology of closely related species or in individuals of the same species which have been submitted to stress such as exposure to pesticides. In this context, the objective of this study was to describe the anatomy and histology of the alimentary canal and ovarioles of *C. cubana* adult females.

Materials and methods

Insects

Six female of *C. cubana* 5-7 days old were obtained from a third generation culture kept in the Acarology Laboratory of the Federal University of Viçosa at 25 ± 2 °C, 60 ± 10% RH and 12 h photoperiod and fed with yeast and honey *ad libitum*. Larvae were fed on *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) eggs.

Anatomy

Females were cryo-anesthetized at 4 °C, dissected in NaCl 125 mM with aid of scissors and forceps and the alimentary canal and ovarioles were isolated and photographed with stereomicroscope.

Histology

The alimentary canal and ovarioles after dissection were transferred to Zamboni fixative solution (Stefanini *et al.*, 1967) for 12 h at 4 °C. After, the organs were de-

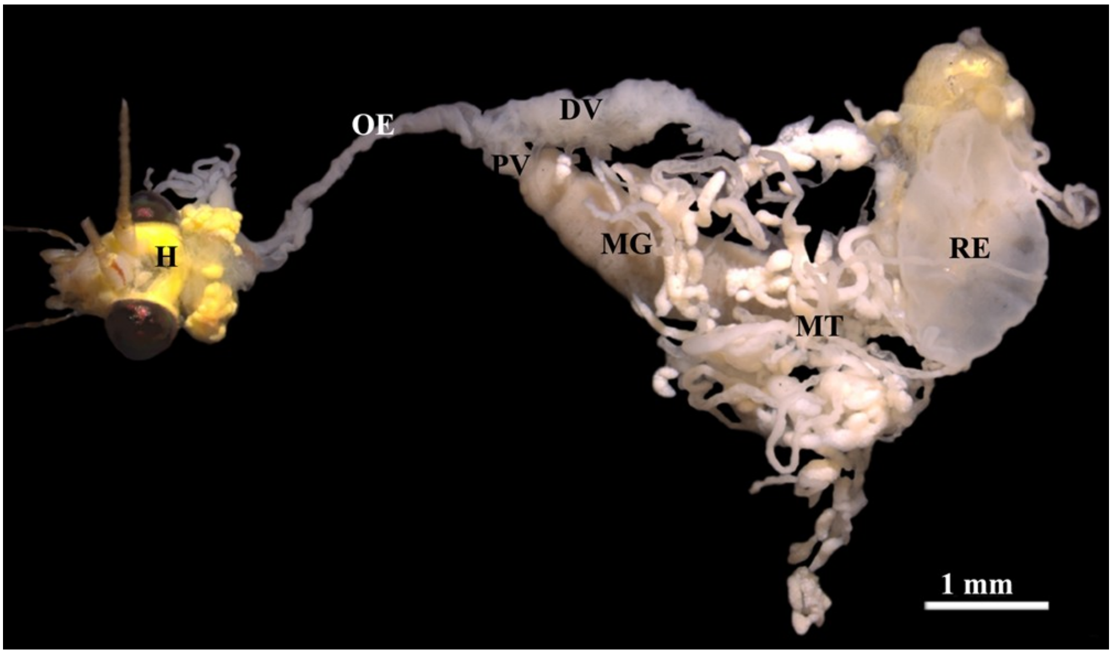

Figure 1. Alimentary canal of *C. cubana* showing the oesophagous (OE), crop diverticulum (DV), proventriculus (PV), midgut (MG), rectum (RE) and Malpighian tubules (MT). H - head.
(In colour at www.bulletinofinsectology.org)

hydrated in a graded ethanol series (70, 80, 90 and 95%) for 10 minutes each bath and embedded in historesin (Leica) for 18 h. Slices 2 µm thick were obtained with rotatory microtome using glass knives, placed on microscopic slides and subsequently stained with hematoxylin (10 min), eosin (30 s) and examined by light microscopy. Images were obtained with digital camera using the computer program Q-capture (Olympus).

Results

Alimentary canal: anatomy

The foregut was anatomically divided into a short pharynx something dilated, connected to a long and narrow oesophagus (figure 1). The posterior portion of the foregut was dilated in a crop which showed a convoluted diverticulum (figure 1). The distal region of the crop had a constriction characterizing the proventriculus that occurs in the transition to the midgut. The midgut was an enlarged tube with constant diameter (figure 1). In the transition mid-hindgut opened many Malpighian tubules characterizing the pylorus, the first hindgut region, followed by a narrow ileum and an enlarged saclike rectum with six rectal pads (figure 1).

Histology

The wall of the pharynx and oesophagus had a single layer of flattened cells, lined by a thin cuticle. Externally to the epithelium there were well-developed circular and longitudinal muscles.

The crop and its diverticulum showed folded wall with a single layered epithelium with flattened cells, covered internally by a thick cuticle and with external muscle layers (figures 2A and 2B). The crop and diverticulum lumen was filled with yeast cells (figure 2A).

The proventriculus was a more differentiated foregut region, with a bulb into the crop lumen and a stomodeal valve that protruded into the midgut joined by a short neck. The proventriculus bulb was conic-shaped with eight wall folds (figure 2C) with a single layered flattened epithelium , which was lined by a thick cuticle containing spine-like projections (figure 2D). The circular and longitudinal muscles were well developed (figure 2D). The stomodeal valve was characterized by a projection of the foregut epithelium into the midgut lumen, which fold and form two layers (figure 2E). The inner layer had flattened cells and the external one cuboidal ones which become smaller in the transition to the midgut epithelium.

Midgut

The midgut epithelium had a single layer of columnar digestive cells with well-developed nucleus containing decondensed chromatin (figure 3A). In the middle and posterior midgut portions was frequent the occurrence of apical cytoplasm protrusions to the lumen (figure 3B). The apical surface of the columnar cells showed a short striated border (figure 3A). In the midgut lumen there was a thin peritrophic matrix forming the endoperitrophic space containing food rich in yeast cells, and the ectoperitrophic ones with homogeneous aspect (figure 3B). Scattered among the base of the digestive cells there were regenerative cells nidi (figure 3A). Externally the midgut wall showed a layer of circular muscle followed by another longitudinal layer (figure 3B).

Hindgut

The mid-hindgut transition was characterized by the opening of numerous Malpighian tubules that were thin and formed by a single layer of cuboidal cells with small nucleus (figure 4A). Posterior to the Malpighian

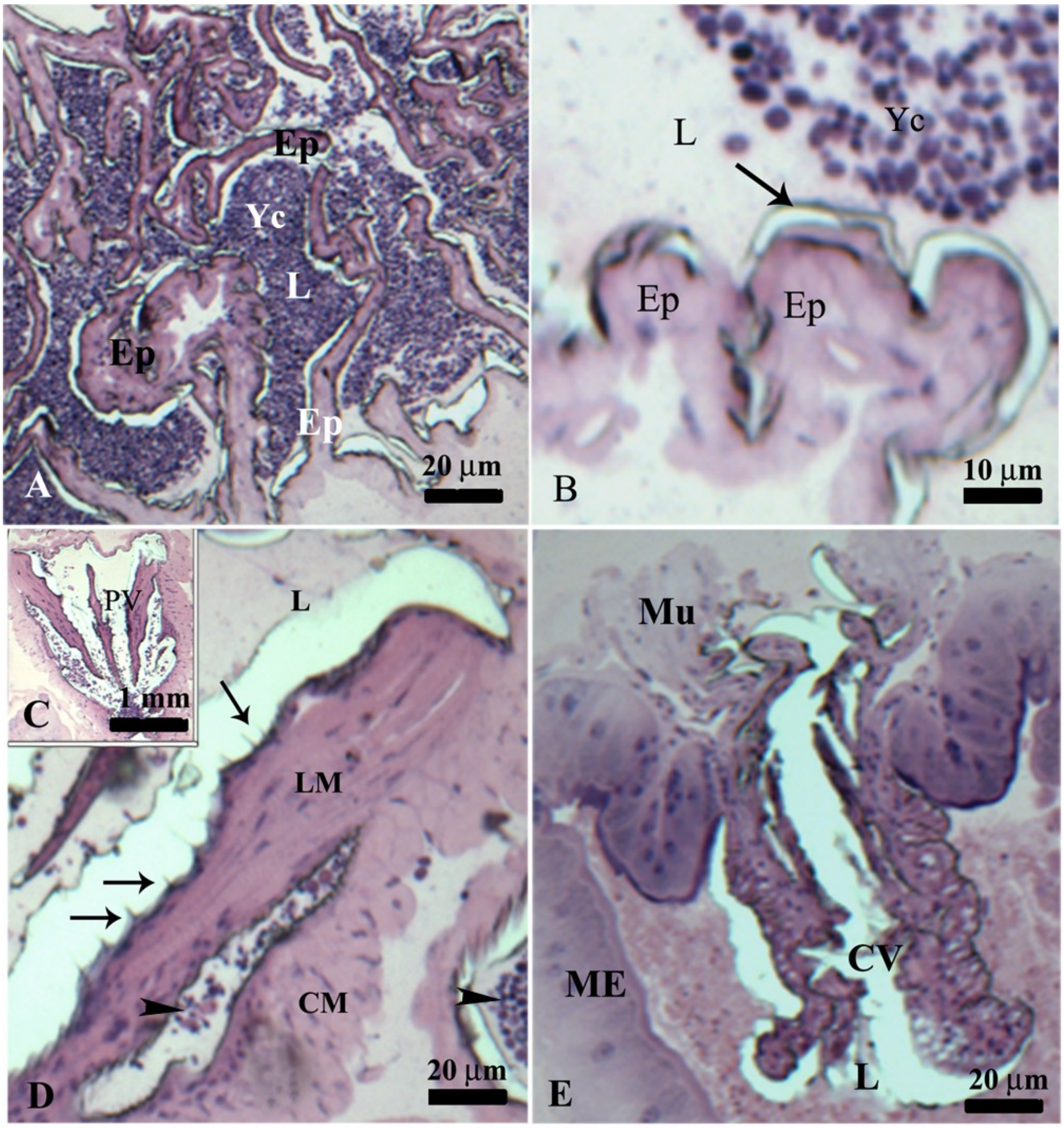

Figure 2. Light micrographs of the foregut of *C. cubana*. **A)** Cross section of the crop showing a folded epithelium (Ep). Note the lumen (L) filled with yeast cells (Yc). **B)** Detail of the crop epithelium showing a cuticle (arrow) lining a narrow epithelium (Ep). **C)** Longitudinal section of the proventriculus (PV) showing three folds of the wall. **D)** Detail of a proventricular fold showing well developed longitudinal muscles (LM) and circular muscles (CM). Note a narrow epithelium lined by cuticle with spine-like projections (arrows) and yeast cells (arrowhead) in the lumen. **E)** Longitudinal section of the fore- midgut transition showing the folded cardiac valve (CV) and the midgut epithelium (ME). L - lumen, Mu - muscle.
(In colour at www.bulletinofinsectology.org)

tubules insertion was the ileum with an epithelium containing a single layer of tall cells with apical and basal regions weakly stained with a striated aspect (figure 4B). The lumen of the organ was lined by a thin cuticle (figure 4B). The final portion of the alimentary canal of *C. cubana* was formed by an enlarged sac-like rectum that showed the wall with a layer of flattened cells (figure 4C) and six rectal pads characterized by an inner layer of columnar cells and an external one of flattened cells, separated by a narrow space (figure 4D).

Ovarioles

The ovaries of *C. cubana* had eight ovarioles each (figure 5). The ovarioles of all dissected specimens from five to seven days old showed oocytes in different developmental stages (figures 5 and 6A). Each ovariole was lined by a peritoneal sheath (figure 6B). The distal portion of ovarioles had a short and narrow terminal filament filled with small cells, followed by a short germarium and a long vitellarium (figure 6A). The germarium was filled by globular cells with well-developed nucleus (figure 6A). In the vitellarium, each follicle had

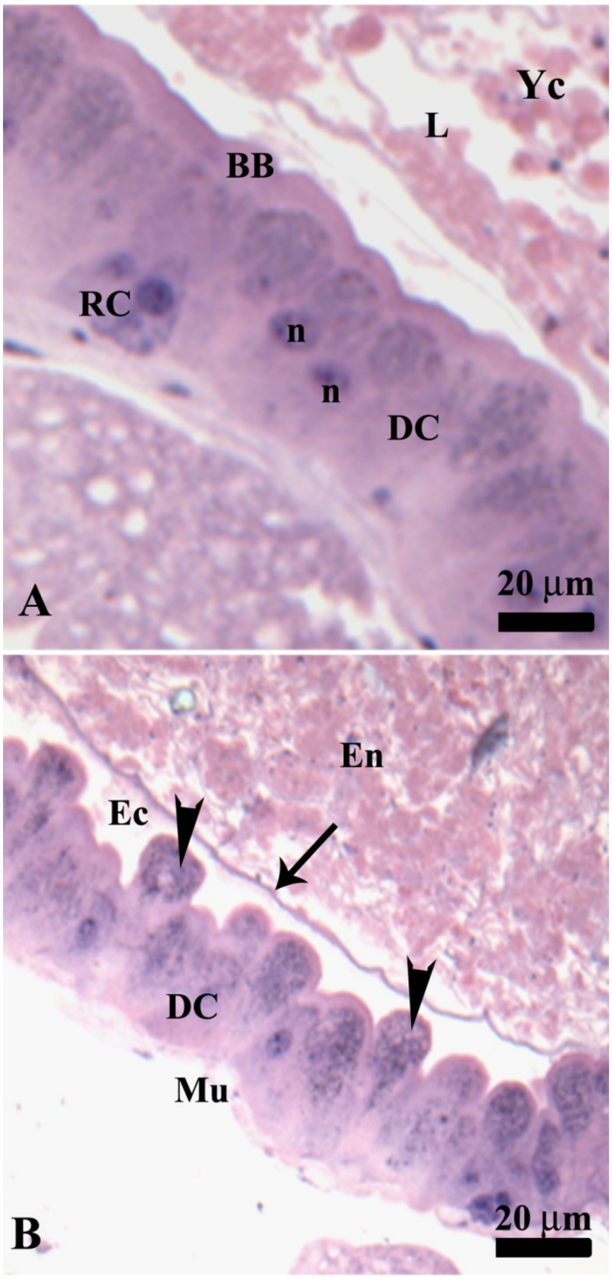

Figure 3. Light micrographs of the midgut of *C. cubana*. **A)** Longitudinal section of the anterior midgut region showing the epithelium with digestive cells (DC) with well-developed nucleus (n) and brush border (BB). Note a nest of regenerative cells (RC) and the lumen (L) with yeast cells (Yc). **B)** Longitudinal section of the posterior midgut region showing the digestive cells (DC) with apical protrusions (arrowheads) to the lumen. Note the peritrophic matrix (arrow) and the endoperitrophic (En) and ectoperitrophic (Ec) spaces. L - lumen, Mu - muscle layer.
(In colour at www.bulletinofinsectology.org)

an oocytic chamber with an oocyte, associated with a nurse chamber containing the nurse cells (figures 6A and 6B). In the young follicles, closely to the germarium, the oocytes showed homogeneous basophil content and a well-developed germinal vesicle (nucleus) with predominance of decondensed chromatin (figure 6B). In this ovary region, the nurse cells showed acidophilus cytoplasm and large nucleus with decondensed chromatin and evident nucleolus (figure 6B). In these follicles, the nurse chamber was lined by a single layer of flattened follicular cells, whereas the follicular cells lining the oocytic chamber were cuboidal (figure 6B).

In the proximal portion of ovarioles, oocytes were found in vitellogenic stage characterized by the storage of granules with different staining affinities, and the lining follicular cells with enlarged intercellular spaces (figure 6C). In this stage of oocyte maturation the nurse chamber showed atrophied nurse cells, characterized by their volume decrease in and nucleus with chromatin highly condensed similar to pyknosis.

Discussion

The anatomy of the alimentary canal of *C. cubana* adults is similar to those described for others Chrysopidae. The alimentary canal of Chrysopidae adults is characteristic the presence of a crop diverticulum of course also present in *C. cubana.* This diverticulum has a folded wall similar to the crop of other insects that store food temporarily in this organ, allowing a great expansion such as in bees and ants (Snodgrass, 1935). In Chrysopidae adults, this crop diverticulum seems to store symbionts yeasts that proliferate in this organ (Hagen *et al*., 1970; Woolfolk *et al*., 2004) similar to found in this study. The yeasts in the digestive tract of adult honeydews Chrysopidae have been suggested to provide some additional amino acids such as valine (Hagen *et al*., 1970) indicating a symbiont relationship. However, we report that yeast cells were found, also, into the midgut lumen of *C. cubana*, suggesting that they may be a nutrient source in addition to a mutualistic role, because the midgut is the organ responsible for the digestion and absorption in insects (Terra, 1988). In *Chrysoperla rufilabris* (Burmeister) adults, yeasts have been supposed to serve as food source in addition to a mutualistic symbiosis (Woodfolk *et al*., 2003; 2004).

The proventriculus bulb of *C. cubana* has eight folds with strong muscular wall and covered by a cuticle with spine-like projections. The proventriculus of insects has been reported as a food triturating with strongly sclerotized plaques and/or teeth as in some Orthoptera (Snodgrass, 1935; Fontanetti *et al*., 2002; Szinwelski *et al*., 2009). However, in *C. cubana* the proventriculus seems to have a filter function due to the presence of spine-like projections, such as in social Hymenoptera (Peng and Marston, 1986; Serrão, 2001), in which muscle contraction promote the movement of cuticular spines that transfer the particulate food to the midgut lumen (Bailey, 1952; Peng and Marston, 1986). *C. cubana* likely other Chrysopidae adults feed on nectar and pollen grains and proventriculus action may transfer the pollen grains to the midgut.

The midgut digestive cells of *C. cubana* adults have features of high metabolic activity, as the presence of well-developed nucleus with decondensed chromatin and nucleolus and apical brush border, similar to that reported for *Caraeochrysa claveri* (Navas) adults

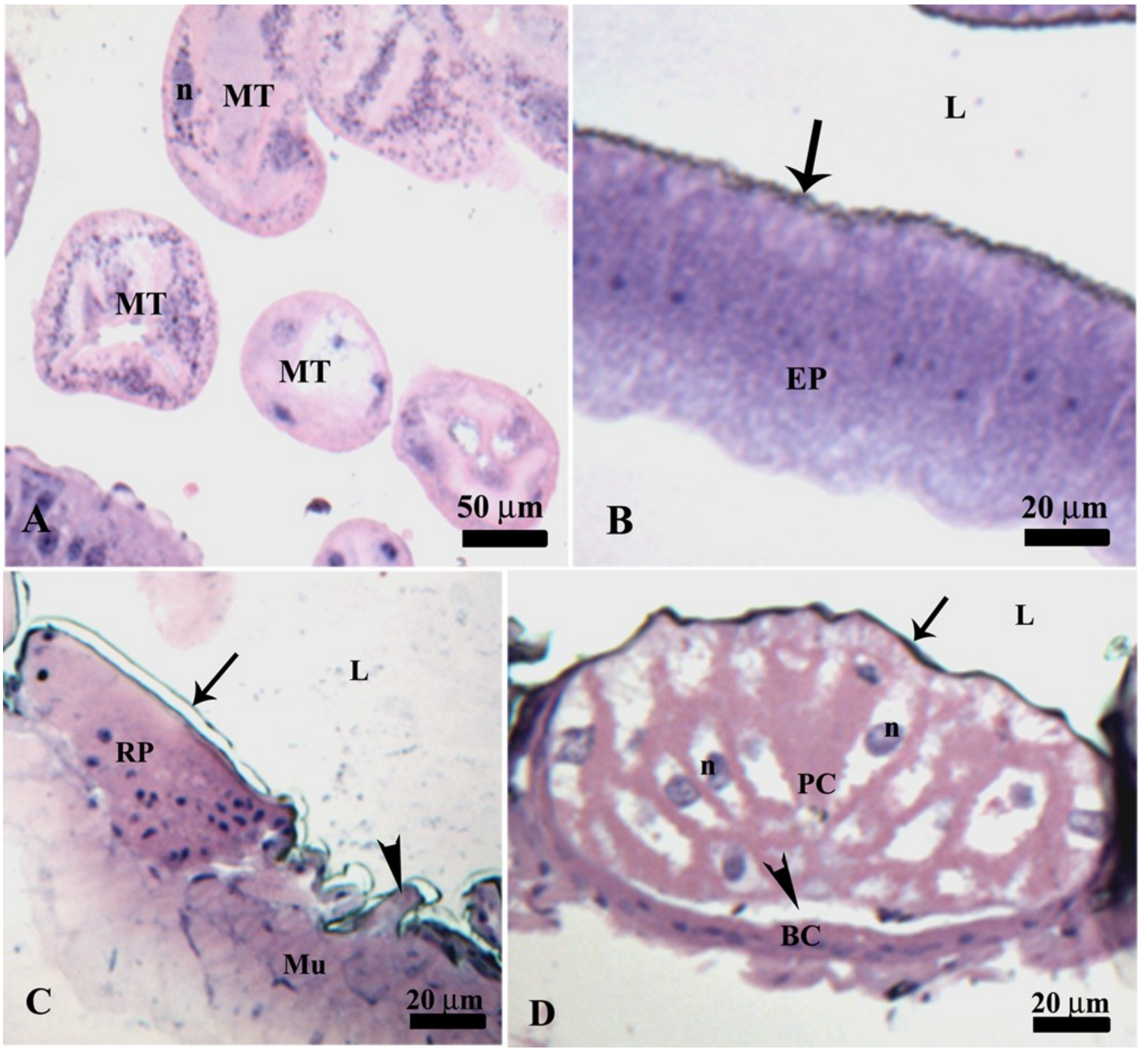

Figure 4. Light micrographs of the hindgut of *C. cubana*. **A)** Cross section of Malpighian tubules (MT). **B)** Longitudinal section of ileum showing columnar epithelium (Ep) lined by a thin cuticle (arrow). **C)** cross section of the rectum showing a flattened epithelium (arrowhead) lined by cuticle (arrow) and a rectal pad (RP). **D)** Detail of a rectal pad showing columnar principal (PC) and basal flattened cells (BC) separated by a narrow space (arrowhead). L - lumen, Mu - muscle, n - nucleus.
(In colour at www.bulletinofinsectology.org)

(Scudeler *et al*., 2016). Although in this latter occurs different digestive cells between the anterior and posterior midgut regions (Scudeler *et al*., 2016), our results show that this does not occurs in *C. cubana.* This may be due to the fact that *C. claveri* (Scudeler *et al*., 2016) was studied immediately after adult emergence, without a first feeding, which may have digestive cells in differentiation process from the pupal stage. Newly emerged insects have digestive cells with some features of the previous developmental stage, and may be in development process (Serrão and Cruz-Landim, 1996; Teixeira *et al*., 2013).

The occurrence of digestive cells with apical protrusions suggests that *C. cubana* adults have intense synthesis of digestive enzymes and / or compounds of the peritrophic matrix. The apical protrusions in digestive cells generally contain digestive enzymes (Cruz-Landim *et al*., 1996; Jordão *et al*., 1996; Fialho *et al*., 2009) and peritrophic matrix compounds (Marques-Silva *et al*., 2005; Teixeira *et al*., 2015).

Figure 5. Ovaries of *C. cubana* showing ovarioles (OV) with oocytes (OO) in different developmental stages.
(In colour at www.bulletinofinsectology.org)

In addition to the digestive cells, the midgut of *C. cubana* adult has regenerative cell nidi that are responsible for the replacement of the digestive cells (Martins *et al*., 2006; Nardi and Bee, 2012). This suggests that Chrysopidae adults have intense feeding, which results in stress of digestive cells that need to be replaced (Hakim *et al*., 2001; Tettamanti *et al*., 2007; Rost-Roszkowska *et al*., 2010).

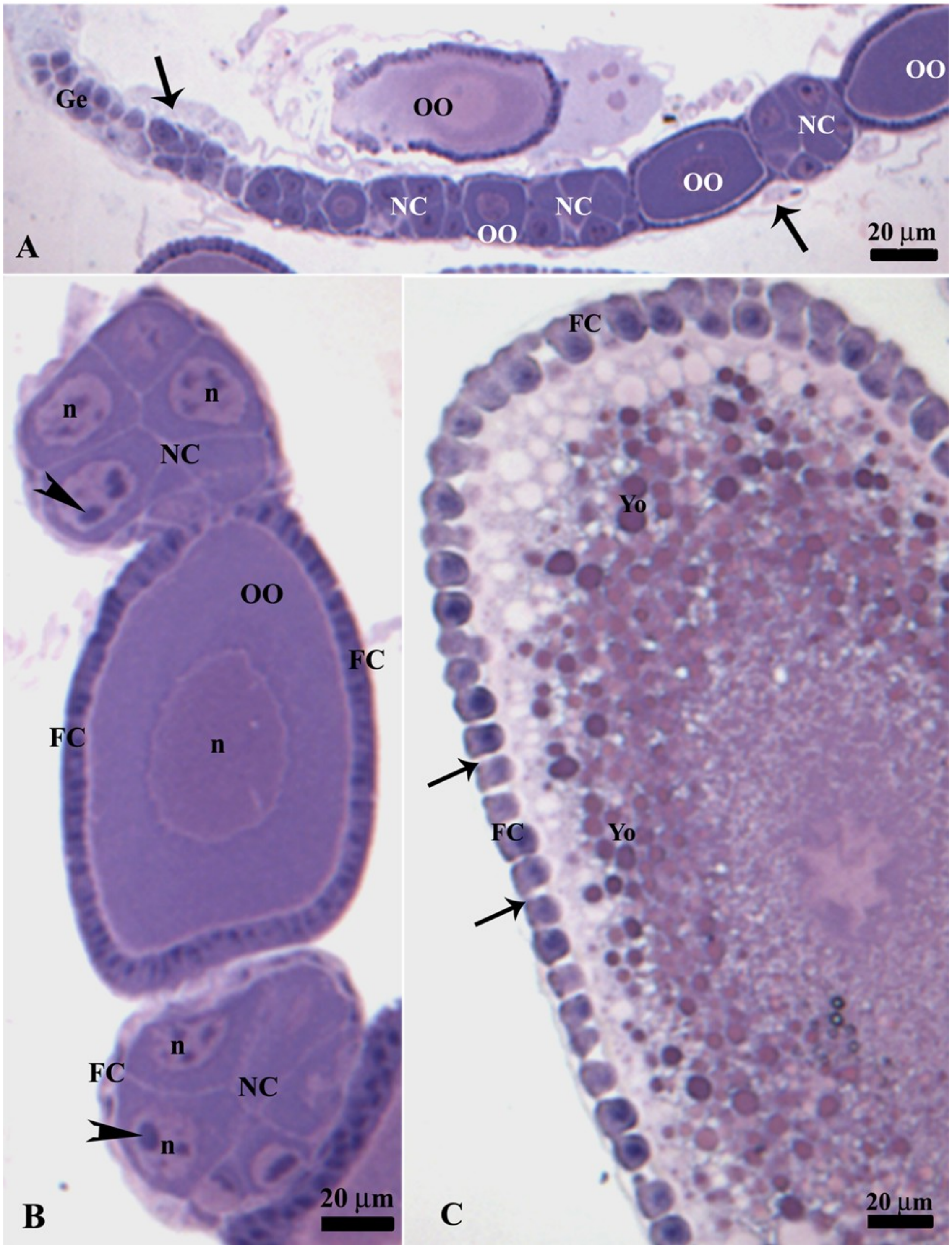

Figure 6. Light micrographs of the ovariole of *C. cubana*. **A)** Longitudinal section showing the germarium (Ge) and the follicles with nurse (NC) and oocytic chambers (OO) enveloped by a peritoneal sheath (arrows). **B)** Details of nurse cells (NC) with well-developed nucleus (n) containing nucleolus (arrowheads), oocyte with homogeneous cytoplasm and large nucleus (n) with decondensed chromatin. Note the cuboidal follicular cells (FC) in the oocytic chamber and flattened ones in the nurse chamber. **C)** Detail of vitellogenic oocyte with yolk granules (Yo) and follicular cells (FC) with enlarged intercellular spaces (arrows).
(In colour at www.bulletinofinsectology.org)

The features of the epithelial cells of the ileum of *C. cubana* adults is similar to that described for other insects, suggesting an important function in ions and water absorption from the primary urine released by the Malpighian tubules in this organ (Peacock, 1986; Cruz-Landim, 1994; Woolfolk *et al*., 2004; Santos and Serrão, 2006, Gonçalves *et al*., 2014).

The sac-like rectum of *C. cubana* with six rectal pads is similar to that described for *C. rufilabris* (Woolfolk *et al*., 2004) that seems to be a characteristic number for insects, although the number can vary between species (Berridge and Gupta, 1967; Jarial, 1992; Serrão *et al*., 2004). The presence of two cell layers separated by a space in the rectal pads of *C. cubana* is similar to that reported for Neuroptera (Snodgrass, 1935), which play a role in the final absorption of water and ions (Berridge and Gupta, 1967).

In the females of *C. cubana* analyzed there are eight ovarioles per ovary, different to found in *Chrysopa perla* (L.) and *Chrysoperla carnea* (Stephens) that have from eight to 12 ovarioles per ovary and in *Chrysopa pallens* Rambur with 20 ovarioles (Principi and Canard, 1984).

The ovary of *C. cubana* is of meroistic polytrophic type, with oocytes in different developmental stages as reported for other Neuroptera, which are more archaic holometabolous insects with this ovary type (Kubrakiewicz *et al*., 1997; Garbiec and Kubrakiewicz, 2012).

The vitellogenic follicles of *C. cubana* have follicular cells with enlarged intercellular spaces, suggesting the occurrence of patency, which allows the transport of vitellogenin yolk precursor from the hemolymph to the oocyte surface as occurs in Hemiptera, Lepidoptera and some Hymenoptera (Anderson and Telfer, 1970; Davey *et al*., 1993; Ronnau *et al*., 2015).

The morphology of the alimentary canal and ovarioles in many Chrysopidae adults remain yet unknown and our data on these organs in *C. cubana* provide more information for future studies, such as on the effect of stress agents as insecticides in these non-target insects.

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